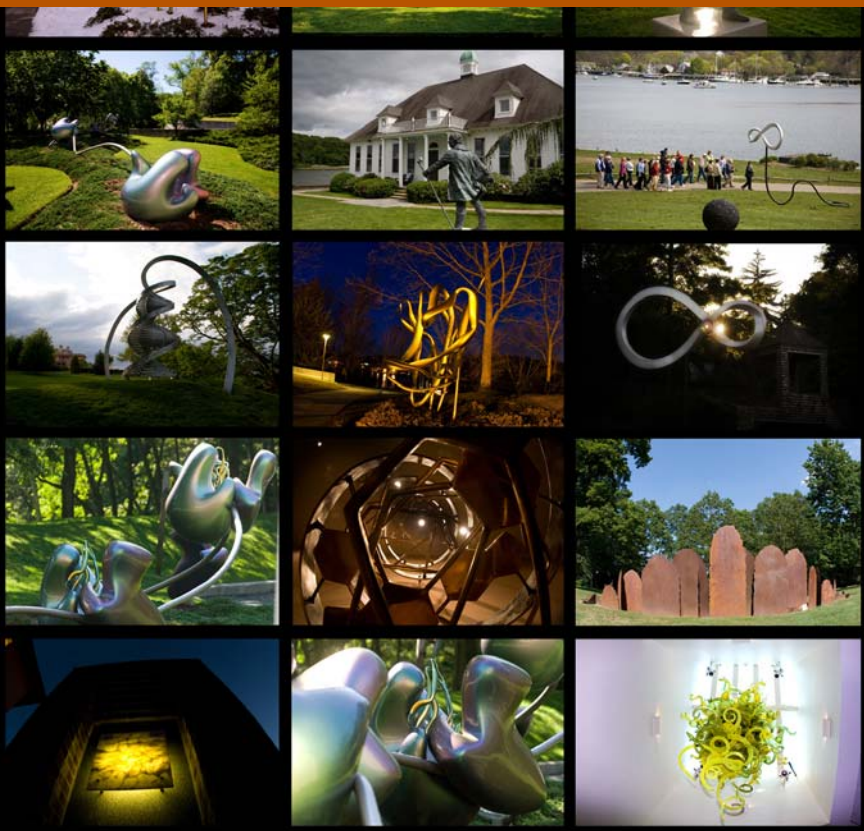
Abstracts of papers presented
at the 2010 meeting on

MECHANISMS & MODELS OF CANCER

August 17–August 21, 2010



Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

Abstracts of papers presented
at the 2010 meeting on

MECHANISMS & MODELS OF CANCER

August 17–August 21, 2010

Arranged by

Dafna Bar-Sagi, *New York University Medical Center*
Jacqueline Lees, *MIT Center for Cancer Research*
Charles Sherr, *HHMI/St. Jude Children's Research Hospital*
William Weiss, *University of California, San Francisco*

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MECHANISMS & MODELS OF CANCER
Tuesday, August 17 – Saturday, August 21, 2010

Tuesday	7:30 pm	**Keynote Speaker** **1** Apoptosis and Autophage
Wednesday	9:00 am	**2** Signaling Mechanisms I
Wednesday	2:00 pm	**3** Poster Session I
Wednesday	4:30 pm	Wine and Cheese Party *
Wednesday	7:30 pm	**4** Mouse Models
Thursday	9:00 am	**5** Rb and p53 Networks
Thursday	2:00 pm	**6** Poster Session II
Thursday	7:30 pm	**7** Microenvironment, Inflammation and Metastasis
Friday	9:00 am	**8** Experimental Therapeutics
Friday	2:00 pm	**9** Genomics, Proteomics, Epigenetics
Friday	6:00 pm	Banquet
Saturday	9:00 am	**10** Signaling Mechanisms II

Poster sessions are located in *Bush Lecture Hall*
* *Airslie Lawn*, weather permitting

Mealtimes at Blackford Hall are as follows:
Breakfast 7:30 am-9:00 am
Lunch 11:30 am-1:30 pm
Dinner 5:30 pm-7:00 pm
Bar is open from 5:00 pm until late

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PROGRAM

TUESDAY, August 17—7:30 PM

SESSION 1 APOPTOSIS AND AUTOPHAGY

Chairperson: **E. White,** Rutgers University, New Brunswick, New Jersey

KEYNOTE SPEAKER

Joan Brugge
Harvard Medical School

Eileen White.
Presenter affiliation: Rutgers University, New Brunswick, New Jersey.

Cyclopamine and the sonic hedgehog pathway in melanoma maintenance and chemoresistance
Agnieszka Checinska, Fernando García, Keith Ashman, Maria Soengas.
Presenter affiliation: CNIO Spanish National Cancer Research Centre, Madrid, Spain. 1

Interplay between oncogene-induced DNA damage response and heterochromatin in senescence and cancer
Fabrizio d'Adda di Fagagna.
Presenter affiliation: IFOM Foundation, Milan, Italy. 2

The pro-apoptotic function of the retinoblastoma tumor suppressor protein
Alessandra Ianari, Tiziana Natale, Eliezer Calo, Elisabetta Ferretti, Edoardo Alesse, Isabella Screpanti, Kevin Haigis, Alberto Gulino, Jacqueline Lees.
Presenter affiliation: MIT, Cambridge, Massachusetts; University of Rome, La Sapienza, Rome, Italy. 3

WEDNESDAY, August 18—9:00 AM

SESSION 2 SIGNALING MECHANISMS I

Chairperson: **R. Shaw,** The Salk Institute for Biological Studies, La Jolla, California

Essential kinases for human metastatic cells pertaining to c-Met signaling.
Wenliang Li, Nandita Bhattacharya, Michael Collins, Sabina Signoretti, Yanhui Hu, Randolph Watnick, Ed Harlow.
Presenter affiliation: Harvard Medical School, Boston, Massachusetts. 120

Cdk2 and Cdk4 regulate the switch from proliferation to differentiation in neural stem cells.
Shuhui Lim, Philipp Kaldis.
Presenter affiliation: Institute of Molecular and Cell Biology (IMCB), Singapore, Singapore. 121

Transient exposure of HCT116 to MLN4924 induces re-replication, apoptosis and cellular senescence
jie J. lin, Michael A. Milhollen, Peter G. Smith, Usha Narayanan, anindya dutta.
Presenter affiliation: University of Virginia, Charlottesville, Virginia. 122

EphA2-Vav3-Rac1 signaling mediates migratory and invasive behavior of prostate cancer cells
Kai-Ti Lin, Jianli Gong, Te-Hsuan Jang, Huei-Jane Chen, Lu-Hai Wang.
Presenter affiliation: National Health Research Institute, Miaoli County, Taiwan. 123

Activation of CREB by PI3 kinase and p38 MAPK is essential for elevated expression of TGFβ2
Youzhong Wan, Maojing Yang, Sunny Kolattukudy, George R. Stark, Tao Lu.
Presenter affiliation: Cleveland Clinic Foundation, Cleveland, Ohio; Case Western Reserve University, Cleveland, Ohio. 124

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SESSION 4 MOUSE MODELS

Chairperson: **C. Abate-Shen,** Columbia University Medical School, New York, New York

SESSION 5 Rb AND p53 NETWORKS

Chairperson: **K. Vousden,** Beatson Institute for Cancer Research, Glasgow, United Kingdom

SESSION 6 POSTER SESSION II

THURSDAY, August 19—7:30 PM

SESSION 7 MICROENVIRONMENT, INFLAMMATION AND METASTASES

Chairperson: **L. Parada,** University of Texas Southwestern Medical Center, Dallas

SESSION 8 EXPERIMENTAL THERAPEUTICS

Chairperson: **R. DePinho,** Dana-Farber Cancer Institute, Boston, Massachusetts

Ronald DePinho.
Presenter affiliation: Dana-Farber Cancer Institute, Boston, Massachusetts.

CD95/Fas and NFκB signaling modulate dependence of lung cancers on mutant EGFR
Trever G. Bivona, Haley Hieronymus, Joel Parker, Kenneth Chang, Kimberly Brown, Vincent Miller, Gregory Hannon, Charles Sawyers.
Presenter affiliation: Memorial Sloan Kettering Cancer Center, New York, New York. 257

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Presenter affiliation: Genentech, South San Francisco, California. 258

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Presenter affiliation: The Netherlands Cancer Institute, Amsterdam, Netherlands. 259

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Presenter affiliation: Brigham and Womens Hospital, Boston, Massachusetts; Harvard Medical School, Boston, Massachusetts. 260

Innate chemotherapy resistance and "cancer stem cells" are enriched in overlapping but distinct populations in murine lung cancer
Yanyan Zheng, Cecile De La Cruz, Leanne Sayles, Chenwei Lin, Erica Jackson, Alejandro Sweet-Cordero.
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Copper promotes tumor growth by increasing oxidative phosphorylation
Seiko Ishida, Frank McCormick, Douglas Hanahan.
Presenter affiliation: University of California, San Francisco, San Francisco, California. 262

Ubiquitination as a novel mechanism of oncogenic transformation in leukemia
Iannis Aifantis.
Presenter affiliation: Howard Hughes Medical Institute, New York University School of Medicine, New York. 263

Therapeutic effect of γ-secretase inhibition in a mouse model of lung adenocarcinoma
Antonio Maraver, Manuel Serrano.
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Forest M. White.
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Presenter affiliation: Massachusetts General Hospital, Boston, Massachusetts. 273

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Synthetic lethal interaction of Ark5 depletion with deregulated Myc suggests that the Hippo pathway mediates Myc-induced apoptosis
Lidan Liu, Jannes Ulbrich, Daniel Murphy, Lars Zender, Martin Eilers.
Presenter affiliation: University of Würzburg, Würzburg, Germany. 274

Identification of Myc-regulated genes during mitogenic stimulation and lymphoma onset
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Presenter affiliation: University California San Francisco, San Francisco, California. 276

CYCLOPAMINE AND THE SONIC HEDGEHOG PATHWAY IN MELANOMA MAINTENANCE AND CHEMORESISTANCE

Agnieszka Checinska[1], Fernando García[2], Keith Ashman[2] Maria Soengas[1]

[1]CNIO Spanish National Cancer Research Centre, Molecular Pathology, Madrid, E- 28029 , Spain, [2]CNIO Spanish National Cancer Research Centre, Proteomics Core Unit, Madrid, E- 28029 , Spain

Melanoma progression is invariably associated with the acquisition of multiple defects in apoptotic pathways. For example, overexpression of anti-apoptotic Bcl-2 family members, dysregulation of caspase inducers, and deficiencies in p53-dependent death programs are all common features of aggressive melanomas. Anti-melanoma therapeutic strategies often aim to overcome these negative apoptotic signals, yet the execution of death programs remains slow and inefficient, even when using pleiotropic agents. These results suggest the existence of yet unidentified mechanisms of melanoma cell survival. Here we present a molecular characterization of protective responses that precede and can interfere with the activation of apoptotic caspases by anticancer drugs. Electron microscopy analyses of melanoma cells revealed the presence of autophagosomes after treatment with various chemotherapeutic agents. Multillamelar structures surrounding cellular organelles were particularly evident upon treatment with cyclopamine, an inhibitor of Smoothened (a central modulator of the Sonic Hedgehog pathway). Cyclopamine-induced autophagy was further confirmed by the lipidation of LC-3 and the relocalization of this protein to autophagosomes. Notably, aberrant phagocytic organelles were sustained for a long time (up to 70 h) while cells blocked their proliferative capacity but remained viable. In contrast to classical genotoxic-induced stress programs, the response to cyclopamine was independent of p53. Instead, we found that cyclopamine targets and deregulates lysosomal functions. These results were unexpected because the Sonic Hedgehog pathway can control the proliferative capacity of a variety of tumor cell types (including melanoma), but it was not previously known as an inducer of autophagy. Interestingly, forcing autophagy with hypoxia or low serum switched the outcome of cyclopamine treatment from a protective response to the induction of cell death. These data reveal a novel role of autophagy in the control of melanoma cell survival, acting independently of the classical tumor suppressor p53. Understanding this previously unexplored autophagy pathway may reveal new targets for drug design.

INTERPLAY BETWEEN ONCOGENE-INDUCED DNA DAMAGE RESPONSE AND HETEROCHROMATIN IN SENESCENCE AND CANCER

Fabrizio d'Adda di Fagagna

IFOM Foundation, Telomere&Senescence group, Milan, 20123, Italy

Oncogene-induced cellular senescence is a permanent proliferative arrest triggered by exogenous or endogenous stresses. Two major mechanisms have been causally implicated in the establishment of the senescence state: the activation of the DNA Damage Response (DDR) pathway, which enforces a permanent checkpoint, and global induction of heterochromatin resulting in the formation of Senescence-Associated Heterochromatic Foci (SAHF), which have been proposed to suppress the transcription of genes involved in proliferation. If and how they interact is unclear. We have recently observed that SAHF are preferentially triggered by oncogene activation, in a manner dependent on DNA replication and controlled by ATR. ATM or p53 inactivation allows the de-repression of proliferative genes and proliferation of oncogene-expressing cells bearing SAHF-like structures, thus revealing that SAHF and transcriptional repression can be uncoupled. In a wide collection of human cancers the levels of heterochromatin markers were found to be higher than in normal tissues and this is independent of their proliferative index or cancer stage. Pharmacological and genetic perturbation of heterochromatin increases DDR signalling specifically in oncogene-expressing cells and leads to DDR-dependent apoptosis. In vivo, systemic administration of a histone deacetylase inhibitor causes heterochromatin relaxation, increased DDR activation, apoptosis and tumor regression.
We propose that oncogenic stress-induced heterochromatin restrains DDR and this may provide the rationale for the use of chromatin modifying drugs, including histone deacetylase inhibitors, as cancer therapies guided, however, by the study of the status of heterochromatin and DDR competence of the tumor.

THE PRO-APOPTOTIC FUNCTION OF THE RETINOBLASTOMA TUMOR SUPPRESSOR PROTEIN

Alessandra Ianari[1,2], Tiziana Natale[2], Eliezer Calo[1], Elisabetta Ferretti[2], Edoardo Alesse[3], Isabella Screpanti[2], Kevin Haigis[4], Alberto Gulino[2], Jacqueline Lees[1]

[1]MIT, Koch Institute, Cambridge, MA, 02139, [2]University of Rome, La Sapienza, Experimental Medicine, Rome, 00161, Italy, [3]University of L'Aquila, Experimental Medicine, L'Aquila, 00176, Italy, [4]Massachusetts General Hospital, Center for Cancer Research, Charlestown, MA, 02129

The tumor suppressive properties of the retinoblastoma protein (pRB) rely largely on its ability to regulate the E2F transcription factors. The resulting pRB-E2F complexes can bind, and actively repress, the promoters of cell cycle genes. E2F1, among other E2F family members, also promotes the transcription of proapoptotic genes in response to both genotoxic and oncogenic stress. Prevailing models suggest that pRB inhibits this apoptosis by binding to E2F and inducing cell cycle arrest. However, while there is strong evidence that pRB promotes arrest in response to DNA damage, the interplay between pRB and E2F1 in proapoptotic gene regulation has not been elucidated. We address this question here. Remarkably, we found that genotoxic agents caused pRB to associate with E2F1 and the transcriptional coactivator P/CAF. The resulting complex formed irrespective of the cell cycle phasing and it occupied the transcriptionally active form of proapoptotic gene promoters. Moreover, pRB was required for the maximal activation of DNA damage-induced apoptosis both in vitro and in vivo. Notably, genotoxic stress also caused recruitment of pRB, E2F1 and histone deacetylase to the cyclin A2 promoter, coincident with its repression. Thus, we conclude DNA damage induces two distinct pRB-containing complexes that mediate activation of apoptotic genes or repression of cell cycle genes. We also examined the effects of E1A, a potent proapoptotic oncogene that is thought to act by sequestering pRB and releasing active E2F. Contrary to this model, our data suggest that E1A participates in a multimeric pRB-E2F1-E1A complex that activates both apoptotic and cell cycle genes. Taken together, our data reveal a previously unappreciated, positive role for pRB in the apoptotic response to both genotoxic and oncogenic stress. This is entirely consistent with pRB's tumor suppressive functions and opens new perspectives for cancer therapies. Altogether these data indicate that pRB has the ability to promote not only cell cycle arrest but also apoptosis. Our current efforts are now directed at the understanding of the molecular mechanisms regulating the formation and the activity of the different pRB-E2F1 complexes.

"SENESCENCE SURVEILLANCE" SUPPRESSES CARCINOGENESIS IN THE LIVER AND INVOLVES AN ADAPTIVE IMMUNE RESPONSE AGAINST PREMALIGNANT SENESCENT HEPATOCYTES

Tae-Won Kang[1], Tetyana Yevsa[1,2], Norman Woller[2], Lisa Hoenicke[2], Torsten Wuestefeld[1,2], Thomas Longerich[3], Peter Schirmacher[3], Stefan Kubicka[2], Lars Zender[1,2]

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To determine whether immune surveillance of premalignant senescent cells plays a significant role in tumor suppression, oncogene induced senescence (OIS) was triggered by oncogenic NrasG12V in otherwise normal mouse hepatocytes in vivo. Cytokine profiling of mouse livers after senescence induction revealed expression of various cytokines known to be involved in the senescence associated secretory phenotype (SASP) but also an increased expression of cytokines regulating the attraction, activation and proliferation of T lymphocytes. In accordance with the observed cytokine profiles, using histology and FACS profiling, we found a strong influx of innate immune cells (neutrophils, macrophages and NK cells) but also of adaptive immune cells (CD4+ and CD8+ lymphocytes and dendritic cells) into mouse livers harboring senescent hepatocytes.Time course analyses revealed clusters of senescent hepatocytes surrounded by immune cells and a decrease in the number of senescent hepatocytes over time, thus suggesting an immune-mediated clearance of premalignant senescent hepatocytes. Experiments with mice harboring either defects in the innate or adative immune system side by side with their syngenic wildtype counterparts showed that a defective adaptive immunity abolishes the immune clearance of premalignant senescent cells. Interestingly, when these mice were followed up long term, defective senescence surveillance resulted in murine hepatocellular carcinomas (HCC). To delineate the mechanism of immune surveillance of premalignant senescent hepatocytes in the liver, we took advantage of immunological assays and also studied senescence surveillance in an expanded panel of mice with defined immune defects and in mice which were treated with different immune cell depleting antibodies. Interestingly, experiments in CD4+ knockout mice showed a complete abrogation of senescence surveillance and Elispot assays revealed the presence of NrasG12V specific T-lymphocytes in mice where liver specific senescence had been triggered by NrasG12V. Taken together, our data show that senescence surveillance represents an important extrinsic component of the senescence anti-tumor barrier which involves an adaptive immune response against premalignant senescent cells.

ABROGATION OF ONCOGENE-INDUCED SENESCENCE BY PI3K PATHWAY ACTIVATION CONTRIBUTES TO HUMAN NEVUS-TO-MELANOMA PROGRESSION

Liesbeth Vredeveld[1], Patricia Possik[1], Chrysiis Michaloglou[1], Wolter Mooi[2], Daniel Peeper[1]

[1]Netherlands Cancer Institute, Molecular Genetics, Amsterdam, 1066cx, Netherlands, [2]VU University Medical Center, Pathology, Amsterdam, 1066cx, Netherlands

Human melanocytic nevi (moles) are benign neoplasms commonly harboring activating mutations in BRAF or NRAS. Although nevi are considered to be precursors of melanoma, a highly aggressive type of (skin) cancer, little is known about the mechanism underlying progression from nevus to melanoma. We and others have previously shown that, although initially, activated BRAF and NRAS oncogenes act mitogenically, eventually oncogene-induced senescence (OIS) ensues. This has led to the hypothesis that abrogation of OIS of nevus cells acts as a rate-limiting event in melanomagenesis. Favoring this model, nevi and melanomas are commonly, and significantly, histologically associated. Indeed, malignant melanoma can emerge within a nevus.
In addition to the activation of the ERK pathway, other frequent genetic events include the loss of the CDKN2A and ARF genes. Although the involvement of p16 in melanomagenesis is undisputed, there is little evidence to support a non-redundant role for p16 in BRAF(E600)- or NRAS(K61)-induced senescence, neither in vitro nor in vivo. Another common genetic event in melanoma is the activation of the PI3K pathway, which is seen in ~60% of cases. This is achieved by loss of PTEN expression, increased AKT3 activity or mutations in PIK3CA. Interestingly, some 20% of melanomas show concurrent mutation in BRAF and diminished expression of PTEN.
We have found that in cultured human melanocytes, BRAF(E600)-induced senescence is accompanied by suppression of AKT3. Activation of the PI3K pathway by ectopic expression of AKT or PIK3CA, or depletion of PTEN, abrogates senescence. Correspondingly, in a series of contiguous human nevus-melanoma specimens, we observed a decrease in PTEN and/or an increase in AKT3 in the melanoma relative to the adjacent nevus in >50% of the cases. In several of these, laser microdissection-guided genetic analysis revealed identical mutations in BRAF or NRAS in the nevus and contiguous melanoma, including a rare double mutation, which is in support of a nevus-to-melanoma progression model. These findings indicate that PI3K pathway activation serves as a rate-limiting event in human melanoma progression, acting at least in part by abrogating OIS. This provides an explanation for the frequent co-occurrence of mutations in BRAF and the PI3K pathway in melanoma.

CDK2 INHIBITION DELAYS MYC-DRIVEN LEUKEMIA *IN VIVO* THROUGH INDUCTION OF CELLULAR SENESCENSE

Eduar Hejll[1], Per Hydbring[1], Kari Högstrand[2], Alf Grandien[2], Lars-Gunnar Larsson[1]

[1]Karolinska Institutet, Dept. of Microbiology, Tumor and Cell Biology (MTC), Stockholm, SE-171 77 , Sweden, [2]Karolinska University Hospital-Huddinge, Dept. of Medicine, Stockholm, SE-141 86 , Sweden

The Myc oncoprotein is a fundamental component of many crucial cellular processes, such as cell cycle progression, cell growth, apoptosis, metabolism and it has been widely implicated in many human cancers. In non-transformed cells, Myc expression is tightly regulated and excessive Myc activation triggers apoptosis as a safe guard mechanism. This anti-tumorigenic response needs to be overcome in tumors. Myc-driven cell proliferation is partly achieved by the induction of cyclins and cyclin-dependent kinases (Cdks). We have recently found that Myc represses oncogene-induced senescence, which is another important barrier of tumor development. This Myc function requires Cdk2-mediated phosphorylation of Myc at serine 62, as shown by inhibition of Cdk2. To investigate whether pharmacological inhibition of Cdk2 delays Myc-driven tumor onset *in vivo* trough senescence, we used a bone marrow transduction/transplantation model. Hematopoietic stem cells (HSC) purified from bone marrow cells of BALB/c mice were transduced with lentiviral vectors containing Myc and Bcl-xl cDNA and then transplanted into lethally irradiated mice. The mice developed massive CD11+/Gr+ acute myeloproliferative leukemia after 2-3 weeks. To investigate the importance of Cdk2 in this process, mice transplanted with Myc and Bcl-xl-expressing HSC were treated with the Cdk2 inhibitor CVT-2584 or vehicle on a daily basis. Analysis of isolated bone marrow, liver and spleen cells of mice by flow cytometry revealed a significant decrease of CD11+/Gr+ leukemic cell population in the treated mice. To gain insight into the effects behind Cdk2 inhibition and to corroborate our previous *in vitro* studies, cellular senescence was assayed in spleen and liver tissues from control and treated mice by immunostaining with antibodies directed against trimethylated histone H3 lysine 9 (H3K9me3). H3K9me3-positive senescence-associated heterochromatinic foci were more abundant in all analyzed tissues of the treated mice. Further, Cdk2 targeting significantly delayed onset of disease and improved mice survival. In summary, the selective inhibition of Cdk2 i*n vivo* delayed Myc+BclxL driven leukemia and induced cellular senescence in analyzed liver and spleen tissues. Our results provide a new rationale for treatment of Myc-driven neoplasia by exploiting Cdk2 inhibition as a strategy to induce senescence in such tumor cells.

MUTANT N-RAS ACTIVATES MAPK AND STAT3 FROM LIPID RAFTS TO SUPPRESS APOPTOSIS: A NEW PARADIGM FOR FUNCTIONAL SPECIFICITY WITHIN THE RAS ONCOPROTEIN FAMILY

Kevin Haigis[1,2,3,4]

[1]Massachusetts General Hospital, Molecular Pathology Unit, Charlestown, MA, 02129, [2]Massachusetts General Hospital, Center for Cancer Research, Charlestown, MA, 02129, [3]Massachusetts General Hospital, Center for Systems Biology, Charlestown, MA, 02129, [4]Harvard Medical School, Department of Pathology, Boston, MA, 02115

The RAS oncoproteins (N-RAS, H-RAS, K-RAS4A, K-RAS4B) have been studied extensively for almost three decades. Although activation of RAS represents a key feature of malignant transformation for many cancers, we are only now beginning to understand the complex underpinnings of RAS biology. And while biochemical studies have failed to identify significant differences between members of this enzyme family, genetic and cellular studies are beginning to reveal non-overlapping functions for RAS family members. For example, we have found that mutationally activated N-RAS (G12D) is unique among the family members in its ability to suppress stress-induced apoptosis in epithelial cells. To do so, mutant N-RAS must simultaneously activate both canonical and non-canonical effectors. Like other family members, mutant N-RAS potently activates the MAPK pathway, but N-RAS is the only family member that can regulate the phosphorylation and transcriptional activity of STAT3. Interestingly, activation of both MAPK and STAT3 is mediated by RAF-1 and activated RAF-1 phenocopies mutant N-RAS in the suppression of apoptosis. Why is N-RAS the only family member that can feed into STAT3? Consistent with it being the only RAS protein localized to lipid rafts in its GTP-bound state, mutant N-RAS activates both ERK and STAT3 from this membrane microdomain. Disruption of lipid rafts completely abrogates the anti-apoptotic phenotype associated with mutant N-RAS. Our studies provide a formal demonstration that sub-cellular localization underlies the functional differences between the highly related RAS GTPases. These data also establish a new paradigm in RAS biology – that functional differences among family members derive from specific engagement of non-canonical effector pathways.

DISTINCT BIOLOGICAL OUTPUTS OF ONCOGENIC N-RAS AND K-RAS EXPRESSION IN THE HEMATOPOIETIC COMPARTMENT

Jin Xu, Kevin Shannon

UCSF, Pediatrics, San Francisco, CA, 94158

The three Ras proto-oncogenes encode four Ras isoforms (K-Ras 4a, K-Ras 4b, N-Ras and H-Ras) that function as signal switch molecules to couple extracellular signaling with activation of its effectors. Ras activation is crucial for modulating cell fate responses, namely proliferation, differentiation and survival. The Ras isoforms are highly conserved, diverging only in the last 24 amino acids. This variation in their C-terminal "hypervariable" domains leads to differential subcellular localization, which may contribute to variable effects on downstream effectors. Although RAS mutations occur in ~30% of all human cancers, there is an isoform specific mutation spectrum in distinct tumor types. For example, Kras mutations predominate solid tumors, while Nras mutations are frequently found in myeloid malignancies. Previous studies from our laboratory demonstrated that endogenous expression of oncogenic K-Ras^{G12D} in hematopoietic cells causes an aggressive myeloproliferative disorder, whereas endogenous N-Ras^{G12D} expression causes a diverse spectrum of hematologic cancers with much longer latencies. My overall goal is to understand the molecular basis of the differential effects of oncogenic N-Ras and K-Ras in leukemogenesis. I have pursued this general question through two aims:
1) To characterize a series of Nras^{G12D} and Kras^{G12D} alleles in which I have modified their respective hypervariable regions. I have engineered MSCV retroviruses encoding chimeric N-Ras and K-Ras molecules and will assess the effects of expressing these proteins on myeloid progenitor growth and Ras effector activation; and,
2) To generate and characterize a new mouse strain that will express an oncogenic N-RasG12D chimeric protein that contains the K-Ras 4b domain from the endogenous Nras locus. These studies will facilitate biochemical and cell biologic studies to understand the mechanism of isoform-specfic differences and may suggest new strategies for targeting oncogenic Ras in leukemias.

THE 8P21.3 LUNG TUMOR SUPPRESSOR *DOK2* OPPOSES ONCOGENIC *EGFR* AND *KRAS*

Alice H Berger[1,2], Alessandro Morotti[1], Katerina Politi[3], Justyna A Janas[4], Masaru Niki[5], Barry S Taylor[3], Cameron Brennan[3], Roderick T Bronson[6], Marc Ladanyi[3], Linda Van Aelst[4], Harold E Varmus[3], Pier Paolo Pandolfi[1,2]

[1]Cancer Genetics Program, BIDMC, Depts of Medicine and Pathology, Harvard Medical School, Boston, MA, 02115, [2]Weill Graduate School, Cornell University, NY, NY, 10021, [3]Memorial Sloan-Kettering Cancer Center, NY, NY, 10021, [4]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 11724, [5]Dept of Internal Medicine, University of Iowa, Iowa City, IA, 52242, [6]Rodent Histopathology Core, Dana Farber/Harvard Cancer Center, Boston, MA, 02115

DOK family proteins are adaptor proteins that modulate receptor tyrosine kinases (RTKs), RAS, and SRC signaling pathways. Our group recently identified *DOK2* as a human lung tumor suppressor gene that is frequently downregulated in human lung adenocarcinoma due to broad-scale genomic losses encompassing the *DOK2* locus at chromosome 8p21.3. Here we find that genomic loss of *DOK2* in human lung adenocarcinoma is significantly associated with both *EGFR* and *KRAS* mutation. These associations are found to be functionally relevant, because loss of *Dok2* in vivo enhances the lung tumorigenesis and lethality caused by doxycycline-induced expression of oncogenic *EGFR* or *Kras* in the murine lung.

Dok2 knockout (KO) mice expressing an oncogenic deletion mutant of *EGFR* had significantly more tumors, greater lung mass, and shorter survival than *Dok2* wild-type (WT) $EGFR^{DEL}$ animals. Knockout of *Dok2* could similarly exacerbate the tumor phenotype induced by lung-specific expression of an oncogenic *Kras* G12D allele. Lungs from $Kras^{G12D}$ animals without *Dok2* had significantly more tumor nodules and a higher percentage of Ki67-positive cells than lungs from *Dok2* WT $Kras^{G12D}$ mice.

Co-immunoprecipitation and immunofluorescence analysis showed that EGFR and DOK2 interact and co-localize after EGF stimulation. Under these conditions, DOK2 is phosphorylated and bound to downstream effector proteins. Whereas DOK2 interacts with wild-type EGFR in an EGF-inducible manner, DOK2 is constitutively bound to the L858R mutant form of EGFR found in human lung cancer.

These data identify *DOK2* as a negative regulator of lung cancer initiated by oncogenic *EGFR* or *KRAS*. Furthermore, these findings suggest the existence of novel RasGAP-independent functions of DOK2 that are involved in suppression of $KRAS^{G12D}$-induced transformation.

RAS ONCOGENE-INDUCED ROS DETOXIFICATION PROMOTES TUMORIGENESIS

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Reactive oxygen species (ROS) are mutagenic and may thereby promote cancer. However, somatic mutations that activate the Nrf2 pathway have recently been described in human cancer, suggesting that enhanced ROS detoxification may be pro-tumorigenic. Here, we demonstrate that ROS are actively suppressed by the oncogene K-Ras to stimulate neoplasia. K-Ras^{G12D} engages an antioxidant program mediated by the transcription factor Nrf2 to lower intracellular ROS and confer a more reduced intracellular environment. Activation of the Nrf2 antioxidant program is evident in primary cells and tissues of mice expressing K-Ras^{G12D}, and in human pancreatic cancer. Activation of Nrf2 occurs independently of mutations in Nrf2 and Keap1 and is instead mediated by the MAP kinase Ras effector pathway, which promotes transcriptional upregulation of Nrf2 through c-Jun. Pharmacological and genetic targeting of the MAPK-Nrf2 pathway impairs proliferation and tumorigenesis *in vivo*. Thus, the Nrf2 antioxidant program represents a previously unappreciated mediator of Ras tumorigenesis. Furthermore, Nrf2 is also activated by oncogenic B-Raf and c-Myc to lower ROS, and thus our findings may represent a general feature of cancers with deregulation of these pathways. These findings warrant a re-evaluation of the role of ROS in carcinogenesis.

TUMOUR SUPPRESSION BY NRBP1 THROUGH REGULATION OF THE WNT PATHWAY

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Development and tumor suppression are intimately linked and the genes that function in these processes are highly conserved throughout evolution. Here we describe the identification of NRBP1 as a key regulator of the Wnt pathway, intestinal differentiation, and tumor suppression. A kinome-wide screen in let60 gain-of-function mutant Ras C. elegans revealed that H37N21.1, the worm ortholog of NRBP1, co-operates with mutant Ras to promote a multivulval phenotype. Suppression of NRBP1 in mouse NIH3T3 cells together with expression of RasV12 resulted in enhanced transformation. Likewise human BJ-ET-st p53kd,p16kd cells transfected with shRNAs against NRBP1 were transformed, but without activation of the Ras pathway suggesting NRBP1 functions downstream or in parallel with Ras in transformation. Germline deletion of Nrbp1 in the mouse resulted in embryonic lethality, while somatic deletion in all tissues of adult mice resulted in profound changes to the proliferation and differentiation of intestinal cell lineages with 80% of mice dying within 10 days. Expression analysis of intestinal tissues from these mice revealed transcriptional activation of Wnt pathway targets. Knockdown of NRBP1 in human HCT-116 and SW480 cell lines using siRNA confirmed activation of Wnt reporters. Mosaic somatic deletion of Nrbp1 in the mouse circumvented the lethality and resulted in tumorigenesis, with tumors staining strongly positive for Wnt targets such as c-Myc. We reveal that NRBP1 is downregulated in a range of human tumors and in lung cancer low NRBP1 expression is associated with reduced survival. Thus, we have established that NRBP1 is a novel tumor suppressor that functions in the Wnt pathway.

FUNCTIONAL DISSECTION OF THE CHR. 6Q DELETION REVEALS EPHRIN SIGNALING AS A TUMOR SUPPRESSIVE MECHANISM IN FOLLICULAR LYMPHOMA

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Follicular lymphoma (FL) is the most common histological type of Non-Hodgkin's lymphoma and characterized by the t(14;18)(q32;q21) rearrangement and increased expression of the anti-apoptotic Bcl2 protein. Lymphomagenesis and ultimate transformation towards aggressive lymphoma are driven by additional genetic events. Cytogenetic studies have linked deletions of Chr. 6q to progression and reduced survival in one third of FL patients. To pinpoint genetic suppressors of FL we conducted a targeted shRNA screen based on array-CGH analyses 70 FLs. Here we show, that a truncated variant of the Ephrin receptor (EphA7TR) acts as a secreted tumor suppressor in vitro and in a murine FL model. EphA7TR is targeted by the 6q deletion in ~25% of FL and its promoter is uniformly silenced. Strikingly, administration of purified EphA7TR suppresses Src and Mapk signaling and produces tumor responses in human lymphomas in vivo. Hence, the EphA7TR is a secreted lymphoma suppressor that is epigenetically silenced and targeted by the Chr. 6q deletion in FL.

ABERRANT EXPRESSION OF NF-?B INDUCING KINASE UNDERLIES CONSTITUTIVE NF-?B ACTIVATION IN CANCER CELLS

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The Nuclear factor-κB (NF-κB) family of transcription factors is known to regulate expressions of diverse cellular genes related to immune response, cell growth and development. Activation of NF-κB is controlled by either canonical and/or non-canonical signaling pathways depending on the stimuli. NF-κB has been reported to be constitutively activated in a variety of cancer tissues and cell lines and to associate with the manifestation of malignant phenotypes of cancer cells, such as cell cycle deregulation, resistance to apoptosis, invasion and metastatsis. However, molecular mechanisms of constitutive activation of NF-κB in cancer cells are in most cases remain to be studied. We previously described that adult T-cell leukemia (ATL) cells show constitutive NF-κB activation although human T-cell leukemia virus type I Tax, a potent viral NF-?B activator, was undetectable in these cells. Previous studies demonstrated post-translational regulation of NIK in cytokine receptor-mediated signaling and mutations in multiple myeloma cells leading to aberrant NIK activation. Stimulation by cytokines such as B cell-activating factor or CD40 ligand induced degradation of TNF receptor-associated factor 3 (TRAF3), which binds to NIK and leads to proteasome-dependent NIK degradation in unstimulated cells, thereby stabilizing NIK to trigger the non-canonical pathway. Multiple myeloma cells achieve increased autonomy from the bone marrow microenvironment by mutations that activate either NF-κB pathway. We demonstrate here that NIK was overexpressed at the pre-translational level in ATL, Hodgkin/Reed-Sternberg, lung cancer and breast cancer cells. NIK overexpression was not simply due to amplification of the *NIK* locus or stabilization of the NIK mRNA. Depletion of NIK by RNA interference in these cancer cells reduced NF-κB-dependent gene expression, DNA-binding activity of NF-κB and the abilities of cancer cells to form colonies in soft agar or tumor in NOD/SCID/γc^{null} mice. Our results indicate that NIK is responsible for constitutive NF-κB activation in a wide range of cancer cells, contributing to manifestation of malignant phenotypes and suggest that NIK can be an attractive molecular target for cancer therapy.

TELOMERE-DRIVEN TETRAPLOIDIZATION AND ITS RELEVANCE TO CANCER

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Polyploidy is frequently associated with the early stages of human solid tumors and has been proposed as an intermediate step toward aneuploidy in cancer. However, a general mechanism for the induction of tetraploidy during tumorigenesis is lacking. We recently reported that tetraploidization occurs in p53-deficient cells experiencing a prolonged DNA damage signal due to persistent telomere dysfunction (Davoli et al., Cell, 2010). Live-cell imaging revealed that these cells have an extended G2 phase due to ATM/ATR- and Chk1/Chk2-mediated inhibition of Cdk1/CyclinB kinase and eventually by-pass mitosis. Despite their lack of mitosis, the cells showed APC/Cdh1-dependent degradation of the replication inhibitor geminin, followed by accumulation of Cdt1, which is required for the licensing of replication origins. Cells then entered a second S phase resulting in whole-genome reduplication and tetraploidy. Upon restoration of telomere protection, these tetraploid cells resumed cell division cycles and proliferated.

We next examined the role of telomere-driven endoreduplication in cellular transformation and during natural telomere erosion in human cells. An inducible system for the expression of the telomeric protein POT1a was used to derive diploid and tetraploid cell populations after telomere-driven endoreduplication. As compared to the diploid counterpart, tetraploid cells showed higher transformation potential in vitro in soft-agar colony formation assay and increased in vivo tumorigenic potential after injection in mice. Furthermore, in the context of natural telomere attrition, we found that after prolonged replication-dependent telomere shortening (crisis stage), p53- and Rb-deficient human fibroblasts show DNA damage signaling, extended G2 phase and the ability to undergo endoreduplication-dependent tetraploidy. Expression of telomerase in these cells silenced the DNA damage response and restored mitotic division cycles, indicating that in this context endoreudplication is dependent on the presence of short dysfunctional telomeres. Altogether, these observations suggest a general mechanism for the induction of tetraploidization in the early stages of human cancer after excessive telomere shortening and a role of this process during tumorigenesis.

SATB2 AUGMENTS ΔNP63A IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Head and neck squamous cell carcinoma (HNSCC) is a malignancy of the squamous epithelium lining the oro- and nasopharynx. Molecularly, ΔNp63α, a truncated and oncogenic form of the p53-family of proteins, is highly expressed in the vast majority of HNSCC and promotes cancer cell survival by inhibiting TAp73β – a potent, apoptosis-inducing p53-family member. HNSCC cancer cells are dependent upon ΔNp63α for survival. In addition to having a critical role in the development of HNSCC, ΔNp63α function is also important in treatment response of HNSCC. Current anti-cancer drugs exert their effects by downregulating pro-survival ΔNp63α while inducing pro-apoptotic TAp73β. Although overexpression of ΔNp63α is frequently observed in HNSCC, these tumors, nonetheless, vary in their responsiveness to radiation and chemotherapies. In particular, advanced-stage HNSCC tumors are highly resistant to current treatments, suggesting additional factors exist that influence the function of ΔNp63α and chemoresponsiveness. Here, we identify Special AT-rich Binding Protein 2 (SATB2) as a protein promoting chemoresistance of SCC cells of the head and neck. SATB2 forms a stable molecular complex with ΔNp63α and augments its DNA-binding capability and, thereby, represses apoptotic gene expression. RNAi-mediated knockdown of SATB2 sensitizes HNSCC cells towards chemotherapy- as well as radiation-induced apoptosis. Interestingly, SATB2 expression was preferentially upregulated in advanced HNSCC tumors that are typically unresponsive to chemotherapy. Taken together, our results suggest that SATB2 promotes survival of SCC cells by modulating the pro-survival function of ΔNp63α.

STRUCTURAL REARRANGEMENTS IN GENETICALLY ENGINEERED MOUSE MAMMARY TUMORS

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Recently massively parallel sequencing has been used to identify somatic mutations and structural rearrangements in human cancer genomes. Here we present the paired-end massively parallel sequencing of tumors from genetically engineered mouse models of mammary cancer to determine how faithfully these models recapitulate the landscape of somatic mutations found in human mammary tumors. This analysis involved tumors from mouse models of Brca1- and Brca2-associated hereditary breast cancer, a model in which Trp53 was deleted in the epithelial compartment of the breast, and a mouse model of E-cadherin (Cdh1) mutated lobular breast cancer. We show that although Brca1- and Brca2-deficient mouse mammary tumors have a defect in the homologous recombination (HR) pathway there is no apparent difference in the type or frequency of somatic rearrangements found in these cancers when compared to HR-proficient cancers, and both HR-deficient and HR-proficient tumors show evidence of micro-homology mediated repair and non-homologous end-joining processes. Analogous to our recent analysis of human breast cancers mouse breast cancers carry expressed in-frame fusion genes, but like human these fusion genes do not appear to be recurrent. One mouse tumor was found to contain an internal deletion of exons of the Lrp1b gene, which leads to a smaller in-frame transcript. We found the human ortholog of this gene (LRP1b) also shows internal in-frame deletions in a significant number of human cancer cell lines, underscoring the potential of mouse cancer genome sequencing for finding human cancer-relevant genes.

CHARACTERIZATION OF THE JUMONJI DOMAIN CONTAINING HISTONE DEMETHYLASE JMJD2C IN NORMAL DEVELOPMENT AND CANCER

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Tri- and di-methylation of lysine 9 on histone H3 (H3K9) is associated with heterochromatin formation and transcriptional repression in a variety of species. These methylation marks has been shown to be associated with normal as well as oncogene-induced senescence and genomic stability. Consistent with a role for these marks in tumor suppression, mice lacking one of the key H3K9 tri- and di-methylating enzymes are tumor prone. Others and we have recently isolated a gene family, called JMJD2, encoding for enzymes that demethylate tri- and di-methylated H3K9. Interestingly, we have found that the expression of these genes is highly elevated in a subset of human cancers, suggesting that they may contribute to tumor formation. Supporting this suggestion is the fact that JMJD2C (a.k.a. GASC1) was originally identified as a gene amplified in esophageal cancer, and that we have shown that inhibition of GASC1 expression slows down growth of tumor cell lines. At the meeting data will be presented characterizing the function of Jmjd2c during mouse development and its role in transcriptional regulation.

β-CATENIN ACTIVATION SYNERGISES WITH RAS ACTIVATION TO CAUSE BLADDER CANCER FORMATION

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Although deregulation of the Wnt signalling pathway has recently been implicated in urothelial cell carcinoma (UCC), the functional significance of this is unknown. To test the importance of deregulated Wnt signalling in bladder tumorigenesis, we have targeted the expression of an activated form of β-catenin (β-cateninexon3) to the urothelium of transgenic mice using Cre-Lox technology (UroIICRE+ β-catenin exon3/+). Expression of this activated form of β-catenin (where β-catenin cannot be phosphorylated, and thus degraded) led to the formation of localised hyperproliferative lesions by 3 months of age, which did not progress to malignancy over 18 months.

We also expressed oncogenic K-Ras and H-Ras in the urothelium alone, and in urothelial cells expressing an activated β-catenin. Although Ras activation was not sufficient to drive tumourigenesis, Ras activation combined with β-catenin activation in UroIICRE+ β –catenin exon3/exon3 K-RasG12D/+ and UroIICRE+ β –catenin exon3/exon3 H-Ras mice rapidly developed UCC. These tumours had massive upregulation of pERK1/2.

Importantly in human UCC, there was a significant correlation between high levels of β-catenin and pERK1/2. Taken together these data definitively show that deregulated Wnt signalling plays a critical role in driving bladder cancer formation.

SSECKS/AKAP12 MAINTAINS CELL CYCLE CHECKPOINT CONTROLS THAT PREVENT ONCOGENESIS BY ATTENUATING PKC ACTIVATION

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SSeCKS/AKAP12 (SSeCKS) is a tumor suppressor which regulates cell cycle progression by scaffolding key mitogenic signaling molecules such as PKA and PKC. SSeCKS is downregulated in many human cancers, and we have shown previously that SSeCKS-null mice develop prostatic hyperplasia exhibiting the hallmarks of senescence. In order to understand the molecular mechanism of SSeCKS-mediated cell proliferation, we have isolated SSeCKS-null mouse embryonic fibroblasts (KO-MEFs) and found that KO-MEFs exhibit multinucleation and premature senescence. Although both p53 and Rb pathways were upregulated upon the loss of SSeCKS, the senescence was inhibited by E7 or siRNA-Rb, indicating that the senescence in KO-MEFs is Rb-dependent. Since SSeCKS is a scaffolder of PKC isozymes, we examined the activity of PKC isozymes. We demonstrated that PKCα and δ are activated in KO-MEFs, and the senescence is driven by PKC hyperactivation in the absence of SSeCKS scaffolding. We provided the evidence that activated PKCα induces p16/Rb activation through a MEK-ERK-dependent downregulation of Id1, whereas activated PKCδ induces cytokinesis defects through the downregulation of the mitotic exit network kinase, Warts/Lats1. In addition, we demonstrated that KO-MEFs are prone to be immortalized and the cells have increased susceptibility to oncogenic transformation. Our data suggest that SSeCKS prevents oncogenic progression by attenuating mitogenic and cytokinetic functions of PKC isozymes. In order to address the physiological relevance of these findings, we performed carcinogenesis experiment using DMBA/TPA skin treatment method. We found that papilloma formation occurs earlier and more frequently in KO mice, and in KO mice, there is a greater transition to squamous cell carcinoma. We also found induced levels of focal adhesion kinase (FAK) in KO epidermal layers, notably because FAK is required for carcinogen-induced progression to skin cancer malignancy. Altogether, this study envisions major mechanisms by which SSeCKS maintains cell cycle checkpoint controls to prevent oncogenesis by attenuating PKC activation.

CONEXIC: AN INTEGRATIVE APPROACH TO UNCOVER DRIVERS OF CANCER

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Systematic characterization of cancer genomes has revealed a staggering complexity of aberrations among individuals, such that the functional importance and physiological impact of most tumor genetic alterations remains poorly defined. A major challenge involves the development of analysis methods to uncover biological insights from the data, including the identification of the key mutations that drive cancer and how these events alter cellular regulation.

We have developed CONEXIC, a novel Bayesian Network-based framework to integrate chromosomal copy number and gene expression data to detect driving mutations that promote cancer progression, and to model how these alterations perturb normal cell growth/survival. The underlying assumption is that significantly recurring copy number change, coinciding with its ability to predict expression patterns varying across tumors, strengthens the evidence of a gene's causative role in cancer. This method not only pinpoints specific regulators within a large aberrant region, but can shed light on the way in which regulation is altered.

We demonstrated the utility of the CONEXIC framework using a melanoma dataset (Lin et al., Cancer Research, 2008) that includes paired measurements of gene expression and copy number from 62 samples. Our analysis correctly identified known drivers in melanoma (e.g. MITF) and connected these to many of their known targets, as well as the biological processes they regulate.

In addition, it predicted multiple tumor dependencies not previously implicated in melanoma. Two such dependencies involving TBC1D16 and RAB27A were confirmed experimentally. The identity of these drivers suggests that abnormal regulation of protein trafficking is important for cell survival in melanoma and highlights the importance of protein trafficking in this malignancy. Together, these results implicate a new tumorigenic process in cancer and demonstrate the ability of integrative Bayesian approaches to identify novel drivers with biological, and possibly therapeutic, importance in cancer.

THE ROLE OF THE TRANSCRIPTION FACTOR NR4A1 IN MAMMARY EPITHELIAL CELL ADHESION AND MIGRATION

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Breast cancer currently accounts for more than a quarter of all female cancers and despite the great progress in treatment observed in the last few years, the need for the identification of new gene targets that can be used for diagnosis, prognosis and therapy is evident. A previous study identified the transcription factor NR4A1 as one of the upregulated genes in primary breast cancer compared to normal tissue by microarray analysis and sequencing technologies. The purpose of the current study was therefore to identify the role of NR4A1 in mammary epithelial cell biology.
NR4A1 belongs to the family of the NR4A orphan nuclear receptors. It is a transcription factor shown to be involved in both apoptosis and cell proliferation. The current study as well as several previous ones, have shown that NR4A1 is expressed in several types of tumours, including breast, while its expression is downregulated in higher grade and metastatic tumours.
To study the role of NR4A1 in mammary epithelial cell biology, normal mammary epithelial cells (MCF-10A) were infected with a retroviral construct containing the full length NR4A1 cDNA. Ectopic expression of NR4A1 led to altered ability of the cells to adhere to the extracellular matrix, altered integrin cell surface expression and reduced migration in scratch wound and transwell migration assays. The initial activation of the Erk1/2 MAPK pathway, which is required for the EGF-mediated migration of MCF-10A cells, was found to be identical in the control and NR4A1 overexpressing cells. However, the levels of active Erk1/2 in response to EGF stimulation during migration were found to be downregulated faster in the cells ectopically expressing NR4A1.
The above data highlight for the first time the involvement of the transcription factor NR4A1 in mammary epithelial cell adhesion and migration. Further studies are underway in order to determine the role of NR4A1 in the migration of breast cancer lines and to delineate the pathways involved.

BIOMARKER IDENTIFICATION USING KINASE SUBSTRATE IDENTIFIER PROTOARRAY® BY INVITROGEN®

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The Kinase Substrate Identifier (KSI) Protoarray (Invitrogen®) was used to identify potential upstream biomarkers for a kinase of interest (which was expressed and purified in house). The technology allows rapid screening of thousands of target proteins as potential protein kinase substrates. Because potential targets are so quickly identified from 9600 purified full-length proteins, it is a highly desired technology if it can be validated as a method to identify direct kinase biomarkers. Nine substrates of interest were chosen from the Protoarray® analysis and were generated to be tested for in vitro phosphorylation by the kinase of interest. Mass Spectrometry is used to analyze the molecular weight of the substrate with and without incubation of the kinase of interest. The phosphorylation state as well as whether these substrates are phosphorylated at multiple sites with the addition of the kinase of interest are assessed. Limited proteolysis is used to identify the phosphorylation sites of the substrate and will be followed up with cell culture analysis.

CONDITIONAL DELETION OF *DROSOPHILA* ACTIVATOR *DE2F1* IN POSTMITOTIC CELLS

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Regulation of cell proliferation and differentiation are critically important developmental events, which when lost can lead to cancer as well as a variety of defects. The importance of the retinoblastoma (pRB) tumor suppressor protein pathway is underscored by its deregulation in most types of cancer. The pRB pathway regulates cell cycle progression by controlling the activities of the E2F transcription factors; downstream targets of pRB. Cell cycle regulation although remarkably conserved is markedly simplified in *Drosophila*. Similar to their mammalian counterparts, the products of *Drosophila* E2F genes predominately act as transcriptional activators or repressors and can be classified as such. However, unlike the mammalian genome, which consists of eight E2F genes, the *Drosophila* E2F family consists of only two members; a single activator (dE2F1), and a single repressor (dE2F2). Thus, using *Drosophila* as a research tool has largely eliminated the issues which arise from functional redundancy and compensation. E2F transcription factors are best known for controlling progression through the cell cycle by regulating the transcription of genes important for the G1/S transition. While most E2F research has focused on its role in the cell cycle, there is emerging evidence that E2F has functions that are distinct from the regulation of cell cycle progression, such as differentiation and development. While loss of the repressor, dE2F2, has relatively mild phenotypes on its own, loss of the activator, dE2F1, leads to a severe reduction in cell proliferation. This precludes recovery of *de2f1* mutant tissue, thus complicating the analysis of *de2f1* function in postmitotic cells. In order to overcome this problem we have developed a new conditional knock-out strategy in *Drosophila* which allows us to delete the *de2f1* gene specifically in postmitotic cells. Details of the method and results of *de2f1* deletion follow.

POTENTIAL ROLE FOR RNF8 IN BREAST CANCER DEVELOPMENT

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Background. Women of Mexican descent have a higher mortality and higher proportion of triple negative or basal-like breast cancers. Basal-like breast tumors exhibit similar characteristics to tumors arising in the rare carriers of BRCA1 mutation. The molecular nature of this “BRCA-ness” in the sporadic disease is unknown. Recently, it was shown that knockdown of ring finger 8 (RNF8), an E3 ubiquitin-protein ligase, by siRNA inhibited BRCA1 focus formation at double strand break sites suggesting that RNF8 is an important upstream regulator of BRCA1 function. Methods. We are investigating the hypothesis that RFN8 is a tumor suppressor gene and acts as a novel regulatory protein of BRCA1 expression in human breast cancers. We tested whether breast cancers taken from a population of Mexican women enriched for basal-like cancers show altered expression in RNF8. We analyzed the effect of RNF8 at the gene expression and protein levels in 27 formalin fixed paraffin embedded human breast cancers and compared expression to normal breast tissue. Results. RNF8 was found to exhibit varied expression at the gene level across human breast cancers. Among the 27 tumors, one-third of tumors showed decreased expression of RNF8, one-third showed no change and the remaining exhibited increased expression including cases with > 5-fold increases in the RFN8 levels compared to healthy control tissue. These data suggests that RNF8 is differentially altered in human breast cancers. Conclusion. This is the first report demonstrating differential expression of RNF8 in a series of human breast cancers. Given the potential importance of RNF8 in the integration of BRCA1 to DNA damage sites, additional studies are needed to understand the relation between RNF8 and BRCA expression and functional consequence in human breast cancers.

Authors contributed equally.

TRANSFORMATION OF PANCREATIC EPITHELIAL CELLS BY B-RAF V600E

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Pancreatic cancer is the 4th leading cause of cancer related death in the United States with a median survival time of less than 6 months. Pancreatic ductal adenocarcinoma (PDAC) accounts for 85% of all pancreatic cancer, and is marked by early and frequent mutation of the K-Ras oncogene, with activating K-Ras mutations present in over 90% of PDAC. Activation of K-Ras has been shown to stimulate multiple signaling pathways, including the MEK-Erk and PI3K-Akt signaling cascades, but the role of downstream effectors in pancreatic tumor initiation and progression remains unclear. Interestingly, one third of K-Ras wild type PDACs harbor activating mutations in B-Raf, and K-Ras and B-Raf mutations appear to be mutually exclusive. We therefore sought to determine the effect of activated B-Raf (B-Raf V600E) on primary pancreatic ductal epithelial cells (PDECs). B-Raf V600E expressing cells displayed increased proliferation relative to GFP expressing cells, as well as increased survival when treated with apoptotic stimuli, such as ultraviolet irradiation. This enhanced survival is dependent on both the MEK-Erk and PI3K-Akt signaling cascades, as inhibition of MEK or PI3K results in decreased survival. Surprisingly, this survival is also dependent on IGF-1R, as inhibition of IGF-1R results in a loss of survival similar to that of MEK and PI3K inhibition, indicating that IGF-1R may be an important mediator of signaling downstream of B-Raf. Western blot analysis confirmed that expression of B-Raf V600E results in the activation of both the MEK-Erk and PI3K-Akt signaling cascades, as shown by phosphorylation of Erk at Thr202 and Akt at Ser473. Finally, we found that the expression of B-Raf V600E in cells lacking both the Ink4a/Arf and Trp53 tumor suppressors was sufficient for tumor formation following orthotopic transplant. In addition, preliminary results suggest that IGF-1R inhibition impairs B-Raf-induced tumor formation in this model. These studies demonstrate that the expression of mutant B-Raf results in the transformation of pancreatic epithelial cells, and indicate that tumor formation as a result of B-Raf expression may depend not only on signaling through the MEK-Erk signaling cascade but also on signaling through the PI3K-Akt signaling cascade and the IGF-1R.

CHARACTERIZATION OF AN *EX VIVO* PRIMARY MULTICELLULAR RENAL CELL CULTURE AS A MODEL SYSTEM FOR RENAL CELL CARCINOMA TUMORIGENESIS

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Sporadic and germline mutations and/or loss of the von Hippel-Lindau (*VHL*) tumor suppressor gene have been linked to varying levels of risk for a highly prevalent malignancy, Renal Cell Carcinoma (RCC). To better understand the genetic changes associated with RCC tumor formation, primary epithelial kidney cells from newborn conditional *Vhl* null and *Vhl* G518A mutant genetically engineered mouse lines are undergoing investigation. This *ex vivo* system can provide insight into both the cellular characteristics and molecular events that contribute substantially to RCC tumor formation. Initial observations of primary neonatal epithelial kidney cells cultured under mild hypoxia show that these cells preferentially grow in an organized multicellular spherical culture. Current work is aimed at identifying the cellular makeup of these spheres and determining any role individual cellular components may have in tumorigenesis. Given that VHL type 2B missense mutations (of which Arg167Gln, represented by the murine G518A mutation, is one hotspot example) confer a high risk for RCC, this project also aims to identify a hypoxia inducible factor (HIF) gene target profile commensurate with the level of risk for RCC. This will be done by comparing levels of gene expression when HIFα subunits are differentially regulated based on *VHL* status as compared to primary kidney epithelial cells displaying wild type *VHL*. This project will identify the most at-risk cell population of the kidney and analyze the molecular changes in this at-risk cell population that promote tumor growth as well as identify a gene target profile for these changes in order to have greater insight into RCC tumorigenesis.

EXPLOITING GENETICALLY ENGINEERED MICE FOR THE ASSEMBLY OF PROSTATE CANCER INTERACTOMES TO IDENTIFY NEW DRUGGABLE TARGETS FOR ADVANCED PROSTATE CANCER.

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Men diagnosed with castration-resistant prostate cancer eventually succumb to lethality since treatment options are limited. Our laboratory has been developing new genetically-engineered mouse models based on inducible prostate-specific deletion of Pten alone or together with activation of oncogenic Kras. These models recapitulate all stages of prostate tumorigenesis, including occurrence of distant metastases, and model the concurrent deregulation of the Akt/mTOR and Erk/Map kinase pathways, observed in more than 25% of advanced prostate cancers. We have been exploiting these models to identify fundamental mechanisms and signaling pathways deregulated in prostate cancer progression through the assembly of human and mouse prostate cancer interactomes, which are genome-wide networks of transcriptional and post-translational interactions. Interactomes are predicated on the availability of large gene expression profile (GEP) datasets representative of the natural phenotypic variability and genetic perturbations of prostate cancer. We are generating GEP using a collection of mouse models from our laboratory, as well as from the mouse model community. We have validated a strategy based on the perturbation of the transcriptome in vivo with a collection of compounds able to generate drug-specific signatures. Our goal is to identify the key regulatory genes causally driving progression to advanced prostate cancer, as well as druggable targets, through the comparison of independent mouse and human prostate cancer interactomes. Based on our findings that concurrent deregulation of the Akt/mTOR and Erk/Map kinase pathways is a driving force for castration-resistant prostate cancer, we have been pursuing preclinical studies, and have found that combination therapy using Rapamycin, a mTOR inhibitor, and PD0325901, a MEK inhibitor, is anti-tumorigenic, improves survival, and inhibits metastases in these models. In summary, we are undertaking a comprehensive approach to identify new molecular pathways and druggable targets for advanced prostate cancer, as well as to evaluate these targets in preclinical studies that will ultimately be translated to clinical trials.

GENOMIC AMPLIFICATION OF *C-MYC* LOCUS IN CANCER: THE USUAL SUSPECTS AND MORE

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The advances in sequencing technologies in the last decade have provoked us to re-examine our understanding of cancer genetics. Of the myriad genetic mutations that contribute to tumorigenesis, recurrent copy number alterations, mainly deletions and amplifications of large genomic intervals, are considered major drivers of the disease. Amplification of human chromosome 8q24 has been among the most common mutations in epithelial tumors. Interestingly, the well studied oncogene *c-MYC* maps at 8q24 and is often included in this amplicon. Overexpression of c-MYC has been implicated in several cancers, mainly of hematological origin. Curiously, in epithelial cancers, *c-MYC* is mainly found to be co-amplified with the 8q24 amplicon. Patients with 8q24 amplification often have poor outcomes. Mouse models for studying the effects of c-myc overexpression have been helpful in elucidating the role of c-myc in neoplastic transformation. However, these models rarely mirror the aggressive cancer often seen in the patients harboring 8q24 amplification. This suggests that other genetic elements may be present in the amplicon which contribute to the poor prognosis in the patients either independently or in synergy with c-MYC.

To investigate the true impact of the 8q24 amplification in human epithelial cancers, we are developing functional models which harbor similar genetic mutations found in human cancers with 8q24 amplification. We focused on a core 4.3 Mb region in human 8q24 region which is frequently amplified in many carcinomas. In addition to *c-MYC*, this region contains several genes and non coding RNAs that have been implicated in various cancers. The synteny of this genetic interval is nearly identical to a 4.5 Mb in mouse chromosome 15. Using chromosome engineering technology, we are developing functional models harboring amplifications of this genetic interval in mouse. By accurately mimicking the true nature of the mutation, the carcinomas in these mouse models will give us insights to the molecular mechanisms involved in tumorigenesis and subsequent progression of cancer in these patients, and will contribute to the development of more targeted therapies.

SPONTANEOUS DOMINANT LEUKEMIA (SDL): A NOVEL MOUSE MODEL OF LEUKEMIA

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Survival rates for T-cell lymphoblastic leukemia have increased dramatically in recent years. However, treatment is often highly cytotoxic, relapse remains difficult to treat, and prognosis remains poor in adults. Identification of novel proto-oncogenes and tumor suppressor genes in mouse models of leukemia may provide new drug targets useful in treating human disease. A spontaneous mutation arose in our mouse breeding colony that results in highly penetrant T-cell leukemia in the mice that inherit this mutation, so we named this model *Spontaneous dominant leukemia (Sdl)*. Approximately 90% of mice that inherit the mutation become moribund between 6 and 14 weeks of age, and peripheral blood smears show a large increase in the number of circulating leukocytes at necropsy. Positional cloning has mapped the mutation to a 1.6Mb region on chromosome 16, but the mutation and affected gene have not yet been identified. Microarrays comparing gene expression in the thymus of wild-type mice at 21 days and *Sdl* carrier mice at 21 days show a trend toward an increase in *Notch1* expression and activation of downstream targets in the carriers, but the changes were not statistically significant. Comparison of 21 day wild-type thymus to thymic tumors reveals a significant increase in expression of *Notch1* and its downstream targets. Preliminary analysis of the microarray data failed to produce any candidate genes affected by the mutation; however, further analysis is ongoing. We hypothesize that *Sdl* can serve as a preclinical model of human leukemia, and that identification of the mutation may reveal a novel proto-oncogene or tumor suppressor gene. We further hypothesize studying *Sdl* will provide insight into normal T-cell development and progression to leukemia. Experiments are ongoing to determine the clonality and cell type of origin of the disease, as well as flow cytometry to assess the immunophenotype of white blood cells in the bone marrow and peripheral circulation prior to leukemia development. In conclusion, the short disease latency and our ability to determine which mice inherit the *Sdl* mutation make this an ideal model to study leukemia initiation and progression.

SKP2-CYCLIN A INTERACTION IS DISPENSABLE FOR NORMAL DEVELOPMENT BUT ESSENTIAL IN PRB LOSS-INDUCED PITUITARY TUMORIGENESIS

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Although Skp2 was best known as the F-box protein in the SCFSkp2 E3 ubiquitin ligase targeting T187 phosphorylated p27, Skp2 was first discovered as a cyclin A binding protein and the Skp2-cyclin A complex is abundant in various cancer cells. Our lab has conducted more detailed studies of the Skp2-cyclin A interaction (Ji et al. 2006 JBC). We determined that amino acid residues 40-60 in the Skp2 N-terminus are required for Skp2 binding to cyclin A and a peptide spanning residues 40-60 can specifically block Skp2-cyclin A interaction but not p27-cyclin A interaction. We further showed that Skp2 binding to cyclin A competed cyclin A binding to p27 and, as a consequence, Skp2 protects cyclin A from inhibition by p27 and p21. When this 40-60 peptide was delivered into cells with a membrane permeable sequence, it could induce apoptosis in various cancer cells but not in non-transformed cells, suggesting that the Skp2-cyclin A interaction is important for survival of tumor cells (Ji et al. 2007 Mol Can Ther). Following these findings, we have now generated KI mice in which the 4 required residues in the 40-60 sequence were mutated to alanine, the Skp2AAAA KI mice. We have determined that disrupting the Skp2-cyclin A binding does not hinder normal murine development and obtained initial results showing that Skp2AAAA KI can block pituitary tumorigenesis following Rb1 deletion, providing the first evidence for an important tumorigenic role of the Skp2-cyclin A interaction. We are also generating mice combining Skp2AAAA KI and p27T187A KI (which abolishes Skp2's function in mediating p27 ubiquitination) to determine whether inactivation of these two best-established functions of Skp2 will be equivalent to Skp2 KO. Findings from these experiments will be presented at the meeting.

ELECTRONICALLY IDENTIFYING AND EVALUATING MOUSE MODELS OF CANCER USING THE MOUSE TUMOR BIOLOGY (MTB) DATABASE

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Cancer is a diverse and complex array of diseases that involves a large variety of cell types, genetic lesions and interconnecting molecular mechanisms. The use of new molecular techniques and development of novel tumor models, commonly mouse models, to investigate cancer has generated data at an expanding rate. As more and more of these data are published it is becoming increasingly difficult to locate and analyze information regarding mechanisms of cancer and identify potential therapeutics. The Mouse Tumor Biology (MTB) Database is designed to provide researchers with access to and the tools to analyze mouse tumor data allowing the identification and evaluation of potential mouse cancer models (http://tumor.informatics.jax.org).

MTB includes data from peer-reviewed literature and health surveillance data from production mouse colonies at The Jackson Laboratory (JAX) and colonies of aging mice from the Jackson Aging Center. Data from public databases, including Pathbase and the SKY/M-FISH & CGH Database, and direct submissions from cancer researchers and tumor pathologists are also collected. MTB contains data from tumors arising in genetically defined mice (inbred, hybrid, mutant and genetically engineered mice), both spontaneous and endogenously induced. Data include classification, incidence and latency of mouse tumors, pathology reports and images and somatic genetic changes in tumors and in the genetic (strain) background. Pathology images are submitted by the scientific community, from primary literature with permission and from JAX. A web based submission form is available. New features in MTB include searching MTB using human gene symbols or Entrez Gene IDs, a Mouse Cancer Quantitative Trait Loci (QTL) viewer, the ability to customize the Tumor Frequency Grid and the inclusion of somatic cytogenetic images.

MTB uses standard nomenclature, controlled vocabularies and inclusion of literature citations to facilitate data searches. MTB is integrated with the Mouse Genome Informatics database (MGI, http://www.informatics.jax.org) and provides links to other related online resources such as the Mouse Phenome Database (MPD, Festing's Listing of Inbred Strains of Mice, the JAX® Mice Web Site, and the Mouse Models of Human Cancers Consortium's (MMHCC's) Mouse Repository.

MTB provides a web based, integrated resource for identifying information on molecular mechanisms in mouse tumors and existing mouse models of cancer and also facilitating the discovery and development of new mouse cancer models.

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RNA POLYMERASE II SER2 CTD PHOSHORYLATION IN CANCER CELLS AND ITS RELATION TO POLY (A) SITE CHOICE

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Dynamic phosphorylation of the C-terminal domain (CTD) of RNA pol II as it transcribes a gene is thought to direct the traffic of pol II-associated proteins including those that perform co-transcriptional pre-mRNA processing. The CTD comprises conserved heptad repeats (YS2PTS5PS7). Phosphorylation of the heptads on Ser2 residues is maximal at 3' ends of genes and this modification is important for recruitment of cleavage/polyadenylation factors to the transcription elongation complex. Cancer cells make abnormal poly(A) site choices; usually preferring to process at ORF-proximal upstream sites resulting in shorter mRNA 3' UTRs 1-3. As a result normal controls of translation and mRNA stability can be corrupted. It is not known if altered poly (A) sites in cancer cells result from a co-transcriptional or a post-transcriptional decision. To investigate this question, we asked whether abnormal poly (A) site choice in cancer cells is associated with altered dynamics of RNA pol II CTD phosphorylation on Ser2 residues. If this were the case it would strongly implicate a co-transcriptional mechanism for abnormal poly (A) site choices in cancer cells. To this end we have surveyed genome-wide distributions of Ser2 phosphorylated RNA pol II by ChIP-seq using a phospho-specific antibody. We will report results for a panel of human breast cell lines that differ in their tumorigenic potential.

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THE DNA REPLICATION CHECKPOINT PROMOTES E2F-DEPENDENT CELL-CYCLE TRANSCRIPTION.

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The primary regulation of cell proliferation in mammalian cells is imposed during the G1-phase of the cell cycle. Activation of G1/S transcription is the earliest indicator of cell cycle initiation. G1/S transcriptional regulation depends on the E2F family of transcription factors (E2F1-8) and their regulators, the tumor suppressor pocket protein family members (pRb, p107, p130). The high frequency of genetic alterations in human tumor cells that affect proteins involved in E2F-dependent transcriptional regulation suggests that inactivation of this pathway may be necessary for tumor development. For example inactivation of the pocket proteins are often associated with cancer; whereas, unrestrained E2F1 activity has been associated with excessive apoptosis. In addition, DNA damage induced E2F1 activity is coupled to cell death, through the activation of pro-apoptotic genes. Therefore, understanding how E2F activity is modulated both during the cell cycle and in response to activation of cell cycle checkpoints will offer insight into cancer biology and medicine.
Although the activation of E2F-dependent transcription during the G1 phase of the cell cycle has been extensively studied, much less is known about inactivation once cells progress into S-phase. Our data suggests that the mechanism of transcriptional inactivation likely involves E2F6 binding to E2F-dependent promoters during S phase, replacing the activating E2Fs. Furthermore, we show that in response to DNA replication stress E2F-dependent transcription is derepressed, which depends on the DNA replication checkpoint protein kinase Chk1. We present evidence that the mechanism of this regulation likely involves direct Chk1-dependent interference of E2F6-dependent repression of E2F-target genes.
Our work uncovers a simple but elegant mechanism by which checkpoint activation can override the regular periodic transcriptional program by directly regulating a transcriptional repressor. As a putative target of cell cycle checkpoints that regulate genomic stability, E2F6 is expected to play a central role in avoidance of DNA damage and chromosomal aberrations, phenomena that directly contribute to the genesis of human cancer. Elucidating the mechanisms through which E2F transcription is regulated both during the cell cycle and in response to activation of cell cycle checkpoints, and the relevance of such regulation, will be the focus of our future work.

THE DYNAMIC ROLE OF RBP2 DURING DIFFERENTIATION

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Cellular differentiation is disrupted in cancer, resulting in the appearance of poorly differentiated cells. The pRB tumor suppressor protein is inactivated in the majority of human cancers and is known to potentiate differentiation. pRB cooperates with tissue-specific transcription factors (e.g. MyoD in skeletal muscle) to execute a transcriptional program. Rb-/- cells are deficient in transcriptional activation and fail to terminally exit the cell cycle. A number of transcription factors have been linked to the establishment or maintenance of this program, but the precise mechanism is unknown. Restoring differentiation or specific elimination of abnormally differentiated cells is a challenge in cancer therapy. Here we present data where the loss of at least one copy of the gene encoding RBP2 histone demethylase restores differentiation potential in cells with inactivated pRB. RBP2 catalyzes the removal of methyl groups from lysine 4 in histone 3, leading to transcriptional repression. Previously we showed that pRB and RBP2 physically interact, and pRB mutants that do not form complexes with RBP2 are unable to induce differentiation. Early in differentiation, RBP2 works primarily in opposition to pRB by maintaining the proliferative state. We have shown by transient RBP2 knockdown in Rb-/- cells that lowering RBP2 protein levels increases transcription factor activity and gene expression associated with differentiation, and induced characteristic morphological changes reminiscent of the effects seen by reintroducing pRB. Later during differentiation RBP2 binds to promoters of genes targeted by RB and E2F. To further address the epistatic relation between RB and RBP2 during differentiation and to eliminate confounding effects that may arise due to altered function of low levels of Rbp2 in knockdown models we generated knockout mice. We can induce differentiation in mouse embryonic fibroblasts (MEFs) down the myogenic lineage into myotubes by overexpressing MyoD. We found that loss of Rbp2 fully rescues expression of early markers and at least partially rescues expression of late markers of differentiation. The tube-shaped morphology and multinucleation of differentiating cells is partially rescued. Differentiating Rb-/-Rbp2-/- cells are death prone and loss of Rbp2 does not rescue the inability of Rb-/- cells to exit the cell cycle, likely due to the cooperative effect of pRB and RBP2 on E2F target genes. This is consistent with the idea that the capacity of pRB to promote differentiation can be genetically separated from its role as a repressor of E2F. Our results suggest that RBP2 can be a therapeutic target in cancer since inhibition of RBP2 may generate death prone progenitors in cells with an inactivated pRB pathway.

EPIGENETICALLY REGULATED GENES IN B CELL LYMPHOMA

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Epigenetic changes have been increasingly recognised as an important mechanism for altered gene expression in malignant cells, and they have been suggested to be even more common than genetic changes in human cancer. Altered DNA methylation patterns may serve as biomarkers for cancer detection, assessment of prognosis, and prediction of response to therapy. In general, many epigenetic changes occur early in tumor development. Thus, they can be of value for early diagnosis and possibly as sensitive markers for relapse. Importantly, identification of key molecules which are deregulated by methylation in cancer is also of therapeutic interest, as methylation is potentially reversible with demethylating agents. Our group used in vitro models to detect upregulated genes after an epigenetic treatment with 5-aza-2'deoxycytidine and trichostatin A. After gene expression analysis using microarray technology and normalization, we found 2199 upregulated genes. Since we are interested in potential tumor suppressor genes, we compared gene expression profiles from patients and healthy donors and were able to exclude 32% of the responding genes. Genes showing the highest responses to treatment where then examined for CpG islands in their promoter regions, thus further excluding some of the genes.
A methylation specific PCR was performed on the main responding genes, revealing that 63% of the tested genes are methylated within the promotor region. 21% of the methylated genes were also methylated in healthy donors, excluding them from further studies. Using these analyses, we now have a list of interesting genes showing cancer-specific hypermethylation and these genes will be further validated by methylation-specific PCR in lymphoma patients at our hospital. Currently, we are performing real-time PCR to investigate whether there is a correlation between promoter hypermethylation and gene expression. Preliminary results indicate that genes showing promoter hypermethylation are also transcriptionally inactivated compared to normal B cells.

STABILIZATION OF BCL2L10 (BCLB) BY THE UBIQUITIN-ASSOCIATED PROTEIN UBQLN1 (PLIC1) LEADS TO INCREASED ONCOGENIC POTENTIAL

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The processes that regulate apoptosis are universally altered in cancer cells and the BCL2-like proteins are critical and direct inhibitors of apoptosis. Aberrant expression of BCL2 proteins is a well-known mechanism that can block apoptosis and contribute to tumorigenesis. As an example, we have previously shown that all six members of the BCL2 family can cooperate with MYC in a mouse model of leukemia. The least well-studied BCL2-like protein, BCLb (or BCL2L10), has recently been shown to be highly expressed in a large proportion of primary human cancer samples by immunohistochemistry, and the protein levels inversely correlated with prognosis and survival outcome in a number of cancer types. We examined the protein and mRNA levels of BCLb in a panel of human cancer cell lines and did not observe extensive variation in mRNA that would explain the vast differences in protein levels. We therefore set out to determine if we could identify interacting factors that affect the post-translational stability of the BCLb protein. We identified a protein, Ubiquilin1 (or PLIC1) that specifically interacts with BCLb, and not with any of the other five BCL2-like proteins. PLIC1 expression dramatically stabilizes the steady-state levels of the BCLb protein. The presence of PLIC1 leads to the mono-ubiquitination, on multiple lysine residues, of BCLb. Interestingly, when all of the lysine residues of BCLb were mutated to arginines, the BCLb protein is also stabilized. Stabilization of BCLb, by either the presence of PLIC1 or by mutation of lysine residues, enhanced the ability of BCLb to potentiate tumorigenesis, in vivo. These findings suggest that any factor that can affect the steady-state protein stability of BCL2 family proteins could potentially play a direct role in neoplastic transformation.

THIOCTANS, FIRST-IN-CLASS LIPOATE DERIVATIVES, ATTACK SPECIFIC CANCER MITOCHONDRIAL METABOLIC PROCESSES INDUCING MULTIPLE, REDUNDANT CELL DEATH PATHWAYS

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Tumor cell metabolism is now recognized to present extremely promising new opportunities for chemotherapy. While several agents targeting these processes have been described, no drug with high clinical efficacy has yet emerged. We have developed a first-in-class group of non-redox-active lipoate analogs, designated thioctans, allowing a novel attack on tumor metabolism. These drugs are in human clinical trials and our pre-clinical translational studies (below) indicate that they may have unique promise. >Specifically, we have used extensive metabolomics analysis and other assays to characterize the effects of thioctans on tumor cell metabolism (below). Thioctans show high efficacy and very low toxicity in human tumor xenograft models and extraordinarily broad spectrum cancer cell killing in cell culture assays, efficiently targeting all tested tumor cells independently of type (carcinoma, sarcoma, liquid tumor), tissue of origin, or genotype. Our new results indicate that these properties arise from thioctan targeting of specific metabolic steps in an unprecedented way. The blockage of these specific tumor cell metabolic steps, in turn, commits cells to multiple, redundant cell death pathways, including both apoptosis and non-apoptotic death.

Central to cancer-specific metabolic properties are the mitochondrial enzymes pyruvate dehydrogenase (PDH) and alpha-ketoglutarate dehydrogenase (KGDH). PDH and KGDH are two of four enzyme complexes using lipoate as a covalent cofactor. Moreover, virtually all tumor cell mitochondrial carbon and reducing potential enter through PDH and KGDH. Further, the regulatory properties of the PDH complex (and probably KGDH) are substantially altered in tumor cells relative to non-cancerous cells. Finally, PDH and KGDH lipoate residues play a central role in regulating these enzymes, in addition to their catalytic functions. >Our new results demonstrate that both PDH and KGDH activities are powerfully inhibited in tumor cell mitochondria by thioctan treatment, apparently reflecting regulatory effects of thioctans. This inhibition is highly selective, as other metabolic steps are unaffected by initial thioctan treatment, apparently becoming altered only secondarily with the onset of thioctan-induced cell death. Assessing clinical efficacy of this new approach will be important.

AUTOPHAGY CONTRIBUTES TO THERAPY-INDUCED DEGRADATION OF THE PML/RARA ONCOPROTEIN

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Treatment of acute promyelocytic leukemia (APL) with all-trans retinoic acid (ATRA) and/or arsenic trioxide (ATO) represents a paradigm in targeted cancer therapy as these drugs cause clinical remission by affecting the stability of the fusion oncoprotein promyelocytic leukemia (PML)/retinoic acid receptor alpha (RARA). Previous studies have implicated the ubiquitin-proteasome pathway as the main mechanism involved in therapy induced PML/RARA degradation. Here we have investigated a role of autophagy, a protein degradation pathway that involves proteolysis of intracellular material within lysosomes. We found that both ATRA and ATO induce autophagy via the mTOR pathway in APL cells and that autophagic degradation contributes significantly both to the basal turnover as well as the therapy induced proteolysis of PML/RARA. In addition, we observed a correlation between autophagy and therapy-induced differentiation of APL cells. Given the central role of the PML/RARA oncoprotein in APL pathogenesis, this study highlights an important role of autophagy in the development and treatment of this disease.

KEY MECHANISMS UNDERLYING THE STEM CELL OVERPOPULATION THAT CONTRIBUTES TO THE INITIATION AND PROMOTION OF COLON TUMORIGENESIS

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Purpose: Since cancer stem cell (SC) overpopulation appears to drive tumor growth in the colon (Boman & Huang, JCO 2008), effective or even curative treatments will likely require targeting mechanisms that underlie SC overpopulation. To identify such mechanisms, the organization and distribution of colonocyte cell subpopulations in human crypts were investigated as a way to evaluate crypt cell maturation. We hypothesized that maturation of these subpopulations becomes dysregulated during colon tumorigenesis. **Methods:** Quantitative immunohistochemical mapping of crypt cell populations was done using markers for: SC (ALDH1); cell cycle transitioning (DNA-synthesis licensing proteins CDT1/geminin/MCM2); cell-cycle arrest (P21); apoptosis (M30/TUNEL). The indices of cell subpopulations were then used to evaluate maturation, i.e., transitions between cell phenotypes in the crypt – from stem (unlicensed) to proliferating (licensed) to terminally-differentiated (cell-cycle arrested) to apoptotic cells. **Results:** In normal colon, the proportion of cells expressing licensing proteins was low at crypt bottom (where $ALDH^+$ cells reside), high among proliferating cells, and nil in upper crypt (where terminally differentiated/apoptotic cells reside). This suggests that SC are not licensed for DNA-synthesis (consistent with their relative quiescence) and licensing mediates generation of proliferating cells from SC. In pre-malignant crypts, the population of licensed cells expanded and distributed further up the crypt, while $P21^+$, $M30^+$ & $TUNEL^+$ cell subpopulations in the upper crypt contracted. **Conclusion:** Crypt cell maturation (transitions between cell phenotypes) is regulated by DNA-synthesis licensing; dysregulation promotes delayed maturation, SC overpopulation, and tumorigenesis. Therapeutically targeting licensing mechanisms to accelerate cell maturation may reverse SC overpopulation.

GENETIC AND EPIGENETIC REGULATION OF BREAST CANCER ASSOCIATED GENES, INCLUDING BRCA1.

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Aberrant expression of cancer-associated genes contributes to initiation and progression of the tumourigenic process. Such changes in gene expression result from defects in transcriptional control elements including promoters and enhancers and in post-transcriptional control elements including those found in the 3'UTR. Epigenetic defects include methylation of transcriptional regulatory elements and altered targeting of post-transcriptional regulatory elements by trans-acting factors including microRNAs. Our group has a long-standing interest in elucidating the genetic and epigenetic regulation of breast cancer associated genes, including BRCA1. Our studies have led to the identification of regulatory sequences mapping to promoter, intronic, 3'UTR and extragenic sequences of BRCA1, and the promoter of AR and a number of miRNAs, including miR-200b. We have also identified proteins and miRNAs that target these sequences, including the RNA binding protein HuR which targets the BRCA1 3'UTR. We have shown that genetic and epigenetic changes in these sequences, some of which are associated with breast cancer, affect gene expression and are sometimes associated with altered targeting by proteins or miRNAs. We have also identified a number of molecules that are differentially expressed in the pre-malignant mammary glands of a BRCA1-associated breast cancer mouse model. These include the mammary luminal progenitor marker c-kit and a number of miRNAs. Current studies involve elucidating the mechanism of this regulation and the role of these events in mammary tumourigenesis.

WIP1 PHOSPHATASE AT THE CROSSROADS OF CANCER AND AGING

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The PP2C family serine/threonine phosphatase WIP1 is characterized by distinctive oncogenic properties mediated by inhibitory functions on several tumor suppressor pathways, including ATM, CHK2, p38MAPK and p53. PPM1D, the gene encoding WIP1, is aberrantly amplified in different types of human primary cancers, and its deletion in mice results in a profound tumor-resistant phenotype. Numerous downstream targets of WIP1 have been identified, and genetic studies confirm that some play a part in tumorigenesis. Recent evidence highlights a new role for WIP1 in the regulation of a cell-autonomous decline in proliferation of certain self-renewing cell types, including pancreatic beta-cells, with advancing age. Furthermore, Wip1 phosphatase modulates lifespan. These emerging functions of WIP1 make it a potent therapeutic target against cancer and aging.

P19ARF SUPPRESSES THE PROMOTION OF K-RAS DRIVEN NON-SMALL CELL LUNG CANCER

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Non-small cell lung cancer (NSCLC) is among the deadliest of human cancers. Mutations in the K-ras proto-oncogene are a frequent event in NSCLC, but tumor suppression in the lung remains poorly elucidated. Oncogenes such as K-ras induce expression of the p19arf tumor suppressor gene, which is located along with p16ink4a at the Cdkn2a locus. p19arf antagonizes the p53 inhibitor Mdm2, thereby stabilizing p53 activity and arresting cell growth. Recent findings suggest that a permanent form of growth arrest known as senescence acts as a barrier to tumor growth and malignant progression *in vivo*. This phenomenon, termed oncogene-induced senescence, is theorized to occur in response to oncogene-induced DNA damage signaling through p53. Activated p53 and additional markers of DNA damage signaling and senescence have been observed in a range of benign lesions, including lung adenomas. p19arf is required for Ras-induced senescence to occur in mouse primary cells, and loss of p19arf has been shown to cooperate with oncogenic Ras in skin and liver tumor models. Urethane-induced NSCLC tumors in mice frequently harbor K-ras mutations, and here we use this model to address the role of p19arf in suppressing lung tumorigenesis. By means of immunohistochemistry, we revealed high p19arf expression in lung adenomas but limited expression of p19arf in malignant adenocarcinomas. Germline p19arf deficiency accelerated the growth, but not the initiation, of urethane-induced NSCLC tumors. Lung tumor burden in p19arf knockout mice was substantial, with tumor diameters ranging up to 14mm by 25 weeks post urethane injection. Early findings also suggest that p19arf loss potentiates malignant conversion, as adenocarcinomas were more common in p19arf knockout mice. Furthermore, p19arf knockout mice experienced significantly compromised survival compared to wild-type and untreated controls. This enhanced mortality was largely due to the unexpected development of hemangiosarcomas in p19arf knockout mice under urethane treatment. This tumor type was not observed in wild-type treated mice, nor did it occur spontaneously in untreated p19arf knockout mice. These results suggest that p19arf participates in oncogene-induced signaling to raise a potent barrier against tumor promotion in the lung, and that p19arf may restrain the proliferation of endothelial cells in adult mice. Identifying the roles of p19arf in tumor suppression is an important step toward the development of personalized, targeted therapies for cancer patients.

HELICOBACTER PYLORI DECREASES GASTRIC EPITHELIAL P27 THROUGH INHIBITING HISTONE ACETYLATION OF THE P27 PROMOTER

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Background/Aim: Chronic H. pylori infection is associated with decreased p27 expression in gastric epithelial cells. Low p27 levels cause apoptosis-resistance in gastric epithelial cells and increased tumor susceptibility in murine models. We have previously established apoptosis-resistant gastric epithelial cell lines by chronic H. pylori infection in vivo. These cells have both low p27 protein and low p27 mRNA levels. Because p27 transcription may be regulated epigenetically through histone acetylation from delta-opioid receptor stimulation, we examined whether low p27 levels in H. pylori infection was related to inhibition of histone acetylation of the p27 promoter by H. pylori. Methods: The level of acetylated histone on the p27 promoter was measured by ChIP assay in the parental AGS gastric cancer cell line, AGS cell line following 6 hour infection with H. pylori, and AGS derivatives with low p27 expression that were derived from AGS gastric cancer cells by chronic H. pylori coculture followed by dilutional cloning ("HS3C cells"). p27 mRNA and protein were measured by real-time PCR and western blotting. Results: Low p27 expression in the acutely H. pylori-infected AGS cells and chronically H. pylori-infected HS3C cells were associated with an approximate 20% and 40% respectively decrease in p27 mRNA expression compared with levels in AGS parental cells, and was restored by treatment with the delta-opioid receptor agonist, DPDPE ([D-Pen2,D-Pen5]-enkephalin) and histone deacetylase inhibitor trichostatin A. Low p27 mRNA levels were associated with a 15-60% reduction in p27 promoter histone H4 acetylation by the chromatin Immunoprecipitation (ChIP) assay. The p300, gene specific acetyltransferase, which was examined by ChIP was also decreased markedly in the acutely H. pylori-infected AGS cells and chronically H. pylori-exposed HS3C cells compared with parental AGS cells. Conclusion: These results demonstrate that in the gastric epithelial cells infected with H. pylori, the decrease in p27 expression is at least in part due to epigenetic mechanisms involving histone acetylation of the p27 promoter.

ELUCIDATING METABOLIC SIGNALS THAT CONTROL CELL GROWTH AND PROLIFERATION

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We have established the yeast metabolic cycle as a system to study how cell growth and proliferation are coordinated with metabolism and cellular metabolic state. During continuous growth in glucose poor medium, yeast cells become highly synchronized and undergo robust oscillations of oxygen consumption that are coupled to their growth and cell cycle. From transcription and metabolite profiling studies of these cycles, we found that growth, cell division and quiescence happen at distinct phases of the cycle and are accompanied by fluctuations of specific metabolites. Addition of carbon sources or certain amino acids can induce cycling cells from the quiescent phase to enter growth phase prematurely. We believe these metabolites are signaling cells to enter growth by activating conserved growth control pathways. We are currently investigating the mechanistic basis by which carbon sources and products of glycolytic metabolism (e.g., lactate, ethanol) signal entry into growth and the transcription of growth genes. We believe our studies will provide significant insights into growth control mechanisms as well as the metabolic dysfunctions that contribute to cancer.

UNDERSTANDING CONSEQUENCES OF K-RAS ACTIVATION BY IDENTIFYING SUBSTRATES OF THE KINASE ERK2

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The mitogen-activated protein kinases ERK1 and ERK2 (MAPKs) are major targets of oncogenic signaling pathways such as constitutive activation of K-RAS, B-RAF or receptor tyrosine kinases. MAPKs regulate a wide range of normal and pathogenic processes by phosphorylating hundreds of substrate proteins. While many MAPK substrates are known, incomplete knowledge of targets is a major hurdle to understanding the pathological consequences of constitutive activation. To understand how ERK2 regulates normal and pathological phenotypes we aim to identify direct ERK2 substrates in non-transformed and cancer cells, and to determine how a subset of those substrates influences cell behavior.

We are using analog substrate kinases (AS-kinases) to identify direct substrates of ERK2. AS-kinases have an expanded ATP-binding pocket that accommodates unnatural ATP analogs not used by endogenous kinases. ATP analogs with non-bridging γ-thiol allow AS-kinases to thiophosphorylate their substrates, and thiophosphate can then serves as a "handle" to isolate substrates for identification by HPLC-MS/MS. In an initial experiment we applied AS-ERK2 to lysate from NIH 3T3-L1 pre-adipocytes, using a matched wild-type ERK2 as the negative control. We identified 98 ERK2 substrate sites on 80 proteins. 13 of these proteins are known substrates while the remaining 67 appear to be novel kinase/substrate interactions. Novel substrates span a wide range of biological processes including migration and cytoskeletal organization, nuclear transport, RNA splicing, phosphorylation signaling, and transcriptional regulation. We used quantitative phospho-proteomics to show that splicing factor FOX2 and the RhoGAP ARHGAP10 are phosphorylated in response to ERK1/2 activation in living cells.

We are extending this approach by using isotopic labels to compare MAPK substrate utilization in models of KRAS-driven colon cancer. We are in the process of comparing the colon cancer cell lines DLD1 and HCT116, both of which carry activated KRAS, with their isogenic derivatives lacking activated KRAS. We aim to identify MAPK targets that are enriched in presence of activated KRAS, and to determine whether phosphorylation of those targets is required for cancer phenotypes. Large-scale discovery and quantitative analysis of MAPK targets allows us to map oncogenic signaling in unprecedented detail and will provide a new perspective on the mechanisms of common oncogenic mutations.

DIETARY FOLATE DEFICIENCY AND AGING ALTER THE ADAPTIVE LANDSCAPE, PROMOTING SELECTION FOR ADAPTIVE ONCOGENIC EVENTS.

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The environment plays a critical role in the development of human disease. Various investigators have proposed that low micronutrient intake may accelerate cancer and degenerative diseases of aging through still poorly understood mechanisms. Strikingly and conversely, excessive micronutrient intake has been also linked to overall higher incidence of all cause mortality. Folate is a B vitamin important for cellular nucleotide synthesis, methylation reactions and protection from oxidative stress. Using competitive bone marrow transplantation experiments, our lab has explored how altered folate intake affects the fitness of hematopoietic cells. We have observed that hematopoietic stem and progenitor cells of mice that have been subjected to abnormal folate levels, via high and low folate diets, exhibit persistent decreases in fitness compared to cells from normal diet controls. We have analyzed B-cell progenitor populations under folate deficiency and control diet using microarray and proteomic analyses, as well as metabolic assays. Our results support a model whereby low folate levels appear to promote mitochondrial decay, decreasing electron transport chain protein levels and thus causing an accumulation of NADH and a decrease of ATP levels in the cell. Interestingly, we observe similar changes in B-progenitors from old mice, consistent with well-described mitochondrial defects with aging, which may provide an unappreciated link between dietary folate deficiency and aging.
The Bcr-Abl translocation is associated with chronic myeloid and acute lymphoblastic leukemias, but associations of these leukemias and diet are virtually unexplored. We further demonstrate that either a low folate diet or old age substantially promote leukemogenesis initiated by Bcr-Abl, and that Bcr-Abl provides an advantage specifically in B-cell progenitors and selectively during either dietary folate deficiency or old age. We hypothesize that in folate deficient or aged progenitor cell pools, Bcr-Abl provides a selective advantage to B-cell progenitors by driving higher levels of glycolysis and thus restoring normal ATP amounts. The consequent competitive expansion of Bcr-Abl expressing progenitors, a critical step in leukemogenesis, would then provide a sufficient target size for secondary oncogenic mutations. Thus, oncogene-driven increases in glycolysis may be selected for specifically under contexts (such as dietary folate deficiency or aging) associated with impairment of the more efficient method of energy production (mitochondrial).

THE ACTIVITY OF LIVER-ENRICHED AND GROWTH-SUPPRESSIVE TRANSCRIPTION FACTOR CREB-H IS TIGHTLY REGULATED BY N-LINKED GLYCOSYLATION AND UBIQUITINATION

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CREB-H is a liver-enriched bZIP transcription factor of the CREB3 subfamily. CREB-H has growth suppressive activity and was underexpressed in hepatocellular carcinoma (HCC) cells. CREB-H has also been suggested to be involved in acute phase inflammatory response, iron metabolism and gluconeogenesis. CREB-H is ambiently bound to the endoplasmic reticulum (ER) and its proteolytic activation releases an active N-terminal piece that translocates to the nucleus. In this study we showed that the activity of CREB-H is tightly regulated by N-linked glycosylation and ubiquitination. We found that full-length CREB-H is modified at three N-linked glycosylation sites in the C-terminal luminal region. N-linked glycosylation of CREB-H was prevented if all three sites were disrupted. Both unglycosylated and deglycosylated CREB-H was largely unresponsive to simulation of intramembrane proteolysis by brefeldin A or ER-localized site 1 protease, remained bound to the ER, and unable to activate transcription of target genes such as the gene coding for C-reactive protein. On the other hand, we also found that the active form of CREB-H designated CREB-HΔTC is a rapidly turned-over protein. Poly-ubiquitination of CREB-HΔTC occurred in the nucleus and targeted the protein to proteosome-dependent degradation, preventing the target genes from constitutive activation. Taken together, our findings suggest tight regulation of CREB-H activation in hepatocytes by post-translational modifications. Our work thus reveals novel regulatory mechanisms in CREB-H-dependent transcription which might have implications in hepatocarcinogenesis.

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GENETICS AND MECHANISMS UNDERLYING THE ROLE OF THE FAMILIAL PARKINSON'S DISEASE GENE PARK2 IN HUMAN ONCOGENESIS

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In a search for multisite tumor suppressors, we identified PARK2 as a frequently targeted gene on chromosome 6q25.2-q27 in cancer. PARK2 encodes a gene product with E3 ubiquitin ligase activity. We describe inactivating somatic mutations and frequent intragenic deletions of PARK2 in human malignancies. The PARK2 mutations in cancer occur in the same domains, and sometimes, at the same residues as the germline mutations causing familial PD. Cancer-specific mutations abrogate the growth suppressive effects of PARK2. PARK2 mutations in cancer decrease the gene product's E3 ligase activity, compromising its ability to ubiquitinate cyclin E, and resulting in mitotic instability. Cell cycle dysregulation following PARK2 inactivation requires cyclin E and synergizes with p53 loss. These data strongly point to PARK2 as a tumor suppressor on 6q25.2-q27. PARK2, a gene that causes neuronal dysfunction when mutated in the germline, may instead contribute to oncogenesis when altered in non-neuronal somatic cells.

UNCOVERING GENETIC DETERMINANTS OF THERAPEUTIC RESISTANCE IN PRECLINICAL MODELS OF GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBM) is the most common and rapidly fatal primary brain tumor for which current treatment options provide very limited survival benefit. Approximately 50% of GBMs are associated with hyper-activation of the epidermal growth factor receptor (EGFR). Dysregulated signaling through the EGFR pathway facilitates tumor initiation, sustains tumor growth, promotes infiltration of tumor cells into surrounding tissue and mediates therapeutic resistance. Aberrant EGFR signaling is the result of receptor over-expression which often occurs concomitantly with activating mutations and generation of autocrine/paracrine signaling loops. To explore the pathobiology of GBM tumors, our lab has created transgenic mouse models of human GBM based on conditional over-expression of activated EGFR in the brain of adult mice. Tumors are initiated in the context of PTEN and/or p16Ink4a/p19Arf deficiency, other mutations that are prevalent in human GBM. We show here that expression of activated EGFR generates high-grade, malignant gliomas with histological and molecular characteristics that resemble human GBM. We demonstrate that tumor progression in p16Ink4a/p19Arf null mice is exacerbated by loss of PTEN. Interestingly, ex vivo analysis of primary tumor cultures revealed that PTEN deficient tumor cells are resistant to the EGFR kinase inhibitor, gefitinib. Global phosphoproteome analysis showed that activation of signaling proteins in PTEN deficient cells may mediate survival and account for the differential response to EGFR inhibition between tumors of different genetic backgrounds. These findings provide important insight into the manner by which specific gene mutations confer drug resistance. It is our hope that the preclinical models of GBM described herein will provide a platform to discover mechanisms of drug resistance and enable testing of therapeutic strategies aimed at increasing sensitivity of GBM tumors to targeted therapeutics.

COLORECTAL TUMOR GROWTH NEED NOT BE DRIVEN BY RARE DYSREGULATED STEM CELLS.

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The role of stem cells in solid tumors remains controversial. In colorectal cancers (CRC), this is further complicated by the conflicting 'top-down' or 'bottom-up' hypothesis of cancer initiation. We profiled the expressions of genes from the top and bottom of the colonic crypt in morphologically normal-appearing mucosa (M) at least 20 cm away from the tumor and contrasted this to that of matched mucosa adjacent to tumor (MT) in twenty-three sporadic CRC patients using Affymetrix Human Gene 1.0 ST Array system. Four-way analysis of variance (ANOVA) with position (top or bottom), gender, distance (M or MT) and scan date as factors was performed using intersection of position and distance as linear contrast factors at false discovery rate corrections of 0.0001. The final list of eighty differentially expressed transcripts was used to perform the principal component analysis (PCA) for visualization. In thirteen specimens, the genetic distance (as measured by Euclidean algorithm) between the bottom fractions of M and MT was smaller than the distance between the top fractions of M and MT; while in the rest of the specimens, the reverse was observed. The results imply that it is equally likely that sporadic colorectal tumorigenesis initiate from 'top-down' via dedifferentiated colonocytes or 'bottom-up' via dysregulated intestinal stem cells, suggesting that this is a random process and hence need not necessarily be driven by rare dysregulated intestinal stem cells. Further, regardless of the initiation site, differential expression of forty of these genes persisted till tumor formation, suggesting that these are the driver genes for CRC. By Ingenuity® pathway analysis, these genes were shown to play significant roles in cellular signalling, development, growth and proliferation as well as amino acid and lipid metabolism and transport, indicating that driver genes are not limited to tumor suppressors or oncogenes but encompass a whole gamut of cellular functions.

NOVEL SIRNAS SCREENED FROM RANDOM SIRNA LIBRARY INDUCE LEUKEMIA CELL DIFFERENTIATION

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Cancer cells are characterized by abnormal differentiation. Poor differentiation generally correlates with high malignant degree and poor prognosis. Differentiation therapy is an alternative strategy to control cancer by inducing cancer cell differentiation. However, the differentiation reagents that can be used in clinical practice at present are limited. We have previously reported the construction of a random small interfering RNA (siRNA) library. Such library is versatile as it avoids siRNA sequence bias, and targets both known and unknown genes. With this random siRNA library, here we carried out a high throughput screening on an erythroleukemia cell line K-562, in order to find siRNAs that can induce leukemia cell differentiation. Two novel siRNAs were screened from the random siRNA library and verified to be able to induce K-562 cell toward erythroid differentiation: the expression of CD235, ε-globin, γ-globin was significantly increased, and the cell proliferation rate was slowed down. Non-specific interferon induction was not detected, indicating that the siRNA-induced K-562 cell differentiation was due to specific effect. Our screening process with random siRNA library provides an alternative strategy for search of cancer cell differentiation-inducing reagents. Besides siRNA, growing number and various types of small RNAs are found to regulate gene expression within cells. In addition to random siRNA library, silimilar small RNA libraries can therefore be expected as new resources for search of therapeutic reagents.

KSHV INDUCES TRANSCRIPTIONAL REPROGRAMMING OF LYMPHATIC ENDOTHELIAL CELLS INTO AN INVASIVE CELL TYPE

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Kaposi's sarcoma (KS) tumors consist of spindle-shaped endothelial cells (ECs) that express markers of lymphatic endothelium, smooth muscle cells, macrophages and dendritic cells. Upon infection with KSHV, lymphatic ECs (LECs) develop spindle morphology, the hallmark of KS tumor cells, but do not show other properties of transformed cells.

To better mimic the microenvironment of infected cells in vivo, we have developed a 3-dimensional (3D) organotypic spheroid outgrowth assay for KSHV-infected LECs. Interestingly, our results show that while control LEC spheroids sprout very little if at all, the KSHV-LEC spheroids show extensive sprouting.

We have also analyzed the cellular differentiation status of KSHV-LEC and control LEC spheroids by confocal microscopy and qRT-PCR using a variety of markers for cell differentiation. Our data strongly suggests that KSHV induces transcriptional reprogramming of LECs into a mesenchymal cell type via a process called endothelial to mesenchymal transition (EndMT). We are currently deciphering which cellular pathways are activated and involved in the KSHV-induced EndMT by performing a global gene expression analysis of LEC and KSHV-LEC spheroids. EndMT has recently been recognized as a potential source for cancer associated fibroblasts (CAFs). To assess if the KSHV-induced EndMT could act as a source for KS-associated CAFs we have looked for the presence of KSHV-positive mesenchymal cells in a panel of KS biopsies. Intriguingly, cells positive for both KSHV LANA and mesenchymal markers such as aSMA or vimentin can be readily detected in the majority of the KS biopsies analyzed.

EndMT endows cells with migratory and invasive properties as a result of the loss of tight-junctions and by expression of N-cadherin. We have analysed the invasive potential of the KSHV-LECs, and the results suggest that KSHV-LECs can invade the collagen matrix. Inhibition and siRNA knock-down experiments indicate that invasion and spheroid sprouting are dependent on specific matrix metalloproteinases. We are currently investigating the role of viral genes in the transcriptional reprogramming and increased invasion.

IN VIVO VALIDATION OF CANCER GENES USING TRANSPOSONS

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Large scale genetic screens and cancer genome sequencing strategies have given rise to a growing list of candidate genes and mutations involved in cancer progression. While most strategies for validating cancer genes are constrained by the specificity of the system's cellular, tissue or developmental contexts, we have developed a system that samples multiple tissue- and developmental-contexts in the whole animal. Transposons are used to deliver and ectopically express candidate cancer genes. Animals with both transposase and oncogene-containing transposons develop a broad tumour spectrum, and recapitulate the spectrum seen in humans. Here we present an improved design of the platform and ongoing work in validating kinase mutations identified in human cancer genomes. The new strategy allows us to efficiently assay the *in vivo* oncogenic potential of multiple candidate mutations in the mouse model.

FORCING GLIOBLASTOMA TUMOR INITIATING CELLS TO DIFFERENTIATE: TARGETING TRANSCRIPTION FACTORS AND GTPASES USING RNAI

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Glioblastomas are either purely astrocytic or have mixed astrocytic and oligodendroglial features, suggesting the tumor arises from a glial precursor cell capable of generating multiple cell types. Tumor initiating cells (TICs) isolated from human glioblastomas have properties similar to normal neural stem cells and can, at low titer, fully recapitulate the parent tumors in mice [Singh SK, *et al*. Nature. 2004 Nov 18;432(7015):396-401]. There is evidence that TICs maintain the tumor bed and are more resistant to chemotherapy and radiation then the more differentiated cells in the tumor. *In vitro*, TICs give rise to cells resembling astrocytes, neurons, and oligodendrocytes. We are interested in understanding what governs the transition from TICs to a more differentiated state, as well as what genes are required to maintain the tumor initiating state. We performed an RNAi screen on TICs, targeting 2,368 human GTPases and transcription factors using ~11,800 shRNAs. Using high throughput, high-content imaging, we identified genes whose depletion shifted cells from a sphere forming phenotype, characteristic of TICs, towards a differentiated, flat and elongated phenotype, a feature of differentiated cells. Here, we present the optimization of our high throughput imaging and analysis techniques and the preliminary results of our screen. We found 136 genes whose suppression gave a differentiated phenotype. Of those, suppression of 5 genes significantly decreased relative cell number and/or neurosphere formation suggesting a role in cell proliferation. Additionally, of these 136 strong hits, some are overexpressed in particular GBM subtypes. Lastly, suppression of 25 genes increased neurosphere number and relative cell number suggesting the genes might suppress proliferation of TICs or their daughter cells. Orthotopic xenograft studies are underway to clarify the effect of targeting these genes on tumorigenicity. Our aim is to identify new therapeutic targets and eventually add differentiation therapy to current treatment strategies for GBM.

CONTRIBUTION OF NF-KB TO CELLULAR SENESCENCE AND CHEMORESISTANCE

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Cellular senescence suppresses cancer by arresting cell proliferation in response to potentially oncogenic stimuli. Senescent cells undergo massive changes in their secretory profile, referred to as the "senescence-associated secretory phenotype" (SASP), which reinforces the arrest program and triggers an innate immune response that can signal clearance of senescent cells in vivo. Interestingly, many of the SASP factors are known targets of the NF-κB transcription factors, which are, paradoxically, often considered pro-oncogenic. To explore the function of NF-κB during senescence, we applied short hairpin RNA (shRNA) against NF-κB to address its contribution. We found NF-κB is activated in senescent cells induced by different senescence stimuli, including in response to oncogenic Ras and DNA-damaging chemotherapeutic agents. In addition, inhibiting NF-κB activation in senescent cells abolishes the transcriptional changes linked to the SASP and attenuates the ability of senescent cells to be targeted by natural killer (NK) cells in vitro. While inhibiting NF-κB alone is not sufficient to bypass cellular senescence, co-inhibition of NF-κB and p53 prevent the induction of senescence and targeting by NK cells. By using a murine lymphoma model driven by Myc and the anti-apoptotic lesion Bcl-2, we found that chemotherapy-induced senescence depended on NF-κB, such that loss of NF-κB was associated with senescence failure and enhanced disease progression. These data together suggest a novel tumor suppressive function of NF-κB in senescence that contributes to the outcome of cancer therapy.

INCREMENTAL GENETIC PERTURBATIONS TO MCM2-7 EXPRESSION AND SUBCELLULAR DISTRIBUTION REVEAL EXQUISITE SENSITIVITY OF MICE TO DNA REPLICATION STRESS

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Mutations causing replication stress can lead to genomic instability (GIN). *In vitro* studies have shown that drastic depletion of the MCM2-7 DNA replication licensing factors, which form the replicative helicase, can cause GIN and cell proliferation defects that are exacerbated under conditions of replication stress. To explore the effects of incrementally attenuated replication licensing in whole animals, we generated and analyzed the phenotypes of mice that were hemizygous for *Mcm2, 3, 4, 6,* and *7* null alleles, combinations thereof, and also in conjunction with the hypomorphic $Mcm4^{Chaos3}$ cancer susceptibility allele. $Mcm4^{Chaos3/Chaos3}$ embryonic fibroblasts have ~40% reduction in all MCM proteins, coincident with reduced *Mcm2-7* mRNA. Further reductions of *Mcm2, 4, 6,* or *7* in this background caused various phenotypes including synthetic lethality, growth retardation, decreased cellular proliferation, and early onset cancer. Remarkably, heterozygosity for *Mcm3* rescued many of these defects, including cancer phenotype. Consistent with a role in MCM nuclear export possessed by the yeast Mcm3 ortholog, the phenotypic rescues correlated with increased chromatin-bound MCMs, and also higher retention of nuclear MCM2 at the early of S phase. The genetic, molecular and phenotypic data demonstrate that relatively minor quantitative alterations of MCM expression, homeostasis or subcellular distribution, can have diverse and serious consequences for health, viability and cancer susceptibility. The results support the notion that the normally high levels of MCMs in cells are needed not only for activating the basal set of replication origins, but also "backup" origins that are recruited in times of replication stress to ensure complete replication of the genome.

IDENTIFICATION OF MOLECULAR DRIVERS OF HUMAN GLIOBLASTOMA TUMOR INITIATING CELL FUNCTIONS USING A LARGE-SCALE RNAI SCREEN

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Glioblastoma (GBM) is the most aggressive primary brain tumor, with a median survival of only 14.6 months. The aggressiveness and therapeutic resistance that characterize GBM are due at least in part to the presence of a subpopulation of tumor initiating cells (TICs), a.k.a. cancer stem cells. Like neural stem cells, GBM TICs possess extensive self-renewal ability and multipotency - the capacity to form neurons, astrocytes, and oligodendrocytes under differentiating conditions; they are also highly tumorigenic, able to recapitulate the original patient tumor upon intracranial injection of very few TICs into immunodeficient mice. TICs may also possess enhanced chemotherapy and radiation resistance compared to other cancer cells. To develop effective therapies against GBM, it is imperative to identify the molecular pathways that allow TICs to self-renew, differentiate, and initiate tumor formation.

To systematically investigate these pathways, we have developed and carried out a large-scale image-based RNAi screen. In human patient-derived GBM TICs, we established techniques for high-throughput arrayed lentiviral shRNA screening and image analysis, using accepted morphologic measures of TIC proliferation, self-renewal, and differentiation. With these methods, we screened a library of over 11,000 shRNAs targeting more than 2300 genes encoding all known human GTPases and DNA-binding factors. Statistical analysis yielded a hit rate of approximately 5%, identifying genes whose knockdown inhibited TIC self-renewal and proliferation and forced terminal differentiation. We have gone on to validate several of these genes as true, on-target hits in multiple TIC lines from different patients. The validated hits include known drivers of TIC functions, such as the oncogene Notch2, as well as several novel candidates of previously unknown function. We are currently performing in vitro mechanistic studies and in vivo tumorigenesis experiments to further ascertain the roles of these novel TIC regulators in stemness and tumorigenesis.

THE FIRST TARGETED P53 KNOCKOUT RAT MODEL

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The laboratory rat is the preferred model system in many biomedical studies for its physiology and larger size, including cancer research, specifically in breast, prostate and bone metastasis. However, until recently, the lack of genetic manipulation tools for the rat genome made the mouse the predominant choice for researchers. The creation of the first knockout rats via microinjection of zinc finger nucleases (ZFNs) into single-cell embryos was the beginning of a revolution in the rat research world. Being the only tool to engineer the rat genome in a targeted fashion, ZFN technology allows specific and efficient introduction of desired mutations to the gene of interest.

ZFNs are fusion proteins of a zinc finger protein and the DNA endonuclease domain of a type II restriction enzyme, FokI. ZFNs are engineered to bind and cleave at specific chromosomal locus to generate double strand breaks, repair of which results either insertions/deletions (potentially gene knockouts) by the non homologous end joining pathway or targeted integration/gene replacement by homologous recombination. Using ZFN technology, we created p53 KO rats, carrying deletions in exon 3, where ZFNs target. Western blotting validated the absence of p53 protein in p53-/- animals. The homozygotes have a lifespan of up to four months. Currently, we are breeding up the colony for carcinogenicity test with heterozygotes. We hope the new model will help shorten the time needed for carcinogenicity test for new drugs, as the p53 KO mouse model did. In the meantime, we are developing conditional p53 KO rats by inserting loxP sites to flank exon 3 so that tissue-specific cre expression can be used to induce tumors in predicted pattern. We will report the current progress as well as any available pathologic data from deceased homozygous rats.

ISOPRENYLATION REGULATES *C17ORF37* MEDIATED CANCER CELL MIGRATION AND METASTASIS

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Post-translational modification by covalent attachment of isoprenoid lipids (prenylation) regulates the functions and biological activities of several proteins implicated in the oncogenic transformation and metastatic progression of cancer. The largest group of prenylated proteins contains a 'CAAX' motif (C denotes cysteine, A represents aliphatic amino acids, and X any amino acid) at the carboxyl terminal which serves as a substrate for a series of post-translational modifications that convert these otherwise hydrophilic proteins to lipidated proteins, facilitating membrane localization. A novel gene named *Chromosome 17 open reading frame 37 (C17orf37)* located in the 17q12 amplicon is overexpressed in different forms of human cancer. Several studies have reported that a 280 kB minimal region of 17q12 that contains *ERBB2* and *C17orf37* is frequently amplified in breast and colon cancer. Although *C17orf37* overexpression is linked with genomic amplification of *ERBB2* locus, abundant expression of C17orf37 protein in ERBB2 non-amplified breast and prostate tumors suggest C17orf37 has an independent functional promoter. The *C17orf37* gene encodes a 12kD protein which enhances migratory and invasive phenotype of cancer cells. This function of C17orf37 is due to its ability to act as a membrane-bound signaling molecule modulating the PI-3K/Akt pathway, there by transcriptionally upregulating NF-κB downstream target genes MMP-9, uPA and VEGF. Via this process, C17orf37 overexpression in tumors contributes to the migratory and invasive phenotype, facilitating the malignant progression of the disease. Here we show that C17orf37 contains a functional CAAX motif and is post-translationally modified by protein geranylgeranyltransferase-I. Geranylgeranylation of C17orf37 at the CAAX motif results in translocation of the protein to the inner leaflet of plasma membrane, and enhances migratory phenotype of cells by inducing increased filopodia formation and potentiation of directional migration. A prenylation-deficient mutant of C17orf37 is functionally inactive and fails to trigger dissemination of injected cells in a mouse model of metastasis. These findings demonstrate that prenylation is required for the function of the C17orf37 protein in cancer cells and imply that the modification may functionally regulate metastatic progression of disease.

IN VIVO RNAI SCREENING IDENTIFIES NEW MEDIATORS OF P53 INDEPENDENT TUMOR SUPPRESSIVE FUNCTIONS OF P19ARF IN THE LIVER

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Target cells for transformation in human hepatocellular carcinoma (HCC) are either bipotential liver progenitor cells (oval cells in rodents) or differentiated hepatocytes, whereas up to 80% of all human HCCs are thought to be derived from hepatocytes. We observed striking differences between progenitor cell- and hepatocyte derived hepatocellular carcinomas in mice: Whereas oncogenic Nras (NrasG12V) efficiently triggers hepatocellular carcinomas derived from p53-/- liver progenitor cells, almost no tumor growth is observed, when NrasG12V is delivered into p53-/- hepatocytes. However, aggressive hepatocellular carcinomas develop after short latency, when oncogenic Nras is delivered into p19Arf-/- hepatocytes, thus suggesting that p19Arf mediates p53-independent tumor suppressive functions in differentiated hepatocytes. To identify mediators of such p53-independent tumor suppressive functions of p19Arf in the mouse liver, we set up an in vivo RNAi screen. As a proof of principle we could show that co-delivery of NrasG12V and p19Arf-shRNAs into p53-/- hepatocytes via transposable elements was able to phenocopy NrasG12V-driven hepatocarcinogenesis in p19Arf-/- livers. To perform the screen, a focused shRNAmir library was compiled consisting of shRNAs targeting genes differentially expressed in NrasG12V; p53-/- mouse livers compared to NrasG12V; p19Arf-/- livers. This focused shRNA library was divided into several low complexity pools (n=48) and subjected to an in vivo positive selection screen in a p53-/- background (co-delivery with NrasG12V). From NrasG12V; p53-/- tumors we identified several shRNAs knocking down new candidate genes mediating p53-independent tumor suppressive functions of p19Arf in the mouse liver. Functional validation experiments using single hairpins have already been completed for several candidates, among these are genes involved in the mitotic spindle assembly and the spindle checkpoint. shRNA-mediated knockdown of those candidate genes allows for Ras driven tumorigenesis in a p53-deficient background.

FUNCTIONAL ANALYSIS OF THE PLU1/JARID1B HISTONE DEMETHYLASE IN BREAST CANCER

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Epigenetic mechanisms are important in determining cell fate, including the development and progression of cancer, through the regulation of specific transcriptional programmes involved in development, differentiation, and normal homeostasis. Recent discovery of histone demethylases expands our view of epigenetic mechanisms. For example, trimethylated lysine 4 of histone H3 is a robust epigenetic mark enriched near transcription start sites of active genes and essential to regulation of multiple cellular processes. Regulation of this mark is mediated, in part, by the JARID1 subgroup of JmjC proteins which have been discovered to demethylate tri- and dimethylated H3K4 residues. We identified RBP2/JARID1A as a critical regulator of the switch from cellular proliferation to differentiation; we found that RBP2 looses binding to some genes that are activated during differentiation and recruited to genes that are repressed during differentiation in a Retinoblastoma (pRB)-dependent manner, which is consistent with pRB's positive role in differentiation. RBP2's closest homologue, PLU1/JARID1B, is expressed in most breast cancers, and has been implicated in cancer progression. Because of the high homology to RBP2, we propose that PLU1 also regulates proliferation and differentiation. To study the function of PLU1 in breast cancer, we employed siRNA technology to knockdown PLU1 in MCF7 and MDA-MB-231 breast cancer cell lines. siRNAs to PLU1 in MCF7 cells reduces proliferation and anchorage-independent growth in soft agar assays compared to control siRNAs. Loss of PLU1 in MDA-MB-231 cells induced a prominent morphological change to a more epithelial-like morphology (round and flat). Additionally, we developed an inducible knockdown system to study the importance of both PLU1 and RBP2 in breast cancer. Using a Tet-off inducible system, we knocked-down PLU1, RBP2, and pRB in MCF7 and MDA-MB-231 cells. By BrdU incorporation, Ki67 immunostaining, and colony formation assays in MCF7 cells, we found that loss of RBP2 decreases proliferation while loss of RB increases proliferation. We also found that, like PLU1 knockdown, loss of RBP2 decreases anchorage-independent growth, while loss of RB increases anchorage-independent growth. Thus, we conclude that both PLU1 and RBP2 are critical regulators of proliferation in breast cancer cells, and may represent important therapeutic targets.

GROWTH STIMULATION AND PROLIFERATION BLOCKAGE: DUAL EFFECTS OF FGF2/FGFR IN RAS-DRIVEN MOUSE MALIGNANT CELLS

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Tyrosine kinase receptors' signaling has ordinarily been implicated in mitotic, pro-surviving and sometimes oncogenic signaling pathways which stimulate cell growth and proliferation. However, some groups, including ours, have reported that in malignant cells RTK's signaling can also trigger unexpected antiproliferative effects. The Fibroblast Growth Factor 2 (FGF2) is one these RTK ligands which triggers a dual response in malignant cells. We have previously shown that FGF2 through its RTK receptor (FGFR) restrains the proliferation of Ras-driven mouse malignant cells; here, we aim to elucidate cell and molecular mechanisms underlying the antiproliferative stress response triggered by FGF2 on K-Ras-dependent Y1D1 mouse malignant adrenocortical cells. Cell cycle flow cytometry analyses following DNA content and BrdU pulse-labeling showed that FGF2, in spite of stimulating G0>G1>S transition, causes in these malignant cells a delay in FCS-stimulated DNA synthesis followed by a strong block of the G2/M transition, uncoupling cell growth from cell division. Polyploidization, giant cells formation and cell death were also late effects of FGF2 treatment. Corroborating these results, 48hs growth curves further confirmed that FGF2 stimulates an increase in the average cell size without signs of mitosis and/or increase in cell number. Furthermore, this antiproliferative picture triggered by FGF2 in Ras-dependent malignant cells is accompanied by the arising of DNA damage response markers, pointing to a mechanistic explanation to this stress response triggered by FGF2.
Thus, altogether these results are showing that FGF2/FGFR signaling can uncover a cancer-specific "Achilles' heel" in the K-Ras-dependent malignant phenotype of mouse cells one-time highly resistant to cell death.

MODELING BCL-2 AND BCL-X_L INHIBITION IN THE HEMATOPOIETIC SYSTEM USING TRANSGENIC RNA INTERFERENCE

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Bcl-2 family proteins including Bcl-2, Bcl-x_L, and Mcl-1 promote cell survival during development, however they are often aberrantly overexpressed in cancer. The novel therapeutic compound ABT-263 targets Bcl-2 and Bcl-x_L and has shown single agent efficacy in recent clinical trials for chronic lymphocytic leukemia. However to date no specific inhibitors of Bcl-2 or Bcl-x_L are available, therefore it has been difficult to assess the contribution of each protein to tumour maintenance. To address this question, we have adapted the tetracycline (tet)-regulated expression system to generate transgenic mouse strains allowing inducible expression of short hairpin RNAs (shRNAs) targeting Bcl-2 or Bcl-x_L *in vivo*. Using a reporter strategy, we find that a transgenic strain expressing the rtTA (tet-on) transactivator under control of the CMV promoter drives particularly efficient tet-regulated expression in the megakaryocyte/platelet lineage. Employing this system to induce doxycycline-dependent Bcl-x_L shRNA expression depletes Bcl-x_L in megakaryocytes and triggers severe thrombocytopenia, consistent with a known role for Bcl-x_L in maintaining platelet survival. Shutting off shRNA expression normalizes Bcl-x_L levels and restores platelet numbers, effects akin to those observed with ABT-263. Knockdown of either Bcl-2 or Bcl-x_L in adult mice results in mild lymphopenia, however reporter analysis suggests that shRNA expression is variable in the lymphoid compartment using this transactivator strain. We are now optimising this inducible knockdown strategy to more accurately model systemic therapeutics targeting Bcl-2, Bcl-x_L and other potential drug targets in established lymphomas and leukemias.

INSULIN RECEPTOR PATHWAY HYPERACTIVITY IN IGF-1R NULL CELLS AND SUPPRESSION OF DOWNSTREAM SIGNALING USING THE DUAL IGF-1R/IR INHIBITOR, BMS-754807

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The biology of IGF-1R/IR signaling was studied in normal mouse embryonic fibroblasts (MEFs) that were either wildtype (wt), heterozygous (het), or null for the IGF-1R. The ability of IGF-1, IGF-II or insulin to stimulate serum-starved MEFs was characterized by gene expression profiling and biochemical analyses for activation of downstream signals. Each genotypic group of MEFs exhibited distinct patterns of expression both while resting and in response to stimulation. The insulin receptor (IR) pathway in IGF-1R null MEFs was hypersensitive to insulin ligand stimulation resulting in greater AKT phosphorylation than in wt or het MEFs stimulated with the same ligand. Interestingly, the IR pathway hypersensitivity in IGF-1R null MEFs occurred with no observed changes in the levels of IR isoforms A or B. A new small molecule IGF-1R inhibitor (BMS-754807), having equipotent activity against both IGF-1R and IR, proved effective in suppressing both AKT and ERK phosphorylation from both the IGF-1R and IR pathways by all 3 ligands tested in wt, het and null MEFs. The use of a dual IGF-1R/IR inhibitor addresses concerns about the use of growth inhibiting therapies directed against the IGF-1R receptor in certain cancers. Lastly, comparison of the anti-proliferative effects (IC50s) of various compounds in wt vs. null MEFs demonstrates that genetically characterized MEFs provide a simple and inexpensive tool with which to define compounds as having mostly on-target or off-target IGF-1R activities since off-target compounds affect both wt and null MEFs equally.

REV3 SUPPRESSION SENSITIZES DRUG RESISTANT LUNG TUMORS TO CHEMOTHERAPY

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Platinum-based chemotherapeutic drugs are front-line therapies for the treatment of non-small cell lung cancer. However, acquired or intrinsic resistance limits the clinical efficacy of these agents. Recent evidence suggests that loss of the translesion (TLS) polymerase, Polζ, can sensitize tumor cell lines to cisplatin, although the relevance of these findings to the treatment of chemoresistant tumors *in vivo* remains unclear. Here, we describe a tumor transplantation approach that enables the rapid introduction of defined genetic lesions into a pre-clinical model of lung adenocarcinoma. Using this approach, we examined the effect of impaired translesion DNA synthesis on cisplatin response in aggressive late-stage lung cancers. In the presence of reduced levels of Rev3, an essential component of Polζ, tumors exhibited pronounced sensitivity to cisplatin, leading to a significant extension in overall survival of treated recipient mice. Additionally, treated Polζ-deficient cells exhibited reduced cisplatin-induced mutation – a process that has been implicated in the induction of secondary malignancy following chemotherapy. Taken together, our data illustrate the potential of Rev3 inhibition as an adjuvant therapy for the treatment of advanced non-small cell lung cancer and highlight the utility of rapid transplantation methodologies for evaluating mechanisms of chemotherapeutic resistance in pre-clinical settings.

DEPLETION OF APC CAUSES MULTIPLE DEVELOPMENTAL DEFECTS AND DISRUPTS HAIR FOLLICLE HOMEOSTASIS

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The adenomatous polyposis coli (APC) gene is one of the most frequently mutated genes in colorectal cancer and is often silenced or mutated in other human malignancies. APC is thought to function primarily as a negative regulator of β-catenin and canonical Wnt signalling although recently, various other direct roles have been described. Mice heterozygous for the APC ^{min} allele (a nonsense mutation creating a truncated protein) develop multiple intestinal polyps by 4-5 months of age due to loss of heterozygosity at the wt allele., while APC $^{min/min}$ homozygous mice fail to progress beyond E7.5 of embryogenesis. Tissue specific truncation of APC using Cre/LoxP strategies have indicated a requirement for APC in the development of hair follicles and intestinal and hepatic homeostasis, but thus far it has not been possible to study the role of APC in multiple tissues during early mammalian development or in adult mice. Here, we have used a recombinase-mediated ES cell targeting strategy to derive transgenic animals expressing a tet-regulated shRNA targeting APC. Knockdown of APC in adult animals induces a rapid expansion of the hair follicles and within two weeks, dox-fed mice grow significantly longer hair. Paradoxically, long-term APC knockdown causes significant hair loss, presumably due to depletion of hair follicle stem cells. Knockdown of APC during early post-natal development causes multiple phenotypes including craniofacial defects and severe runting, which cannot be reverted following re-expression of the endogenous APC protein. We are now developing improved tet-transactivator mice to optimise the expression of shAPC in diverse tissues and investigating the consequences of chronic vs. transient disruptions to the Wnt pathway in normal tissue homeostasis and during malignant progression.

IDENTIFICATION OF CJUN NH2-TERMINAL KINASE 2 TARGET MOLECULES NECESSARY FOR RAS TRANSFORMATION OF MOUSE EMBRYONIC FIBROBLASTS.

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Mutations that lead to activation of the proto-oncogene Ras are found in over 25 % of all human cancers, making it the most frequently activated oncogene in cancer. The function of active Ras and the pathways it activates have been studied intensively, but how Ras signaling actually mediates the transformation of a normal cell into a tumor cell is not fully understood.
It is well established that the Ras oncogene can activate several signaling pathways (ERK, NFκB, Ral/JNK and PI3K/AKT) and that they all can contribute to cellular transformation. The aim of this project was to find and characterize those downstream target/s of the Ras signaling pathway that mediate transformation.
While analyzing the cJun NH2-Terminal kinase (JNK) isoform specific knockout mouse embryonic fibroblasts (MEFs), I observed that the Jnk2 knockout cells, opposite to the corresponding Jnk1 knockout cells, cannot be transformed with oncogenic Ras.
The best studied JNK target, c-Jun which is a part of the activator protein 1 (AP-1) transcription factor complex, has previously been shown to be crucial for Ras transformation. I showed that JNK2 is not functioning via cJun/AP-1 (TRE site), since the extent of c-Jun phosphorylation and the activity of the AP-1 transcription factor complex was not impaired in Ras expressing Jnk2-/- MEFs. Instead expression of the ATF2 protein, which is also an AP-1 component was severely impaired in Jnk2-/- MEFs and ectopic expression of ATF2 restored the ability for Ras to transform Jnk2-/- MEFs. In addition, transformation was rescued by increasing c-Myc protein level via ectopic expression of c-Myc or cancerous inhibitor of protein phosphatase 2A (CIP2A) that seems to have oncogenic effect in mediating Ras transformation in Jnk2-/- MEFs. ATF2, CIP2A and c-Myc seem to cooperate in mediating Ras transformation in JNK2-/- MEFs.

TARGETING MITOCHONDRIAL APOPTOTIC PATHWAYS IN C-MYC-INDUCED MAMMARY TUMORS

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Evasion of apoptosis is a hallmark of cancer and a major cause of treatment failure. In breast cancer, the expression levels of Bcl-2 and Bcl-xL are commonly elevated and deregulation of Bcl-2 or Bcl-xL accelerates mouse mammary tumor progression by allowing cells to escape apoptosis. Upregulated levels of Bcl-2 and Bcl-xL confer resistance to chemotherapy and therefore, these proteins are attractive targets for apoptosis sensitization and synthetic lethal strategies. We have previously shown that c-Myc-induced sensitization of mammary cells to apoptosis is tightly linked to conformational activation of Bak. Addition of TRAIL to sensitized cells is required for progression of Bak to oligomerization stage followed by MOMP and strictly Bak-dependent apoptosis. The data infer that pharmaceutical neutralization of Bcl-2/Bcl-xL might specifically promote apoptosis in c-Myc-induced mammary tumors expressing active Bak. We show that WAP-Myc induced mammary tumorigenesis is tightly associated with elevated levels of Bcl-xL but not of Mcl-1. In 3D cultures of WAP-Myc tumors, the BH3 mimetic small molecule inhibitor ABT-737 strongly synergizes with active c-Myc in the induction of apoptosis. Consistently, administration of ABT-737 as a single agent to the WAP-Myc mice with palpable tumors induced significant reduction in the tumor growth rate, which associated with increased levels of apoptosis. Furthermore, treatment of ex vivo prostate tumor tissue slices induced sporadic activation of Bak as a marker of apoptotic sensitization. Notably, previous studies with xenograft-based preclinical models have revealed high Bcl-xL levels and low Mcl-1 levels as indicators for good ABT-737 efficacy. The responses observed in WAP-Myc model endorse the significance of high Bcl-xl/low Mcl-1 as predictors of ABT-737 efficacy. These GEMMs for breast cancer may thus serve as tractable model for search of treatment modalities based on apoptosis sensitization strategies.

INFLUENCE OF CAGA EPIYA MOTIFS VARIATIONS IN *HELICOBACTER PYLORI* STRAINS FROM COLOMBIA ON IL-8 EXPRESSION AND CELL ELONGATION

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Helicobacter pylori is a gram negative bacterium that has been associated with the development of gatroduodenal diseases such as chronic gastritis, duodenal ulcers and gastric cancer. *H pylori* CagA protein is translocated into gastric epithelial cells where it undergoes phosphorylation by cellular kinases on tyrosine residues within EPIYA motifs, present within the C-terminal region of the protein. Phosphorylated CagA activates important cell signaling pathways, inducing the expression of the proinflammatory cytokine interleukin-8 (IL-8) and cell elongation (also known as the "hummingbird phenotype"). EPIYA motifs in Western *H. pylori* strains are classified as EPIYA-A, EPIYA-B and EPIYA-C, based on the sequence flanking the motif. The number of EPIYA motifs can vary from two to six. Previous studies have suggested a positive association between the number of EPIYA motifs, the number of EPIYA-C motifs and the expression of IL-8 and "hummingbird phenotype" formation. The aim of this study was to evaluate the possible association between the variations in the CagA EPIYA motifs in *H. pylori* isolates from Colombia and the expression of IL-8 and cell elongation induction in gastric epithelial cells. 84 *H. pylori cagA* positive strains with different EPIYA motifs patterns, isolated from Colombian patients with different gastric pathologies, were evaluated. IL-8 expression was determined by ELISA after taking supernatants from cocultures of AGS epithelial gastric cells with *H. pylori*. For cell elongation quantification, coculture photographs were taken and the proportion of "hummingbird" cells (>5µm) was determined. There was no association between the number of EPIYA motifs and IL-8 expression, although a variable expression of IL-8 was observed. Furthermore, no significant differences were found in *H. pylori*-induced IL-8 expression between strains isolated from gastric cancer and strains isolated from other gastric pathologies. Similar results were obtained for the "hummingbird phenotype" induction, for which no association was found neither to the number of EPIYA motifs nor to the pathologies from which strains were isolated. These results suggest that other factors (e.g. host or environmental) may play a more important role than *H. pylori* CagA protein EPIYA variations in gastric cancer development in Colombia.

REGULATION OF DYNAMIC C-MYC PROMOTER BINDING AND TRANSCRIPTIONAL ACTIVITY BY THE PEPTIDYL PROLYL ISOMERASE PIN1

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The c-Myc oncoprotein is a transcription factor that regulates most cellular functions by either activating or suppressing downstream target genes. While c-Myc levels are generally low in most normal tissues, high levels of c-Myc expression occur in a wide variety of human tumors. Control of c-Myc expression can occur at many levels, including transcription, mRNA stability, translation, and protein stability. We and others have characterized a complex signaling pathway that helps to control c-Myc protein stability and accumulation through the sequential phosphorylation of two highly conserved sites, Serine 62 (S62) and Threonine 58 (T58). These phosphorylation sites have opposing effects on c-Myc stability, where phosphorylation at S62 can stabilize c-Myc and subsequent phosphorylation at T58 promotes c-Myc ubiquitin-dependent proteolysis. Pin1 is a peptidyl prolyl isomerase that plays an important role in this degradation pathway for c-Myc. As the only known phosphorylation-specific prolyl isomerase, Pin1 plays a key role in the regulation of protein function by alterations in substrate conformation after phosphorylation. Pin1 is known to regulate diverse cellular processes, and it has been linked to several diseases, including cancer, where it is often overexpressed. Here, we show that Pin1 positively regulates c-Myc's transcriptional activity by enhancing the on-rate for c-Myc promoter binding, resulting in changes in the expression of endogenous c-Myc target genes and enhanced c-Myc oncogenic potential. Moreover, a positive correlation exists between Pin1 levels and phospho-Serine62 c-Myc levels in human breast cancer cell lines and patient samples. We have found that the Axin1 scaffold protein coordinates a destruction complex for c-Myc at target gene promoters containing GSK3β, PP2A-B56α, and Pin1. Thus, Pin1 plays a dual role in regulating c-Myc, both enhancing its transcriptional activity and stimulating its degradation, suggesting a mechanism for coordinating the activation of c-Myc with its destruction at the promoters of its target genes. Taken together, this work highlights the function of Pin1 in the dynamic regulation of c-Myc activity and suggests that Pin1 is a viable therapeutic target in human cancers overexpressing c-Myc.

EXPERIMENTAL RESTORATION OF TUMOR SUPPRESSOR FUNCTION INDENTIFIES A CONTEXT SPECIFIC SENSITIVITY TO P53 IN NON-SMALL CELL LUNG CANCER

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Reversing the effects of so-called *driver* mutations to eradicate cancer rests on the premise that activation of oncogenes, or inactivation of tumor-suppressor genes is required not only for tumor genesis, but also for tumor maintenance. Experimental genetic approaches and pharmaceutical inhibition of the effects of driver mutations has supported the notion that a sustained effect of these mutations is indeed required for the viability of tumor cells. In contrast, we present evidence for a context specific sensitivity to restoration of p53 gene function in non-small cell lung cancer. We show that early stage tumors are apathetic to the restoration of p53 gene function, whereas more advanced tumors are sensitive to the anti-tumor effects of p53. Moreover, we find that all tumors of advanced stage maintain varying proportions of less advanced cells within the tumor mass that are also indifferent to p53 restoration. These low-grade tumor cells persist despite significant loss of tumor volume after p53 restoration. Through histological analysis, we provide evidence that p53 opposes tumor progression toward the adenocarcinoma stage but has no effect on tumor initiation. Advanced lung tumors in *KrasLA2; p53* deficient mice characteristically harbor high levels of MAPK signaling, that in some instances is likely caused by copy number gains of the chromosomal region encompassing Kras. Though the mechanisms of MAPK signal amplification are diverse, the increased level of signaling that we observe is universally associated with increased expression of the p53-activating tumor suppressor Arf. These observations have led to a model where increased MAPK signaling drives tumor progression but also leads to activation of p53 through Arf expression. Thus, when p53 gene function is restored, only Arf expressing tumors are capable of activating p53's tumor suppressive effects. These findings suggest that restoration of pathways important in tumor progression, as opposed to initiation, may lead to incomplete tumor regression due to the heterogeneity of cell populations within a tumor.

FUNCTIONAL IDENTIFICATION OF OPTIMIZED RNAI TRIGGERS USING A MASSIVELY PARALLEL SENSOR ASSAY

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Short hairpin RNAs (shRNAs) enable regulated silencing of target genes through their programming of endogenous RNAi pathways. Although widely used to study gene function, the identification of potent shRNAs can be tedious, often involving the empirical examination of dozens of shRNAs to find one that is effective. To overcome this barrier, we developed a functional strategy integrating all aspects of the RNAi process to assess the potency of shRNAs in a high-throughput manner. By interrogating a library of 20,000 constructs comprising every possible shRNA across 9 mammalian transcripts, we demonstrate that our assay reliably identifies highly potent shRNAs that are predominantly missed by existing prediction algorithms. Although potent shRNAs are surprisingly rare, they share strong nucleotide biases that provide insights into the mechanistic basis of RNAi. Overall, this assay can be used to identify potent shRNAs targeting any gene, which will enable the production of validated genome-wide shRNA libraries and may facilitate the development of effective RNAi therapeutics. More broadly, the "Sensor" assay illustrates a general co-optimization strategy in which a library of linked regulators and their binding sites can be screened in a multiplexed fashion.

TUBERIN REGULATES MITOTIC ONSET THROUGH THE CELLULAR LOCALIZATION OF CYCLIN B1

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Tuberous Sclerosis (TS) is a multi-organ disorder characterized by the formation of primarily benign tumours, called hamartomas, which affects more than 1 million people worldwide annually. TS is caused by a mutation in one of two tumor suppressor genes, TSC1 or TSC2 which encode for the protein products Hamartin and Tuberin respectively. This TS Complex (TSC) is known to negatively regulate protein translation and cell cycle progression through G1/S. Herein we demonstrate that Tuberin binds and regulates the G2/M cyclin, Cyclin B1 (CycB1). We have determined that this binding region encompasses a mutational hotspot within Tuberin implicated in some of the most severe cases of TS. Mimicking a mutation frequently found in patients we report a significant acceleration in the nuclear movement of CycB1 and enhanced mitotic index. These results demonstrate a novel and clinically relevant mechanism by which Tuberin may function as a tumor suppressor to prevent premature mitotic onset.

CHARACTERIZATION OF THE POTENTIAL TUMOUR SUPPRESSOR PRDM5

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PRDM5 belongs to PR-domain family of putative methyltransferases. Members of this family are characterized by the presence of a N-terminal PR/SET domain, typically involved in methyltransferase activity, and a variable number of C-terminal C2H2 zinc fingers domains likely responsible for DNA and protein binding.
PRDM5 has been reported to be a tumour suppressor due to its lowered or lost expression in different types of cancer, including breast, ovarian and gastric cancer. The transcriptional silencing of *PRDM5* is caused by promoter hyper-methylation, and is especially frequent among tumours of gastrointestinal tract origin (Watanabe et al., Gastroenterology, 2009). Furthermore, over-expression of PRDM5 in cancer cells induces cell cycle arrest, apoptosis and block of proliferation (Deng et al., Oncogene, 2004), sustaining the notion of PRDM5 as a tumor-suppressor. Moreover, in a zebrafish model, Prdm5 has been reported to be involved in mesoendoderm development and to collaborate with the WNT pathway.
We have initiated a comprehensive project aiming to mechanistically characterize the tumour-suppressive functions of PRDM5 *in vivo*. Towards this, we have generated a *PRDM5* knockout mouse model that we are currently analyzing for spontaneous and chemically induced tumour formation and in crosses with tumour-prone mouse strains.
Interestingly, our preliminary data from knockout MEFs show that loss of Prdm5 results in increased growth and susceptibility to RAS-V12-mediated cellular transformation. Moreover, we are evaluating a potential role of PRDM5 in WNT pathway activation in terms of target genes modulation.
To unveil the molecular mechanism of PRDM5, yeast-two-hybrid and mass-spec analysis of biochemical protein complex purifications have been performed and reveal that PRDM5 participate in large MDa-size complexes also encompassing a range of chromatin-modifying proteins. Despite the presence of a PR/SET domain we have not been able to detect any intrinsic methyltransferase activity of PRDM5, but we have confirmed the interaction of PRDM5 with the HMT G9a and HDACs.
Current efforts focus on a more detailed phenotypic analysis of the *Prdm5* KO mouse strain and on the identification of PRDM5 target genes using a combination of expression array analyses and ChIP-seq.

CDK7 ABLATION *IN VIVO* ABOLISHES CELL CYCLE PROLIFERATION AND CDK ACTIVITY WITHOUT AFFECTING RNA POL II DEPENDENT GLOBAL TRANSCRIPTION.

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The activation of Cdks require two main steps: Cyclin binding and phosphorylation of their T-loop. The latter is mediated by CAK (Cdk Activating Kinase), a trimeric kinase that includes Cdk7, Cyclin H and Mat1. CAK has also been identified as part of the general transcription factor TFIIH. Here, CAK is responsible for the phosphorylation of the carboxy-terminal domain (CTD) of the large subunit of the RNA Polimerase II, a process thought to be essential for the generation of mRNAs. We have generated germ line as well as conditional Cdk7 mutant mice. Ablation of Cdk7 in the germ line results in early embryonic lethality. The elimination of Cdk7 in the epidermis of newborn mice leads to a cytologically and architecturally dysplasic skin, characterized by an improper differentiation of keratinocytes, as demonstrated by an altered cytokeratin pattern. On the other hand, depletion of Cdk7 in adult tissues with low proliferation rate, such as brain, cerebellum or liver, did not produce any histological alteration.
In MEFs, the elimination of Cdk7 arrests them in G1 and G2 phases of the cell cycle, and when serum starved they are no longer able to exit quiescence. We did not observe variation in phosphorylation degree of Ser 5 in the CTD. Moreover, the mRNA levels of all the housekeeping genes that we have analysed, were not modified. Remarkably, we were able to restore the proliferation of cells lacking endogenous Cdk7 by expression of an exogenous Cdk7 mutant that only shows activity towards Cdks. Although less eficiently, proliferation can also be rescued by expression of phosphomimetic mutants in Thr of the T-loop of Cdk1 or Cdk2. We can conclude that the main role of Cdk7 in cell division can be attributed to Cdk activation.
Since Cdk7 depletion would not affect global gene expression in non-dividing cells, thereby limiting undesired side effects, it should be considered as an interesting druggable target. Inhibition of Cdk7 would deprive cancer cells of the Cdk activity required to promote E2F- dependent transcription and orderly progression throughtout the cell division process.

REGULATION OF FOXM1 IN CANCER AND ITS POTENTIAL AS A THERAPEUTIC TARGET

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The oncogenic transcription factor FoxM1 is an attractive therapeutic target in the fight against cancer, because it is overexpressed in the majority of human tumors, while the tumor suppressor p53 is mutated and inactivated in 50% of human tumors. We found that the FoxM1 gene is repressed by p53 and that FoxM1 overexpression in cancers can partially be explained by the absence of p53 mediated repression. In addition to being upregulated in cancers, we found that FoxM1 protein is stabilized by DNA-damage in human cancer cells with mutant p53, and FoxM1 inhibits apoptosis induced by radiation or DNA-damaging agents, making cancer cells resistant to treatment. Knockdown of FoxM1 sensitized human cancer cells to radiation or DNA-damaging drugs.

Earlier, we identified the thiazole antibiotics Siomycin A and thiostrepton as specific inhibitors of FoxM1 transcriptional activity and expression that induce apoptosis in cancer cells. Paradoxically, at the same time Siomycin A and thiostrepton stabilized the expression of a variety of proteins, such as p21, Mcl-1, p53 and hdm-2 and also act as proteasome inhibitors in vitro. In contrast, we found that other known thiazole antibiotics neither inhibit FoxM1 activity, nor act as proteasome inhibitors and did not induce apoptosis in mammalian cells. Moreover, we found that thiostrepton could suppress tumor growth in human cancer xenograft models suggesting that it has anticancer activity in vivo. Interestingly, thiostrepton synergistically induced apoptosis in human cancer cells following combination treatment with another proteasome inhibitor bortezomib.

Furthermore, well-known proteasome inhibitors such as MG115, MG132 and Velcade also inhibit FoxM1 transcriptional activity and FoxM1 expression. Siomycin A/thiostrepton and traditional proteasome inhibitors induced apoptosis and suppressed FoxM1 at the same time in human cancer cell lines of different origin. Our data indicate that proteasome inhibitors generally inhibit FoxM1 expression and this may contribute to their efficacy as anticancer drugs. Since proteasome inhibitors stabilize the majority of cellular proteins, we hypothesize that they stabilize a negative regulator of FoxM1 that inhibits FoxM1 transcriptional activity. Since FoxM1 induces its own expression, then by repressing FoxM1 transcriptional activity negative regulator of FoxM1 will also inhibit FoxM1 expression. Overall, since FoxM1 plays an important role in driving tumorigenesis and may induce resistance to anticancer treatment, it is a viable target for cancer therapies. In the end, we propose a strategy to identify novel FoxM1 inhibitors compounds that are not proteasome inhibitors.
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SOS REGULATION BY THE MEMBRANE-ACTIN LINKER PROTEIN EZRIN

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Small G proteins of the Ras subfamily control the communication between the cellular outside and inside. The level and life time of the GTP-loaded active state is mainly regulated by two factors: GTPase activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs). Upon receptor tyrosine kinase (RTK) activation adaptor proteins recruit the GEF son of sevenless (SOS) to the receptor and thereby into the proximity of Ras, where SOS can catalyze GTP loading of Ras.

SOS carries an allosteric domain whose interaction with Ras-GDP or Ras-GTP is required for effective catalytic action on another Ras molecule. This allosteric site is, however, blocked by autoinhibitory SOS domains. The *in vivo* trigger that releases SOS autoinhibition is currently only partially known. Here we show SOS regulation by the membrane-actin linker protein ezrin (or the related proteins radixin and moesin [ERM]). We can distinguish two steps: interaction of ezrin with the Dbl homology/pleckstrin homology domain of SOS which promotes unfolding of SOS and release from autoinhibition; and more efficient presentation of Ras-GDP by ezrin to the allosteric site of SOS. Moreover SOS/Ras regulation by ezrin is physiologically antagonised by the neurofibromatosis type 2 tumour suppressor merlin. Taken together we propose a new level of SOS/Ras control involving a family of actin-binding proteins that is relevant in human cancer as well as in physiological processes involving Ras.

ONCOGENIC FUNCTION OF MDM4 IN SKIN MELANOMA.

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MDM2 and MDM4 are key negative regulators of p53. Aberrant expression of either MDM2 or MDM4 is thought to impair p53 tumor suppression function and therefore to favor the development of tumors that retain wild-type p53.

p53 mutations (less than 5%) and MDM2 amplification and/or overexpression are rare events in skin melanoma,. We have examined the levels of MDM4 expression in a panel of 16 skin melanoma cell lines and 40 primary melanoma tumors (10 primary lesions, 10 dermal invasive tumors, 10 lymph node metastasis and 10 distant metastasis). As expected, only 4 primary tumors and 3 melanoma cell lines showed elevated MDM2 levels. In contrast, MDM4 protein levels were elevated in about 65% of all primary tumors and 38% of the melanoma cell lines analyzed. Preliminary knock-down experiments indicate that MDM4 prevents induction of p53-mediated growth arrest and is therefore required for the proliferation of these cell lines.

In order to further establish a causative link between MDM4 overexpression and melanoma formation in vivo, we sought to develop a Mdm4 melanoma mouse model. To this end, a conditional Mdm4 transgenic line has been intercrossed with mice expressing the Cre recombinase (Tyr::Cre) and the NrasQ61R mutant specifically in melanocytes (Tyr::NrasQ61R). Data from these crosses are not yet available, however, we will provide compelling evidence that loss of p53 on this specific melanoma background (Tyr::NrasQ61R) accelerates tumor formation.

Together these data, yet preliminary, indicate that MDM4 might contribute to the development of skin melanoma. Consequently, MDM4 might be a potential drug target for the treatment of this disease.

ACTIVATION OF THE HMGA2 PATHWAY AND NOT BI-ALLELIC INACTIVATION OF THE TUMOR SUPPRESSOR GENE TSC2 IS REQUIRED FOR TUMOR FORMATION.

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Tuberous sclerosis complex (TSC) is regarded as a tumor suppressor gene syndrome whereby the classical bi-allelic inactivation of either TSC genes (*TSC1*, hamartin or *TSC2*, tuberin) is said to activate the mTOR pathway and ultimately lead to tumorigenesis. TSC is associated with the widespread development of mesenchymal tumor cell types, manifestations such as lymphangiomyomatosis (LAM) and renal angiomyolipomas (AML). High mobility group A2 (HMGA2) is an architectural transcription factor, that is misexpressed in a number of benign mesenchymal tumors and its tumorigenic ability in differentiated mesenchymal cells *in vivo* studies.

Our experiments were directed at the potential genetic interaction between the TSC2 and HMGA2 pathways. Similar to humans, $Tsc2^{+/-}$ mice display a wide spectrum of tissue tumor cell types in multiple tissues. Our initial, correlative studies demonstrated that the HMGA2 pathway was activated in 100% of human and mouse TSC2 tumor samples. Tumor incidence was examined in $Tsc2^{+/-}$ mice following loss of HMGA2. Most dramatically, there was a complete absence of mesenchymal tumors in the $Tsc2^{+/-}Hmga2^{-/-}$ mice that are observed in the liver, lung, eyes and skin of the $Tsc2^{+/-}$ mice. Therefore, there is an absolute requirement for HMGA2 activation in mesenchymal tumor pathogenesis in the $Tsc2^{+/-}$ mice. In the course of our studies, tuberin expression was analyzed and surprisingly, tuberin was detected in all of the mouse and human tumor cell types with the exception of a subset of kidney tumors that had undergone LOH at the *TSC2* locus.

Our results have important ramifications for the prevailing hypothesis in TSC and by extension, to other tumor suppressor syndromes. These studies demonstrate that haploinsufficiency in a tumor suppressor gene is necessary for tumor initiation but bi-allelic inactivation is not required for tumor formation. Additional pathways therefore are activated and in TSC, the HMGA2 pathway was found to be essential for mesenchymal tumor formation. Furthermore, it appears that Knudson's hypothesis may apply in a highly tissue-specific manner such that in TSC, LOH is observed only in the kidney. In conclusion, the present study has identified the activation of the HMGA2 pathway as an obligate requirement for mesenchymal tumor formation and for TSC specifically the HMGA2 pathway provides novel, potential drug targets for therapy in the broad spectrum of tumors.

EVIDENCE FOR A NOVEL MECHANISM OF LENTIVIRAL VECTOR-INDUCED TUMORIGENESIS

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Safer and more effective lentiviral vectors are presently being used for gene delivery in early phase clinical trials. In contrast to leukemias that developed following gamma-retroviral transduction, as yet, no adverse outcomes have resulted from lentiviral-based therapy. In this study, we focused on prenatal liver-directed gene transfer by transducing fetal mice with a lentiviral vector derived from feline immunodeficiency virus. This procedure resulted in efficient hepatocyte transduction, however, 3/8 transduced mice each developed two hepatocellular carcinomas. To investigate the link between lentiviral transduction and tumor development, we mapped viral integration and chromosomal aberration sites and found them to be unique in each tumor. Gene expression analysis using microarrays did not reveal differential expression of oncogenes or tumor suppressor genes in the vicinity of viral integrations; however, 5/6 tumors exhibited common activation patterns of genes associated with DNA repair, chromosomal instability, oncogenesis, and E2F transcription factors. Thus, we propose that lentiviral transduction may induce a global cellular mechanism entailing the DNA damage response that leads to upregulation of E2F target genes, resulting in oncogene activation and tumor development.

THE ROLE OF ATF2 AND ATF7 IN MODELS OF HEPATOCELLULAR CARCINOMA.

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ATF2 and ATF7 are members of the AP-1 transcription factor family which regulate expression of genes involved in stress and DNA damage response. In cancer, ATF2 has been reported to be involved in tumour suppressing but also in tumour promoting activities. Here we present an analysis of potential roles for ATF2/7 in response to oncogenic Ras transformation in a model of hepatocellular carcinoma (HCC).
Using mouse knockouts we have previously established that the two factors are essential for the embryonic liver development by engaging in antiapoptotic mechanisms to ensure the survival of hepatoblasts. Cultured hepatoblasts can be used to study the onset of hepatocellular carcinoma via transformation with oncogenes and reintroduction into recipient livers through orthotopic transplantation. We found that ATF2/7 double knockout hepatoblasts transformed with the H-Ras oncogene (H-RasG12D) produced significantly more and larger colonies in vitro. After orthotopic transplantation transformed knockout cells developed tumour nodules in recipient livers at significantly faster rates and in greater numbers compared to cells that were normal for ATF2. In addition, deletion of ATF2 and ATF7 in cells isolated from established liver tumours also result in accelerated growth in graft models.
Therefore, in contrast to their antiapoptotic activities in primary hepatoblasts, our data support a growth suppressing role for ATF2/7 in response to oncogenic transformation.

ACTIVATED BRAF INDUCES PILOCYTIC ASTROCYTOMAS IN MICE

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Pilocytic astrocytoma (PA) comprises the most common primary brain tumor in children and is assigned to grade I according to the WHO. Although PA shows a benign biologic behavior, many patients suffer from therapy-related neurologic impairment and up to 20% of cases experience tumor recurrence. Due to the slow growing nature of PA, post-operative radiation and chemotherapy are only moderately effective.
Recent research has led to the discovery of the activation of mitogen-activated protein kinase (MAPK) signaling as a general molecular pathomechanism in PA and a potential molecular target for therapy. Germline NF1 gene mutation is associated with astrocytoma development, which leads to aberrant MAPK pathway activation. Intriguingly, also sporadic cases of PA show similar MAPK target gene signatures. We and others have previously identified duplications or activating mutations (V600E) of the proto-oncogene BRAF at 7q34 or fusion genes including the BRAF kinase domain as a predominant genetic mechanism leading to MAPK pathway activation in more than 50% of PAs.
However, generation of an appropriate model for PA by using xenotransplants or genetically engineered mice has been hampered by the low proliferative character of these tumors and a lack of knowledge about their molecular etiology.
To evaluate the oncogenic potential of BRAF, we used the replication competent avian leukosis virus with splice acceptor (RCAS)/Tv-a system for retroviral gene transfer to introduce different BRAF variants into nestin positive cells in the brain of Ntv-a mice. Thereby, we were able to induce astrocytic tumors, which closely resemble the histology of human PAs, and show a causal role of BRAF activation in the pathogenesis of PA. This model now enables us to perform pre-clinical assessment of novel therapy approaches.

CDK4 IS A CENTRAL MEDIATOR OF CENTROSOME AMPLIFICATION IN HER2 POSITIVE BREAST CANCER CELLS.

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Centrosome amplification, the acquisition of three or more centrosomes per cell, contributes to carcinogenesis by generating multipolar spindles and aneuploidy. Observations in breast tumors show that adenocarcinoma cells have a much higher frequency of centrosome defects when compared to normal breast tissue. Similar phenotypes are also found in pre-invasive in situ ductal carcinoma, suggesting that these aberrations occur early in breast carcinogenesis. Identification of the pathways and regulatory molecules involved in the generation of centrosome amplification is essential to understanding the role of centrosome amplification in breast tumorigenesis. Due to the strong association between Her2 over-expression and centrosome amplification in human breast cancers, our goal is to identify cell and centrosome cycle regulators that are de-regulated by aberrant Her2 signaling in human and mouse mammary epithelial cells. Her2 signals through various pathways, including Ras. Results from our lab show breast cancer cells over-expressing Her2, human mammary epithelial cells over-expressing H-RasG12V, and pre-malignant mouse mammary hyperplasias expressing Neu or K-RasG12D harbor centrosome amplification. Genome-wide and targeted expression screens of human and mouse mammary epithelial cells expressing Her2 and Ras, respectively, show up-regulation of cyclin D1 and other important centrosome regulatory molecules. Abrogation of Cdk4, a catalytic partner of cyclin D1, using siRNA and lentiviral shRNA reduced the frequencies of centrosome amplification in Ras and Her2+ cells without affecting the cell cycle. In contrast, silencing cyclin B2, cyclin E1, or Cdk2 did not affect centrosome profiles. We postulate that Cdk4 is an important link between centrosome amplification, aneuploidy, and mammary epithelial transformation, as ablation of Cdk4 not only abrogates centrosome amplification, but also mouse mammary tumorigenesis triggered by Neu and H-RasG12V. We want to elucidate the mechanism responsible for centrosome amplification in Her2+ breast cancers and determine its involvement in breast carcinogenesis. We propose that in Her2+ cells, Cdk4 signals centrosome amplification through a classical pathway involving hyperphosphorylation of NPM at Thr199, a site that negatively regulates centrosome duplication. Our results challenge the classical view that Cdk2 is critical to oncogene-triggered centrosome amplification. We propose a new paradigm: Oncogenes such as Her2/Ras signal centrosome amplification exclusively through Cdk4, while other oncogenes, such as E7 or ablated p53 signal centrosome amplification through Cdk2 and Cdk2&Cdk4, respectively.

PHYSICAL AND FUNCTIONAL MAPPING OF THE SOMATICALLY MUTATED EGFR PATHWAY

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We are undertaking a comprehensive characterization of signaling elicited by somatically mutated epidermal growth factor receptors (EGFR). These mutations occur in nearly ten-percent of lung cancer and predict EGFR kinase inhibitor sensitivity. Physical maps of EGFR signaling are being created using (i) detailed protein-protein interaction map using tandem affinity purification in conjunction LC-MS/MS and (ii) a global characterization of tyrosine phosphorylated proteins regulated by EGFR using immunoaffinity capture with anti-phosphotyrosine antibodies and LC-MS/MS. By interrelating protein-protein interactions, phosphotyrosine signatures, and data from a RNA interference screen, a map of signaling will be constructed to highlight important components vulnerable to attack. Using EGFR as a bait, we identified 36 interacting proteins in PC9 lung cells including known components such as other Erb members (ErbB2), adaptors (Grb2, ShcA), EGFR regulators (MIG6, Sts1, AP2u1), and chaperones (HSP90, Cdc37, BIP). We selected an additional 9 proteins for additional TAP experiments and produced a protein-protein interaction network consisting of 110 putative proteins. We found PI3K subunits in complex with ErbB3 but not EGFR nor ErbB2. AP2M1 associated with its larger family of AP proteins, EPS15, and proteins involved in cell adhesion icluding E-cadherin and catenin-delta. Cdc37 bound multiple kinases, including EGFR, ARAF, CDK4, and NFκB members, suggesting chaperone requirements of these kinases in partnership with Hsp90 and functional importance in these cells. Consistent with this hypothesis, RNAi mediated loss of Cdc37 reduced EGFR expression and increased apoptosis in EGFR addicted lung cancer cells. Additional selected targets have been functionally validated using RNA interference or overexpression studies. Overexpression of MIG6 over a one month period resulted in reduced EGFR and Erk phosphorylation consistent with its known role as a negative regulator of EGFR kinase. Interestingly, cells overexpressing MIG6 developed gradual resistance to EGFR tyrosine kinase inhibitors and were found to have increased Akt phosphorylation. No evidence exists for upregulation of another receptor tyrosine kinase as a mechanism of increased Akt phosphorylation. These preliminary results suggest that kinase addicted cells could adapt to negative regulators of EGFR through mechanisms that increase signaling through Akt. Current studies on this mechanism are planned and updated work will be presented.

CHARACTERIZATION OF THE AP4-REGULATED TRANSCRIPTOME

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Recently, we identified the AP4 gene as a direct target of the c-MYC proto-oncogene. Similar to c-MYC, AP4 encodes a BR-HLH-LZ transcription factor which binds to so-called E-boxes in the vicinity of target genes. However, the AP4 binding sites have a different consensus (CAGCTG) when compared to the c-MYC E-box (CACGTG). Therefore, AP4 presumably regulates a different set of genes than c-MYC. We previously found that AP4 represses expression of the CDK inhibitor p21 via 4 E-boxes in the p21 promoter. Interestingly, AP4 is expressed in progenitor cells in a number of tissues and human tumors often show high levels of AP4 expression. Here we characterized the set of genes directly regulated by AP4 in colorectal cancer cells by a combination of microarray and global ChIP-Seq analyses. Thereby we found that AP4 induces the expression of 122 genes and represses 129 genes more than 2 fold. The regulation of selected genes by AP4 was confirmed at the mRNA, gDNA and protein level, and in vivo by confocal microscopy of intestinal crypts, in which AP4 is expressed at the base and declines towards the top. The identity of the validated AP4 targets indicates that AP4 is a coordinating regulator of cell cycle progression and differentiation. Therefore, the AP4-regulated transcriptome presumably fulfills important functions during normal and tumorigenic processes.

SIRT1 OVEREXPRESSION IMPROVES HEALTH AND PROTECTS FROM LIVER CANCER BUT DOES NOT AFFECT P53-KNOCKOUT ASSOCIATED TUMORIGENESIS

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Sirt1 is the mammalian closest homologue to yeast Sir2, a protein deacetylase that has been shown to increase the lifespan of lower organisms when overexpressed. Besides, it has been proposed to mediate calorie restriction beneficial effects on health, which include lifespan extension and cancer protection, although genetic evidence is lacking in mammals. We have generated transgenic mice moderately overexpressing Sirt1 under its own regulatory elements (Sirt1-tg mice). We have found that Sirt1-tg mice do not live longer but preserve a better health at old ages according to a variety of physiological indicators. Moreover, Sirt1-tg mice are also protected from metabolic syndrome-associated liver cancer1. We present evidence indicating that the beneficial effects of Sirt1 are due to its impact on metabolism[1,2] and genomic integrity[1]. On the other hand, Sirt1 doesn't affect either 3-methylcolanthrene-induced fibrosarcoma development or spontaneous cancer associated to p53-knockout background, being the latter a tumorigenesis that is known to be delayed by calorie restriction. Together, these results indicate that Sirt1 overexpression improves health and protects from certain type of cancers but it does not fully recapitulate calorie restriction-associated phenotypes.

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[2]Pfluger, P.T*., Herranz, D.*, et al., (2008). Sirt1 protects against high-fat diet-induced metabolic damage. Proc Natl Acad Sci U S A 105, 9793-9798.
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IDENTIFICATION OF A NOVEL CHEMOSENSITIZER BY SCREENING SMALL COMPOUNDS AGAINST HUMAN THYMIDYLATE KINASE

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Thymidylate kinase (TMPK) is an essential enzyme for dTTP formation in either de novo or salvage pathways. Our previous study has shown that silencing human TMPK by shRNA decreases the cellular level of dTTP and sensitizes colon cancer cells to doxorubicin-induceddeath, suggesting that agent that blocks TMPK function can be used as a chemotherapysensitizer. We then further identified a novel inhibitor of TMPK, designated H9805, by a biolueminescence-based screening. H9805 inhibited TMPK enzymatic reaction in a dose-dependent manner with Ki of 0.22 ± 0.03 μM. H9805 treatment decreased the cellular dTTP level and caused a significant synergistic effect with doxorubicin in killing a variety of cancer cell lines but not normal cell lines. Interestingly, doxorubicin sensitivity was increased by H9805 treatment 1.7- and 30-fold in in non-tumorigenic H184B5F5/M10 and tumorigenic MDA-MB-231 cells, respectively. When profiling the cell cycle of these two cell lines in response to doxorubicin treatment, we found that MDA- MB231 cells were accumulated in S and G2 phase, whereas H184B5F5/M10 cells showed more population in G1 phase. The differential checkpoint activation induced by doxorubicin treatment and the sustained checkpoint activation for complete DNA repair, may determine the chemosensitization effect of H9805. Moreover, animal experiments also showed its effectiveness on suppressing tumor growth when combined with low dosage of doxorubicin. These data suggest H9805 compound as a good lead candidate for chemosensitization.

IDENTIFICATION AND INVESTIGATION OF THE POTENTIAL TUMOR SUPPRESSIVE FUNCTION OF *ALPHA B-CRYSTALLIN* (*CRYAB*) IN NASOPHARYNGEAL CARCINOMA

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Regions of chromosome 11 are frequently lost in many cancers including nasopharyngeal carcinoma (NPC). This may reflect the possibility that essential tumor suppressor genes are harbored in the lost regions. Previously, we have transferred the intact chromosome 11 into the NPC cell line HONE1. Functional suppression of tumor formation in nude mice suggests intact chromosome 11 is able to suppress tumor growth. 11q13 and 11q22-23 were identified as tumor suppressive critical regions (CRs). *Alpha B-crystallin (CRYAB)* maps within the 11q22-23 CR and is down-regulated in all NPC cell lines and in approximately 47% of NPC tumors via promoter hypermethylation and allelic loss. In this study, we provide the functional evidence that *CRYAB* suppresses in vivo tumor formation in nude mice. Re-expression of CRYAB protein in the stable transfectants affects cell adhesion and invasion ability. These results suggest that *CRYAB* may be a potential tumor suppressor gene in NPC and play a role in affecting tumor cell adhesion and metastasis.

WILD TYPE P53 CONTROLS CELL MOTILITY AND INVASION BY DUAL REGULATION OF MET IN THE OVARIAN SURFACE EPITHELIUM

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Association of *p53* gain of function mutations with poor prognosis has been well established for cancers of some systems, such as colorectal and hematopoietic. Recently, it has been recognized that such mutations may facilitate carcinogenesis by increasing cell motility and invasion. Intriguingly, these observations have been difficult to reconcile with the poorest prognosis of *p53* null mutations reported in some types of cancer, including ovarian carcinoma. To evaluate the effects of loss vs. gain of function *p53* mutations on the ovarian surface epithelium (OSE) we have taken an advantage of previously established by us conditional mouse model of epithelial ovarian cancer. Cre-*loxP*-mediated *p53* inactivation resulted in immediate upregulation of Met receptor tyrosine kinase and led to increased cell motility and invasion of the OSE. These effects were abrogated by deletion of the *Met* gene. In addition to previously reported Met regulation by p53-transactivated miR-34, we determined that p53 represses Met expression by inhibiting Sp1 transcription factor binding to the proximal region of *Met* promoter. Notably, while miR-34-mediated regulation of Met was inactive in the OSE with both null and missense mutations, mutant p53 retained *Met* promoter suppressive function by interacting with Sp1. Accordingly, conditional activation of *p53* R172H and R270H mutants led to increased OSE motility and invasion, but to a lesser degree than *p53* null mutation. Taken together, our findings identify Met as a critical player in p53-mediated control of motility and invasion and provide a mechanistic explanation for less pronounced effect of mutant p53 on ovarian carcinogenesis. These results indicate that personalized therapeutic approaches tailored to *p53* mutational status may have uneven effect in a cell context -dependent manner.

THE INV(16) FUSION GENE *CBFB-MYH11* HAS ACTIVITIES INDEPENDENT OF *CBFB/RUNX1* REPRESSION THAT ARE IMPORTANT FOR LEUKEMOGENESIS.

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Inv(16) is found in nearly all patients with acute myeloid leukemia (AML) subtype M4Eo. Inv(16) results in the fusion of the transcription factor gene *CBFB*, and the *MYH11* gene, which encodes Smooth Muscle Myosin Heavy Chain. The leukemia fusion gene *CBFB-MYH11* is thought to induce leukemia by repressing RUNX1 and CBFß. Consistent with this model, mice expressing a knocked in *Cbfb-MYH11* allele ($Cbfb^{+/MYH11}$) have a similar phenotype as mice nullizygous for either *Cbfb* ($Cbfb^{-/-}$) or *Runx1* ($Runx1^{-/-}$). Embryos of all 3 genotypes have a complete block in definitive hematopoiesis. Recently we showed that *Cbfb-MYH11* also causes defects during the earlier stage of primitive hematopoiesis. $Cbfb^{+/MYH11}$ embryos have a delay in differentiation that is not seen in either $Cbfb^{-/-}$ or $Runx1^{-/-}$ indicating that *Cbfb-MYH11* has additional activities independent from the repression of *Cbfb* and *Runx1*. We also showed that these new activities are potentially important during leukemogenesis. To determine which domains of the fusion protein may be involved in this new activity, we generated knock-in mice expressing a mutant *Cbfb-MYH11* allele with a deletion of the 95 C-terminal amino acids ($Cbfb^{+/MYH11?95}$). During primitive hematopoiesis, we found that $Cbfb^{+/MYH11?95}$ and $Cbfb^{MYH11?95/MYH11?95}$ mice had no or very mild differentiation defects, statistically significantly less severe than seen in embryos expressing full-length *Cbfb-MYH11*. This finding indicates that *Cbfb-MYH11?95* has lost the *Cbfb/Runx1*-repression independent activities. During definitive hematopoesis, there were no observable defects in $Cbfb^{+/MYH11?95}$ mice, but $Cbfb^{MYH11?95/MYH11?95}$ embryos showed a complete block in definitive hematopoiesis implying that *Cbfb-MYH11?95* is capable of repressing *Cbfb* and *Runx1*, although less efficiently than full-length *Cbfb-MYH11*. Importantly, $Cbfb^{+/MYH11?95}$ mice did not develop leukemia, even after treatment with the mutagen *N-ethyl-N-nitrosourea*, which we've shown previously to quickly and efficiently induce leukemia in mice expression full length *Cbfb-MYH11*. Together, these results indicate that the 95 C-terminal amino acids of *Cbfb-MYH11* are required for leukemogenesis and that the recently described *Cbfb/Runx1* repression independent activities are important during this process.

DIFFERENTIAL IMPACT OF DMP1-LOSS BETWEEN MOUSE AND HUMAN BREAST CANCER SURVIVAL

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Dmp1 (cyclin D binding myb-like protein 1; Dmtf1) is a transcription factor that causes $p19^{Arf}$-, p53-dependent cell cycle arrest in response to oncogenic Ras and HER2 signaling. *HER2/neu* is amplified in nearly 30% of human breast cancers and is associated with metastatic disease, and thus, with poor prognosis of patients. The receptor is activated by overexpression or by making heterodimers with other EGFR family proteins. Both the *Dmp1* and *Arf* promoters are activated by HER2/neu through the PI3K-Akt-NF-?B pathway. Molecular genetic analyses of MMTV-*neu* mammary tumors showed that biallelic *Arf* deletions, Mdm2 overexpression, or *p53* mutations were rare. On the other hand, we found that the *Dmp1* gene was deleted in more than 50% of MMTV-*neu* tumors, suggesting the critical role of *Dmp1*-inactivation in HER2/neu-driven mammary carcinogenesis. Overexpression of Tbx2 and Pokemon was also frequent in *neu* tumors. Tumor-free survival of MMTV-*neu* mice was significantly shortened in both $Dmp1^{+/-}$ and $Dmp1^{-/-}$ with no significant differences between the two cohorts, suggesting haploid-insufficiency of *Dmp1* for *neu* tumor suppression. Tumors from $Dmp1^{+/-}$ or $Dmp1^{-/-}$ mice were significantly larger than those from $Dmp1^{+/+}$ mice when weighed 30 days after detection, and showed more invasive phenotypes than those from wild-type *neu* mice. Analysis of 66 pairs of human breast cancer samples showed loss of heterozygosity (LOH) of h*DMP1* in ~50% of breast carcinomas, the frequency of which was even higher than LOH of the *INK4a/ARF* (~20%) or *p53* (~30%) locus, or amplification of the *Hdm2* gene (~10%) with the same samples. LOH of h*DMP1* was typically found in mutually exclusive fashion from that of the *INK4a/ARF* or the *p53* locus. The hDMP1 protein expression was low or barely detectable in the nuclei of breast carcinomas that showed LOH for h*DMP1*. LOH of h*DMP1* was associated with low Ki67 index and diploid karyotype of tumor cells, suggesting favorable prognosis. Consistently, breast cancer patients with LOH for h*DMP1* showed significantly longer relapse-free survival than those without LOH. Thus, loss of *DMP1* has differential prognostic impact on survival of breast cancer between mice and humans, possibly because of the low frequency of the *Ink4a/Arf*, *Mdm2*, and *p53* locus involvement in murine tumors.

DOWNREGULATION OF THE CYCLIN-LIKE PROTEIN SPY1 IS AN ESSENTIAL COMPONENT OF THE DNA DAMAGE RESPONSE.

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Maintenance of DNA integrity is essential for the viability of the organism. Potentially oncogenic lesions trigger a signalling cascade known as the DNA damage response (DDR) which initiates repair of the DNA as well as essential cell cycle checkpoints, serving as a safeguard against transformation. This research focuses on a cyclin-like protein Spy1 (Speedy; Spdy1) previously shown to prevent DDR-induced apoptosis when ectopically overexpressed and to promote clonal cell growth in cells harboring damaged DNA. Spy1 has been found at elevated levels in several human cancers including breast and liver. Herein we demonstrate that selective degradation of Spy1 is an essential component of the DDR signalling cascade occurring in response to both DNA damage as well as critically shortened telomeres. Interestingly, our work suggests that endogenous expression of Spy1 may play a pivotal mechanism in DDR recovery to promote cell cycle re-entry following DNA repair. This work suggests a mechanism whereby the cell could adapt to the DDR to promote malignant progression.

BRCA2 DEFICIENCY IN THYMOCYTES INSTIGATES P53-DEPENDENT T CELL LOSS AND IMMUNE DYSFUNCTION

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Germline mutation in *BRCA*2 is linked to the familial breast cancer as well as to the progressive bone marrow failure syndrome, Fanconi Anemia. Established mouse knock out models to study the function of Brca2 show embryonic lethality, but ones with truncating mutation at more C-terminus survive to birth and succumb to thymic lymphoma at an early age. To overcome early lethality and investigate the function of Brca2, we utilized T cell specific conditional *Brca2* knock-out mice which develop thymic lymphoma at a moderate penetrance. In the conditional knock out mice, we found that the number of peripheral T cells, especially naïve pools drastically decline with age. The decline was due to the loss of thymic input as well as inapt peripheral T cell maintenance. Furthermore, Brca2-deficient T cells were highly compromised in cell division and in effector cytokine production upon activation. These defects were due to the activation of p53 pathway, as the Brca2 deficient phenotypes were rescued by *p53* heterozygosity. Altogether, our study faithfully records the molecular events occurring in the Brca2-deficient T cells and suggests that mutation in *Brca2* could lead to the malfunction of T cell populations which will make the host more susceptible to cancer.

A FUNCTIONAL GENETIC APPROACH TO ANTI-CANCER DRUG CHARACTERIZATION

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Identifying mechanisms of drug action remains a fundamental impediment to the development and effective use of chemotherapeutics. Here, we describe an RNA interference (RNAi)-based approach to characterize small molecule function in mammalian cells. By examining the response of cells expressing a validated set of short hairpin RNAs (shRNAs) to a diverse selection of chemotherapeutics, we could generate a functional shRNA signature that was able to accurately group drugs into established biochemical modes of action. This, in turn, provided a diversely sampled reference set for high-resolution prediction of mechanisms of action for poorly characterized small molecules. For example, we were able to use this approach to classify two ill-defined cytotoxic drugs as inhibitors of topoisomerase I and II, respectively. We could further reduce the predictive shRNA target set to as few as 8 genes, and by using a newly derived probability based nearest neighbors approach, could extend the predictive power of this limited shRNA set to characterize additional drug categories. Thus, a focused shRNA phenotypic signature can provide a highly sensitive and tractable approach for characterizing new anti-cancer drugs.

THE *CEBPB* 3'UTR SUPPRESSES THE RAS-INDUCED CYTOSTATIC AND PRO-SENESCENCE FUNCTIONS OF C/EBPB BY INHIBITING ITS POST-TRANSLATIONAL ACTIVATION

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C/EBPβ is an auto-repressed protein that is activated by Ras signaling and contributes to oncogene-induced senescence (OIS) in primary fibroblasts. We discovered that Ras-induced post-translational activation of C/EBPβ (LAP) is suppressed in several immortalized and transformed cell lines by the 3' untranslated region (3'UTR) of its mRNA. The *Cebpb* 3'UTR blocked Ras^{V12}-stimulated proliferative arrest by C/EBPβ, as well as DNA binding, transactivation, phosphorylation, and homodimerization, without significantly affecting C/EBPβ expression. The *Cebpa* 3'UTR similarly suppressed the anti-proliferative activity of C/EBPα and that of C/EBPβ when appended to its coding region. An AU-rich element (ARE) and its cognate RNA-binding protein, HuR, were required for *Cebpb* 3'UTR inhibition. The 3'UTR also excluded the *Cebpb* transcript from a juxtanuclear region of the cytoplasm in a manner that depended on the ARE and HuR, suggesting a functional link between mRNA localization and protein activation. Importantly, 3'UTR inhibition was diminished in primary fibroblasts, allowing Ras-induced C/EBPβ activation and OIS to proceed. C/EBPβ expressed from a coding region construct ($C/EBP\beta^{CR}$) induced a gene signature in NIH $3T3^{Ras}$ cells that was biased toward inflammatory and anti-proliferative mediators. In contrast, genes activated by the 3'UTR-containing construct ($C/EBP\beta^{UTR}$) showed associations with cancers and the TGFβ pathway. These results suggest that the 3'UTR instructs C/EBPβ to activate a pro-oncogenic program in immortalized or transformed cells and suppresses its ability to induce genes involved in tumor suppression and senescence. In summary, our findings reveal a novel regulatory mechanism, termed 3'UTR regulation of protein activity or UPA, in which non-coding mRNA sequences control post-translational activation of C/EBPβ and may thereby determine its pro- versus anti-oncogenic functions.

LOSS OF CDK1 IN LIVER CANCER

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In most tumors, the activity of cyclin-dependent kinases (Cdks) is elevated. It is still not clear if this is a cause or consequence of tumorigenesis. We have shown in the past that there is significant functional overlap between Cdk2, Cdk4, and Cdk1. Because of this, $Cdk2^{-/-}$ mice do not display any overt phenotype. Mouse genetics has demonstrated that in $Cdk2^{-/-}Cdk4^{-/-}$ double mutants, the transcriptional expression of Cdk1 is repressed because of hypophosphorylation of the retinoblastoma protein (Rb). Loss of the tumor suppressor p53 induces tumors in mice and we found that concomitant loss of Cdk2 does not alleviate tumorigenesis. Even the loss of Cdk2 & Cdk4 in a p53 null background does not affect cell transformation because the loss of p53 induces the expression of Cdk1 and cyclin B1. All these results indicated an essential role for Cdk1 in controlling cell division in tumorigenesis. Therefore, we generated mice that do not express Cdk1 in hepatocytes. These Cdk1-liver-knockout mice were viable and did not display any obvious phenotype. Liver tumors in mice can be induced by tail vein injection of activated Ras in combination with silencing of p53. Within 3-4 months, wild type mice developed massive liver tumors. Currently, we are currently testing Cdk1-liver-knockout mice for the development of liver tumors induced by activated Ras/shp53. We will report our newest results.

ROLE OF THE RAS EFFECTOR RASSF5/NORE1 MEDIATING TNF-A INDUCED APOPTOSIS IN TUMORIGENESIS

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Epigenetic silencing of Ras-Association Domain Family (*RASSF*) genes, *RASSF1* and *RASSF5* (*also called NORE1*), by CpG hypermethylation is found frequently in many cancers. While the physiological roles of *RASSF1* have been studied in some detail, the exact functions of *RASSF5* are not well understood.

Here, we show that *RASSF5* plays an important role in TNF-α meditated apoptosis. Depletion of *RASSF5* by siRNA significantly reduced the TNF-α mediated apoptosis, most likely through its interaction with proapoptotic kinase MST1, a mammalian homolog of *Hippo*. Consistent with this, siRNA knockdown of *MST1* also resulted in resistance to TNF-α−induced apoptosis. In addition to its interaction with MST1, RASSF5 formed complexes with WW45, LATS1, and YAP1, which are components of the Hippo signaling pathway. Genetic ablation of Rassf5 in mouse embryonic fibroblasts also resulted in resistance to TNF-α induced apoptosis.

Importantly, *Rassf5*-null mice were significantly more resistant to the TNF-α induced apoptosis and failed to activate Mst1 *in vivo*. Furthermore, *Rassf5*-null MEFs underwent spontaneous immortalization at earlier passages than the control MEFs and showed increased susceptibility to oncogenic transformation by *K-RasG12V*. Together, our results demonstrate a direct role for *RASSF5*in TNF-α mediated apoptosis and further suggest an important role for *RASSF5* in tumorigenesis.

DIFFERENTIAL REQUIREMENT OF B-RAF AND C-RAF IN K-RAS^{G12D}-MEDIATED LUNG TUMORIGENESIS

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Mutations in KRAS are frequently occurring genetic lesions in the genesis of human pulmonary adenocarcinoma. Despite extensive efforts, therapeutic strategies targeting Ras have remained unsuccessful so far; thus, Ras effector pathways may present more suitable targets for therapeutic intervention. However, which Ras effector pathways are critical for tumorigenesis is incompletely understood. Here we show that in a K-Ras^{G12D} mutant mouse model of lung cancer, both B-Raf and C-Raf are involved in the regulation of lung tumor development. Genetic deletion of C-Raf drastically reduced the number of lung tumors and overall disease burden, leading to a significantly extended lifespan of these animals. Tumors that developed in these mice showed inefficient recombination of the floxed C-Raf allele, indicating that C-Raf is required for tumor initiation. B-Raf deficiency did not reduce tumor burden at early time-points, but decreased MAPK activation, proliferation and tumor size, resulting in increased survival of these mice. B-Raf was also found to mediate K-Ras^{G12D}-induced proliferation in mouse embryonic fibroblasts, while C-Raf is critical for survival in response to apoptotic stimuli. Thus, B-Raf and C-Raf play important but distinct roles in mediating the oncogenic effects of K-Ras^{G12D}. These results suggest dual targeting of B-Raf and C-Raf as suitable therapeutic option for the treatment of K-Ras mutant lung adenocarcinomas.

SIMVASTATIN INCREASES EXPRESSION AND ACTIVITY OF HUMAN EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1 (HENT1) IN OVARIAN CANCER CELLS SENSITIZING TO GEMCITABINE

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Ovarian cancer is the most lethal gynecological cancer with less than 40% of 5-year overall survival. The major chance of successful therapeutic outcome is obtained when optimal debulking and sensitivity to chemotherapy are achievable. Recently, retrospective studies have supported that those patients who were contemporarily receiving statin treatment -when treated for ovarian cancer- had better survival. Statins, HMG-CoA reductase inhibitors, are used to decrease cholesterol levels and also show pleiotropic effects including antiproliferative and apoptotic effects in several epithelial cancers. So far, it is not well known how statins can improve chemotherapeutic response and survival. The hENT1 is a transporter facilitating the entrance of Gemcitabine into cancer cells. This drug is one of the alternatives chosen to treat recurrent ovarian cancer. Objectives: Studying the effect of Simvastatin in hENT1 expression, transporter activity, and changes in sensitivity to Gemcitabine in ovarian cancer cells. Methods: The A2780 ovarian cancer cell line and primary tissue cultures established from ascites coming from advanced ovarian cancers were both treated with different concentrations of Simvastatin at different intervals. Upon Simvastatin incubation, mRNA hENT1 levels were determined by RT-PCR and its activity measured ENT-1 transport activity assay. Simvastatin effect on Gemcitabine sensitivity was addressed using 6h of statin or vehicle preincubation followed by 48h of drug exposure. Gemcitabine-mediated cell death was studied by MTS and apoptosis confirmed by detection of cleaved form of PARP by WB. Results: An increase in mRNA hENT1 levels was observed after 6h of Simvastatin treatment both in the A2780 cells as well as in the primary cultures. The maximum increase in ENT-1 activity was reached after 24h of Simvastatin incubation. A synergistic effect by the combination was observed in MTS assays and confirmed by fractional inhibition analysis. An increase in the cleaved form of PARP was also observed with this combination compared with each reagent used alone. Conclusions: Simvastatin increase hENT1 expression and activity sensitizing ovarian cancer cells to gemcitabine-mediated cell death. Changes in activity of certain transporters induced by statins could account for better response to chemotherapy in advanced ovarian cancer contributing to better survival. (Funded by FONDECYT 1080163, 1060495 and 11080206)

MICROGLIA- A NEW WEAPON AGAINST BRAIN TUMORS?

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The role of microglia, the resident macrophages of the brain, in brain tumor biology is not well understood. Despite their potent capability of killing tumor cells, microglia seem to support tumor growth rather than suppress it. Clinical data suggest that a higher grade of microglial infiltration into brain tumors correlates with worse prognosis. A better understanding of this tumor supporting phenotype of microglia and putative ways to stimulate microglia to recover anti-tumor properties could pave the way for new immunotherapeutical approaches. Here we report for the first time that human microglia, isolated from brain tumor specimen, secrete factors that support tumor migration in a 3D collagen invasion assay. On the other hand, a stimulation of human microglia with the TLR-3 agonist poly(I:C), but not with any other TLR agonist, leads to a phenotype that suppresses tumor cell growth, as shown by viability assays. Furthermore, this stimulation leads to the secretion of factors that exert a cytotoxic and an anti-migratory effect on tumor cells, as shown by 2D wound healing and 3D collagen invasion assays. In summary, these data show that human microglia isolated from tumor tissue have a tumor supporting phenotype that can be switched to a tumor killing phenotype by application of a proper stimulus.

GROWTH FACTOR INDEPENDENT 1 IS REQUIRED FOR INITIATION AND MAINTENANCE OF LEUKEMIA

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Lymphoma and leukemia represent approximately 8% of cancer incidence in North America and Europe. Here we show that the transcriptional repressor protein Growth factor independent-1 (Gfi1) plays an important role in the initiation, maintenance and progression of lymphoid leukemia and lymphoma. Specifically, GFI1 expression is associated with Notch signaling in human T-ALL and collaborates with Notch to induce murine leukemia. In contrast, constitutive absence or inducible deletion of Gfi1 significantly delayed or completely prevented the initiation of T-cell lymphoma in three independent experimental models, in which T-ALL was elicited either by a carcinogen (N-ethyl-N-nitrosourea (ENU)), by a non acute transforming retrovirus (MoMuLV) or by expressing a truncated Notch transgene (NotchΔCT) mimicking Notch mutations of human T-ALL cases. Furthermore, Gfi1 deletion is counter-selected during leukemia initiation in these models of T-ALL, when the Gfi1 alleles are conditionally deletable. Most striking is that Gfi1 is required for T-ALL maintenance such that inducible deletion of Gfi1 leads to tumor regression, which we could demonstrate by using ultrasound allowing us to follow growth and regression of T-cell lymphoma in mice. A similar observation was made using an Eμ-Myc lymphoma mouse model, in which tumors emerge that resemble human B-cell lymphoma. Mx-Cre, Eμ-Myc , Gfi1fl/fl mice were monitored for B-cell lymphoma development by ultrasound and once the lymphoma was detected, the Gfi1 gene was deleted by pIpC and a regression of the tumor could be observed by ultrasound. Moreover, we attempted to simulate a therapeutic regimen used for human leukemia patients in our lymphoma prone mice. We found that the ablation of Gfi1 enhances the efficacy of a combination therapy including radiation and bone marrow transplantation by sensitizing tumor cells to p53-dependent apoptosis. This effect can be blocked by Bcl-2 and is very likely due to the function of Gfi1 as a repressor of the proapoptotic Bax gene. In conclusion, we have gathered data using several different leukemia mouse models that suggest that lymphoid leukemia and lymphoma are dependent on Gfi1 for survival and that Gfi1 may represent a promising therapeutic target structure for new treatments against lymphoid malignancies.* authors contributed equally

FUNCTIONAL CHARACTERIZATION OF NOVEL TUMOR SUPPRESSOR GENE(S) IN PANCREATIC CANCER

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Pancreatic cancer is perhaps the most lethal form of cancer with low 5 year survival rate (<5%). Surgery and conventional chemotherapy have not led to any improvement in the dismal prognosis necessitating urgent efforts towards development of targeted therapy. DNA copy number alterations (CNAs) are the hallmark of solid tumors and have been suggested to harbor important oncogenes and tumor suppressor genes (TSGs). Previous studies from our laboratory and others have led to the identification of localized DNA amplifications and deletions from pancreatic cancer genomes and studies on a novel localized amplification at 18q11.2 led to the identification of GATA6 as a lineage specific oncogene in pancreatic cancer. In the current study, we report the identification and characterization of two novel TSGs viz. ARID1B and PARD6G located within two independent regions viz. 6q25.3 and 18q23 respectively, which are frequently deleted in pancreatic cancer.
ARID1B is a member of the human ATP-dependent SWI/SNF chromatin remodeling complex, and PARD6G is a member of the epithelial cell polarity complex. ARID1B stable clones were generated in MiaPaCa2 pancreatic cancer cell line (harboring a homozygous deletion of ARID1B). We first analyzed the ability of ARID1B stable clones to exhibit loss of contact inhibition and anchorage independence. There was a significant reduction in the number and size of colonies in ARID1B stable clones as compared to vector stable clones in liquid colony formation and soft agar colony formation assays, indicating that expression of ARID1B may reduce tumor-related characteristics in pancreatic cancer cell lines. 5-Aza2-deoxycytidine treatment of pancreatic cancer cell lines SW1990, CFPAC-1 and Panc10.05 (harboring single copy loss and low level of expression of ARID1B) and SW1990 and CFPAC-1 (harboring single copy loss and low level of expression of PARD6G), resulted in an increase in transcript level of both the genes in a dose dependent manner. A strong hypermethylated CpG island in the putative promoter region of ARID1B in CFPAC-1 and a partial methylated CpG island in the first intron of PARD6G in CFPAC-1 and other cell lines were identified, suggesting thereby that ARID1B and PARD6G may be transcriptionally repressed through CpG island hypermethylation in pancreatic cancer cells. We also identified the correct ARID1B transcript isoform expressed in pancreatic cancer. In addition, a novel 6bp insertion mutation was identified in the promoter of PARD6G gene in CFPAC-1. Further experiments are currently underway to study tumor suppressor function of the two genes in relation to pancreatic cancer.

GWAS-IDENTIFIED PROSTATE CANCER RISK SNP RS10993994 DOWNREGULATES EXPRESSION THE PUTATIVE ANTI-CANCER GENE *MSMB*.

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Recent genome-wide association studies (GWAS) have identified numerous common, inherited single nucleotide polymorphisms (SNPs) that influence an individual's susceptibility to develop cancer. Despite these robust findings, no clear mechanism explains how these alleles confer a modest change in disease susceptibility. One such SNP, rs10993994, is located in the promoter of the gene β-microseminoprotein (*MSMB*), whose gene product (β-MSP) is one of the major secretory products of the prostate and which may have anti-tumor properties. We have shown that the risk allele ("T") correlates with decreased levels of β-MSP in both the semen and serum of healthy young men. The risk allele at rs10993994 also correlates with increased levels of the prostate proteins PSA and hK2, in both younger and older men. We now show that the risk allele at rs10993994 is less able to drive promoter activity than the wild-type allele. The SNP appears to abrogate a CREB binding site, and only the promoter with the wild-type allele is sensitive to the action of forskolin. We conclude that the "T" allele at rs10993994 confers increased cancer susceptibility by destroying a CREB binding site, thereby resulting in decreased production of β-MSP. These data suggest that one mechanism by which a SNP can confer a modest change in cancer risk is by subtly altering expression levels of a gene with tumor suppressive properties. Current experiments are aimed at understanding how decreased levels of β-MSP promote tumorigenesis.

ARF PROMOTES TUMORIGENESIS IN THE CONTEXT OF COMBINED LOSS OF FUNCTION OF *P53* AND *PTEN* IN A MOUSE MODEL OF INVASIVE BLADDER CANCER

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Although most superficial bladder cancers can be resectable transurethrally with an excellent prognosis, clinical outcome becomes much more problematic for patients who develop muscle-invasive disease. Indeed, invasive bladder cancer is a major clinical challenge since it is often associated with regional or distant metastasis, for which there is no effective treatment and, even if radical surgery is curative, it involves removal of the entire bladder, resulting in poor quality of life caused by urinary tract diversion. Clearly, there is a need for the identification of novel prevention and treatment paradigms for invasive bladder cancer; however, our limited understanding of the molecular mechanisms of bladder tumorigenesis has hampered the identification of new targets for therapuetic intervention.
To investigate mechanisms of bladder tumorigenesis, we have developed a genetically-engineered mouse model of invasive bladder cancer based on the combinatorial deletion of *p53* and *Pten* in bladder epithelium using an adenovirus expressing Cre recombinase (Adeno-Cre) delivered to the bladder lumen. *p53* and *PTEN* are frequently inactivated in human bladder cancers especially those with poor prognosis. Additionally, carcinoma in-situ (CIS) is often observed adjacent to invasive tumors in the mouse model. Collectively, these and other data indicate that our mouse model accurately recapitulates the progressive stages of human invasive bladder cancer. In our analyses of cancer mechanisms in these mice, we have found that expression of $p19^{Arf}$ is up-regulated in the *p53*; *PTEN* deficient mouse bladder tumors, which is consistent with the observed up-regulation of $p14^{Arf}$ in human invasive bladder cancers. We have found that depletion of $p19^{Arf}$ in primary cultures from the *p53* and *Pten* deficient mouse bladder tumors resulted in suppression of tumor growth in cell recombination model, while overexpression of $p14^{Arf}$ with concomitant *p53* and *PTEN* knock-down promoted tumor formation in Arf-null human bladder cancer cells. Furthermore, targeted deletion of Arf in the context of p53 and Pten deletion suppresses bladder tumor growth and promoted survival in the context of the mouse model in vivo. Our findings suggest that Arf has a context-dependent tumor-promoting role in bladder tumorigenesis.

GENOMIC SCALE DNA METHYLATION PROFILING OF PROSTATE CANCER

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Prostate cancer is the most common cancer and the second leading cause of cancer mortality for men in the United States with 192,280 new cases and 27,360 deaths in 2009. Aberrant DNA methylation has been implicated in playing a role in tumorigenesis and cancer progression in many tissue types. In prostate, previous candidate-gene studies showed strikingly tumor-specific DNA methylation changes at several promoter CpG-islands. In this study, we profiled DNA methylation at 27,578 promoter region CpG sites in 95 prostate cancer samples and 86 healthy prostate samples. Analysis of these data suggests a large-scale change in DNA methylation across nearly one-third of all the assayed CpG sites. These data, combined with available clinical data, revealed novel candidates for diagnostic markers of prostate cancer as well as prognostic makers of aggressive tumors.

ANALYSIS OF ESCAPE FROM ONCOGENE-INDUCED SENESCENCE IN TUMORIGENESIS

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Oncogenic stimuli, such as a constitutively active form of H-Ras (H-RasV12), induce oncogene-induced senescence (OIS) in normal cells in order to protect cells against transformation. In this study, we show that a population of the human diploid fibroblasts is resistant to H-Ras-induced senescence and escapes OIS defense mechanism. We designated these OIS-escaped cells as OISECs (oncogene-induced senescence escaped cells) and investigated the character of OISECs. OISECs did not exhibit any tumorgenic activity in vivo. By re-introduction of H-RasV12, OIS or stress-induced premature senescence could be induced in OISECs. Further introduction of SV40 early region induced transformation of OISECs with weak potential of tumorigenisity in vivo. Although frequent aneuploidy was observed in H-Ras-transformed fibroblasts, stable ploidy of OISECs may contribute to their limited transforming activity. These results suggest that a population of the cells can escape from OIS and such cells are ready to transformation by additional oncogenic stimulation, however, stable ploidy of OISECs or accumulation of DNA damages suppresses their transforming activity.

THE MK5/PRAK KINASE AND MYC FORM A NEGATIVE FEEDBACK LOOP THAT IS DISRUPTED DURING COLORECTAL TUMORIGENESIS

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Myc oncoproteins are transcription factors that regulate multiple cellular functions, including proliferation and apoptosis. A tight control of Myc levels is therefore essential for sustaining cellular and organismal homeostasis. Mechanisms that stabilize Myc in response to growth factor-dependent signals, e.g. via the MAPK and PI3K pathways, have been identified. However, downregulation of Myc expression in response to stress signals allows cells to cease proliferation and escape apoptosis but the mechanisms involved in this process are poorly understood.
To identify novel mechanisms that regulate Myc at a posttranscriptional level, we have used immunofluorescence based high-content siRNA screening of the human kinome. We identified the MAP kinase activated protein kinase MK5 (MAPKAPK5, PRAK) as a negative regulator of Myc expression. MK5 regulates translation of Myc, since it is required for expression of miR-34b and miR-34c, that bind to the 3'-UTR of *MYC*. Although p53 is a known inducer of the miR-34 family of miRNAs, MK5 regulates Myc translation in a p53-independent manner. MK5 activates miR-34b/c expression via phosphorylation of the transcription factor FoxO3a at multiple serine residues, thereby promoting nuclear localisation of FoxO3a. It enables FoxO3a to bind to the miR-34b/c promoter, induce their expression and arrest proliferation. Conversely, expression of MK5 is directly activated by Myc, forming a negative feedback loop. Immunohistochemical stainings reveal that MK5 is expressed in normal colon mucosa, but downregulated in poorly differentiated colorectal adenocarcinomas, reciprocal to the expression profile of Myc. Our data identify a novel MK5/FoxO/miR-34/Myc feedback loop that is disrupted during colorectal tumorigenesis.

THE DISCOVERY OF NOVEL DNA METHYLATION EVENTS AS PROGNOSTIC MARKERS IN PROSTATE CANCER

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Aberrant DNA methylation in gene promoters causes gene silencing and is a common event in prostate cancer development and progression. While commonly identified methylated genes have been analyzed for their potential clinical utility, few studies have attempted a genome-wide methylation approach to discover new and possibly improved biomarkers. We have investigated the methylation profile of approximately 28,000 CpG islands in prostate cancer to uncover methylated DNA loci that can better serve as diagnostic and prognostic markers.

In order to identify DNA methylation changes associated with aggressive prostate cancer, we have performed a genome-wide analysis of 39 prostate cancers using Agilent human CpG island microarrays. We have verified the array methylation profiles using EpiTYPER mass spectrometry analysis and have validated candidate genes using quantitative MethyLight technology in an independent series of 214 cancers. The resulting methylation profiles were compared with clinicopathological paramaters.

We discovered previously unidentified methylation of HOXD3, TGFβ2, HOXD8 and CRIP3 as potential prognostic biomarkers. Within the independent cohort, we observed a greater proportion of cases with high methylation (HM) in GS 7 versus GS ≤ 6 cancers for HOXD3, HOXD8 and CRIP3 (chi-squared p-values = 0.001, 0.032 and <0.001, respectively). There were also significant increases in methylation from Gleason pattern 3 to 4/5 for both HOXD3 and CRIP3 (Mann-Whitney p-values < 0.001). Furthermore, a combination of TGFβ2, HOXD8 and CRIP3 HM was significant in predicting biochemical recurrence in univariate analysis (p-value = 0.002) and multivariate analysis (p-value = 0.034). In order to determine the consequence of these specific methylation events in prostate cancer, we analyzed the expression level of HOXD3 in fresh frozen tumors and discovered HOXD3 methylation and overexpression as compared to normal tissue. The cause of the paradoxical relationship between methylation and overexpression for HOXD3 is currently being explored. These results indicate that a panel of novel DNA methylation markers including HOXD3, TGFβ2, HOXD8 and CRIP3 distinguish low grade prostate cancers from intermediate and high grade ones, and methylation of these genes in combination can predict poor clinical outcome.

SMALL MOLECULE KINASE INHIBITORS PROVIDE INSIGHT INTO MPS1 CELL CYCLE FUNCTION

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Mps1, a dual-specificity kinase, is required for the proper functioning of the spindle assembly checkpoint and the maintenance of chromosomal stability. As Mps1 function has been implicated in numerous phases of the cell cycle, it is expected the development of a potent, selective small molecule inhibitor of Mps1 would greatly facilitate dissection of Mps1-related biology. We describe the cellular effects and Mps1 co-crystal structures of novel, selective small molecule inhibitors of Mps1. Consistent with RNAi studies, chemical inhibition of Mps1 leads to defects in Mad1 and Mad2 establishment at unattached kinetochores, decreased Aurora B kinase activity, premature mitotic exit, and gross aneuploidy, without any evidence of centrosome duplication defects. However, in U2OS cells possessing extra centrosomes, an abnormality found in some cancers, Mps1 inhibition increases the frequency of multipolar mitoses. Lastly, Mps1 inhibitor treatment resulted in a decrease in cancer cell viability.

A NOVEL GRK2/HDAC6 INTERACTION MODULATES CELL SPREADING AND MOTILITY

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Cell migration is a critical step in tumor invasion and metastasis, being the mechanisms underlying this process extensively studied with the aim to develop proper therapies for treating cancer. Directed cell motility requires the coordinate reorganization of both actin cytoskeleton and microtubules (MTs) in order to promote membrane protrusions, adhesion turnover and traction forces. The regulation of microtubule dynamics by post-translational modifications of tubulin subunits is crucial for different MT-based responses. In particular, control of acetylation levels in α-tubulin by the class III deacetylase enzyme HDAC6 has a functional impact in cell motility and cellular spreading. HDAC6 over-expression enhances invasive motility and a wide range of HDAC6 specific inhibitors have being studied as potential anticancer-drugs, but how the activity of such tubulin modifier is regulated in response to different signaling inputs remains to be determined.

We have identified the G protein-coupled receptor kinase GRK2 as a new regulator of HDAC6-mediated functions. This serine-threonine kinase is a well-known negative modulator of the G protein-coupled receptor (GPCR) activity that also interacts with a variety of non-GPCR "transducing" proteins, contributing to signal propagation. GRK2 binds to and phosphorylates HDAC6 in vitro, increasing its ability to deacetylate α-tubulin, while the presence of kinase-defective mutants towards HDAC6 increases the cellular levels of acetylated tubulin in the lamelipodium. Accordingly, GRK2 co-localization with HDAC6 is detected at the leading edge of migratory cells in wound-healing assays. Moreover, increased tubulin acetylation in Hela cells caused by an impaired GRK2-dependent phosphorylation of HDAC6 correlates with a higher rate of membrane extension during early spreading, a process that is modulated by MT dynamics. These results predict that alterations in GRK levels and/or activity, as those reported in inflammatory or tumoral contexts may have important effects on cell motility by altering the acetylation state of tubulin and perhaps of other HDAC6 substrates related to migration.

CIP2A DOWNREGULATION IS A NOVEL MECHANISM FOR P53-MEDIATED TUMOR SUPPRESSION

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For a long time it has been known that tumor suppressor p53 has an important limiting role in cellular transformation. In addition, inactivation of p53 is frequently found from different kind of cancer types. Nowadays, tumor suppression by reactivation of p53 is in intense focus of drug development. In our study, we propose that recently discovered oncogene, CIP2A, has a critical role in p53-mediated tumor suppression.
CIP2A (Cancerous Inhibitor of PP2A) inhibits activity of Protein phosphatase 2A in human malignancies. We have demonstrated that CIP2A is required for the malignant cellular growth, for *in vivo* tumor formation and that it is overexpressed in common human malignancies, such as breast cancer (Junttila et al. 2007, Come et al. 2009, Khanna et al. 2009). Importantly, overexpression of CIP2A in human immortalized cells results in cellular transformation (Junttila et al. 2007). According to our published results high CIP2A mRNA expression is associated with p53 mutation status in human breast tumors (Come et al. 2009). In addition, p53 reactivation negatively regulates CIP2A expression in breast cancer cells. Also, we show that CIP2A downregulation is necessary for p53 reactivation-induced tumor suppression in breast cancer cells *in vitro*. Data about the functional relevance of p53-mediated regulation of CIP2A will be presented at the meeting "Mechanisms & Models of Cancer". Overall, our study reveals a novel mechanism for p53-mediated tumor suppression.

TARGETING MALIGNANT BRAIN TUMORS

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Gliomas are a class of brain tumors arising from glial cells, especially from astrocytes. The mean life expectancy of high-grade glioma patients has risen from 12 to only 15 months during the past two decades despite of numerous studies on more effective treatments. Therefore it is essential to clarify the mechanisms of the growth and invasion of these tumors and to develop new treatment strategies.
Using phage display method, we have identified a peptide (CooP) that effectively and selectively homes to the co-opted vessels in murine orthotopic glioma models. We have also identified the receptor for CooP (CooP-receptor, CooPR) using the yeast two-hybrid screen. We have generated a breast cancer (MDA-MB-231) cell line stably expressing 143, respectively,± 143 vs. 378 mm3 ±CooPR, and implanted these cells subcutaneously to nude mice. Expression of CooPR resulted in accelerated tumor growth compared to the parental MDA-MB-231 tumors (603 mm3 p<0,8; p± 0,6 vs. 2,7 % ±0,05). Histological analyses of the tumors showed that CooPR expression reduced the relative area of blood vessels in tumors compared to the parental tumors (1,2 % <0,05).
Our protein analyses showed upregulation of vasculature-associated proteins in the CooPR expressing tumors. These include α smooth muscle actin (SMA) and integrin α v (CD51). SMA is expressed in the smooth muscle cells and pericytes of the blood vasculature. CD51 links cells to neighbouring cells and is highly upregulated in the angiogenic vasculature. Its expression has also been linked to the invasiveness in various carcinomas, including high-grade gliomas. Expression of both SMA and CD51 was associated with the vasculature in the parental MDA-MB-231 tumors, while in the CooPR expressing tumors also the tumor cells expressed them at high levels. Intriguingly, our preliminary data indicate that tumors arising from the CooPR expressing cells were more metastatic than the parental tumors. We have now created human U87MG glioma cells stably expressing CooPR to study the effect of CooPR expression on the growth and infiltration of intracranial tumors.
In order to study the potential of our peptide in targeted drug delivery we conjugated the CooP-peptide to a cell-penetrating peptide and Chlorambucil. When we used this compound to treat mice with intracranial gliomas, we were able to significantly prolong the lifespan of mice compared to the control animals treated with only Chlorambucil.

PREVENTION OF EFFECTIVE ARSENIC-INDUCED PML AND PML/RARA PROTEOLYSIS BY MITOTIC CELL DIVISION

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The drugs al trans retinoic acid (ATRA) and arsenic trioxide (ATO) are being used in the clinic to cure the cancer acute promyelocytic leukemia (APL). Both of these components induce complete remission of the disease through a mechanism that involves targeted degradation of the APL associated fusion oncoprotein PML/RARA. Recent publications have revealed that ATO initiates degradation of PML and PML/RARA by contacting a zinc-binding motif present within the PML protein. This interaction leads to modification of PML by the small ubiquitin-like modifier (SUMO) that subsequently causes poly-ubiquitination by the ubiquitin ligase RNF4 and degradation by the proteasome. In the present study we have used biochemical analysis and live cell imaging of cells expressing EYFP-tagged PML to study the fate of PML in ATO-treated cells. We find that ATO-induced SUMO conjugates become effectively removed from the PML protein by SUMO proteases during entry of cells into mitosis. This leads to accumulation of un-degraded PML as stable cytoplasmic aggregates that are refractory to further ATO induced proteolysis. Consequently, we observe a more effective PML and PML/RARA eradication by ATO in cells that are prevented from undergoing mitotic cell division. Our data may, in part, explain the synergy effect observed between ATO and drugs that cause reduced cell proliferation.

SENSITIVE AND SPECIFIC PROBES FOR DISCRIMINATION OF THE THREE MAJOR TYPES OF ABC TRANSPORTERS USING FLOW CYTOMETRY AND FLUORESCENCE MICROPLATE-BASED CYTOMETRY.

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The phenomenon of multi drug resistance (MDR) of tumor cells is a well known problem in oncology. One of the underlying molecular rationales for MDR is the up-regulation of a family of MDR transporter proteins that belong to the family of ATP binding cassette (ABC) proteins and cause chemotherapy resistance in cancer by actively extruding a large variety of therapeutic compounds from the malignant cells. The three major multidrug resistance ABC proteins are MDR1 (P-glycoprotein, ABCB1), MRP1 (ABCC1) and BCRP (ABCG2, MXR).

Because of a significant role that ABC transporters play in cancer multidrug resistance and the body's protection against xenobiotics, sensitive and specific quantitative assays are required for the detection of the activity of these proteins. Functional activity of these proteins may not correlate with their expression levels detected by classical methods, such as Northern blotting, RNA in situ hybridization, RT PCR or immunostaining. Expression levels can be below the detection threshold of some techniques, as relatively few active transporter molecules can cause major alterations in drug transport.

To characterize the actual function expression levels of ABC transporters the cellular uptake of a number of fluorescent substrates can be measured using flow or fluorescence microplate-based cytometry. Typically, live cells are loaded with the MDR probe in the presence or absence of inhibitors. Cells with increased ABC transporter activity demonstrate lower fluorescence in the absence of the specific efflux inhibitors. Increased fluorescent signal in the presence of any of the specific inhibitors indicates activity of the corresponding transporter protein. Most dyes used as indicators (Rho123, $DiOC_2(3)$, Calcein-AM) have limited applicability as they do not detect the three major types of ABC transporters at once. Dyes with broad specificity (such as doxorubicin or mitoxantrone) lack sensitivity due to overall dimness and may yield a significant percentage of false negative results. We developed two fluorescent probes that are substrates for all three common types of ABC transporters and can serve as indicators of MDR in flow or fluorescence microplate-based cytometry assays using live cells. The probes demonstrate fast internalization, favorable uptake/efflux kinetics and high sensitivity of MDR detection. Used in combination with general or specific inhibitors of ABC transporters, both dyes readily identify functional efflux and can detect small degrees of efflux as well as define the type of multidrug resistance. The assay may be applicable to the screening of putative modulators of MDR-ABC transporters. The described assay workflow facilitates rapid, reproducible, specific and relatively simple functional detection of ABC transporter activity, high-volume specimen throughput and can readily be implemented on widely available instrumentation.

14-3-3E PROMOTES SCF^{FBX4}-MEDIATED CYCLIN D1 DEGRADATION AND CONTRIBUTES TO SUPPRESSION OF TUMOR GROWTH

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Cyclin D1, a bona fide oncogene, is frequently over-expressed in human cancer through impaired proteolysis. The SCF^{Fbx4} E3 ligase catalyzes ubiquitylation and subsequent degradation of cyclin D1 following the G1/S transition. GSK3β contributes directly to this regulation through both phosphorylation of cyclin D1 on T286 to generate a phospho-degron and through phosphorylation of Fbx4. GSK3β-dependent phosphorylation of Fbx4 on S12 increases SCF^{Fbx4} activity by facilitating dimerization. Missense mutation of S12 in Fbx4 impairs SCF^{Fbx4} activity; the presence of such mutations in human cancer suggest that impaired Fbx4 function contributes to tumorigenesis. However, a mechanistic understanding of SCF^{Fbx4} activation remains incomplete. We have identified 14-3-3ε as a novel regulator of SCF^{Fbx4} dimerization and activity. Phosphorylation of S12 creates a 14-3-3ε binding motif in Fbx4. Cancer derived mutations that inhibit S12 phosphorylation or target serine 8 directly interfere with 14-3-3ε binding and Fbx4 dimerization. Data to be presented demonstrate that 14-3-3 binding increases Fbx4 dimerization. Inhibition of the Fbx4 S12 kinase GSK3B also inhibits 14-3-3ε-Fbx4 binding. The association of Fbx4 with 14-3-3ε is maximal in the S-phase of the cell cycle, coinciding with the peak of GSK3β-dependent Fbx4 phosphorylation and cyclin D1 degradation. 14-3-3ε enhances SCF^{Fbx4} ubiquitylation of cyclin D1 in vitro and in vivo and loss of 14-3-3ε leads to cyclin D1 stabilization. Reconstitution of Fbx4 in human esophageal carcinoma cell lines harboring inactive Fbx4 leads to the inhibition of cell growth in soft agar, providing evidence for the SCF^{Fbx4}-cyclin D1 pathway as a potential therapeutic target in human cancer.

ABERRANT METHYLATION ON CPG ISLAND OF DKK3 PREDICTS RECURRENCE OF CERVICAL CANCER.

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PURPOSE: This study aimed to assess the quantitative methylation levels of the promoter region of the Dkk3 gene in human uterine cervical carcinoma and determine potential clinical correlations.
EXPERIMENTAL DESIGN: Sixty-two patients with primary uterine cervical carcinoma and 13 patients with uterine leiomyoma, used as normal controls were included in this study if frozen tissues were available for further investigation. Quantitative metylation levels were evaluated by pyrosequencing after bisulfite treatment. Clinical and pathological findings were obtained from the medical records. Correlation or t-test statistics were used to analyze the relationship between the methylation levels and the clinical features. Survival data were estimated using Kaplan-Meier estimates and compared with the log-rank test where indicated. Multivariate analysis was performed using the Cox-regression method. Classification performance, using the adaBoost M1 algorithm in the Weka data mining program was used to estimate the recurrence-prediction value.
RESULTS: Pyrosequencing analysis showed that the methylation level of four out of five CpG positions were aberrantly and strongly methylated in the cervical carcinoma compared to the normal cervical tissue samples ($P<0.005$). The methylation in positions 1 and 2 were stronger in patients with higher serum levels of the SCC tumor marker and larger tumors. The patients with a methylation level above 26.28% at position 1 had a lower survival rate than the patients with methylation levels at position 1 that were below 26.28%. The accuracy of the methylation levels at the five CpG sites for the prediction of disease recurrence in patients with cervical cancer was estimated to be 78.3%.
CONCLUSIONS: The results of this study defined a threshold level of methylation associated with recurrence-free survival. In addition, the findings suggest that the quantitative methylation levels may aid in the prediction of disease recurrence in patients with cervical cancer with an accuracy of 78.3%.

CISPLATIN INDUCES HUMAN EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1 (HENT1) EXPRESSION AND ACTIVITY AND GEMCITABINE-INDUCED CELL DEATH IN OVARIAN CANCER CELLS.

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Ovarian cancer (OC) is the leading cause of gynecologic cancer-related mortality in the United States. Currently, standard primary therapy for advanced disease involves the combination of maximal cytoreductive surgery and chemotherapy with Carboplatin plus Paclitaxel or with Carboplatin alone. Despite high initial response rates, a large proportion of patients relapse, resulting in a therapeutic challenge.
Gemcitabine (2',2'-difluorodeoxycytidine) is one of the most actively investigated drugs in OC. Carboplatin-Gemcitabine doublet is an important therapeutic option for patients with both previously treated and untreated OC. Many molecular mechanisms have been proposed to be involved in GEM sensitivity/resistance including the Equilibrative Nucleoside Transporter-1 (hENT1).
Equilibrative Nucleoside Transporters (ENTs) are facilitative nucleoside transporters. ENTs play a key role in physiology and pharmacology by the modulation of nucleoside concentration and nucleoside analog availability such as anticancer and antiviral agents. The observation that hENT1 expression is an indication of anti-cancer therapy success makes ENTs an important target for study to improve anti-cancer therapy and drug effectiveness, specially the study of hENT1 activity and expression regulation. It is currently unknown how several parameters or treatments modulate the activity or expression of these transporters affecting cell proliferation and most importantly chemotherapy success with nucleoside analogs.
Using ovarian-derived tumor cell lines, A2780 (Cisplatin sensitive) and SKOV3 (Cisplatin resistant), we investigated the effects of Cisplatin on hENT1 expression and transport activity. Cisplatin increases hENT1 expression and hENT1-dependent nucleoside uptake in both cell lines. Under these conditions several kinases were activated such as JNK, ERK and Akt, however only a potent PI3K inhibitor, blocked the Cisplatin effects on hENT1 activity and expression, strongly suggesting the involvement of PI3K-Akt pathway. On the other hand, MTT assays showed an increase in cell death when cells were incubated with the combination of Cisplatin and Gemcitabine.
In summary we show that Cisplatin induces hENT1 expression and transport activity in A2780 and SKOV3 cells, probably through the PI3K-Akt pathway sensitizing to Gemcitabine-induced cell death.

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AN SIRNA SCREEN TO IDENTIFY SYNTHETIC LETHAL INTERACTIONS WITH ONCOGENIC BRAF

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Activating mutations of the protein kinase BRAF are a major cause of melanoma, a disease for which currently available therapeutics show little clinical benefit. A large proportion of BRAF mutant melanomas are highly dependent on BRAF and the MEK signalling pathway. The novel BRAF inhibitor PLX4032 has shown promising results in clinical trials, however, there are already indications of serious side effects and drug resistance. An alternative to the clinical targeting of mutant BRAF or its downstream effectors is to inhibit genes that are essential specifically in the context of this mutation. An effective way to screen for such genes is based on the principle of synthetic lethality, whereby only a combination of two perturbations, but not each individual one, causes loss of fitness.
We have performed an siRNA screen to identify synthetic lethal partners of oncogenic BRAF. We used a genetically defined cell system, which was based on immortalized fibroblasts expressing oncogenic BRAFE600. The screen was performed in a single well format with the Dharmacon kinome library. After hit selection and several rounds of validation, we identified 28 genes whose knockdown by at least two independent siRNAs selectively impaired the proliferation or viability of BRAFE600-expressing cells.
We subsequently set out to confirm the synthetic lethal relationship using shRNA-expressing lentiviral vectors, in the same cell system. We identified a number of genes for which at least three non-overlapping shRNAs gave rise to toxicity only selectively in the context of oncogenic BRAFE600. For two of these genes, we have demonstrated that the ability of the shRNAs to diminish cell viability is associated with efficient downregulation of the genes they targeted.
Currently, we are studying the effect of the depletion of these two genes in multiple melanoma cell lines, in order to determine whether the requirement for these genes is specific to BRAFE600-expressing melanoma cells.

OVEREXPRESSION AND NEOPLASTIC TRANSFORMING ACTIVITY OF ENDOGENOUS BRCA1-IRIS IN SPORADIC, HUMAN BREAST CANCER CELLS AND CELL LINES

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BRCA1-IRIS, a 1399 residue product of alternative splicing of the BRCA1 gene locus, is a proliferation-stimulating, chromatin-associated polypeptide when normally expressed. In this context, it interacts with the replication initiation regulator, geminin, the outcome of which is facilitated origin firing and normal S phase passage timing (ElShamy and Livingston, 2004). When BRCA1-IRIS is overexpressed, cells progress too rapidly through S phase, and the opposite phenotype develops when it is depleted from cells. BRCA1-IRIS does not appear to colocalize in nuclear foci with BRCA1-p220, the primary DNA damage repair and breast/ovarian cancer suppressing product of the gene, and BRCA1-p220 lacks the S phase progression function of BRCA1-IRIS. BRCA1-IRIS is also overexpressed in multiple, established human breast cancer cell lines (ElShamy and Livingston, 2004). Moreover, using qRT-PCR, we have recently detected significant overexpression of BRCA1-IRIS mRNA, by comparison with its expression in normal mammary tissue, in 28% of 78 sporadic human breast cancers. Where tested, the most highly mRNA-overexpressing tumors also overexpressed the protein, as detected by Western blotting. When ectopically overproduced in primary human mammary epithelial cells, BRCA1-IRIS stimulated the RNA and protein expression of two, breast cancer-associated proteins, Her2 and cortactin, the former of which is a known breast cancer oncoprotein. In this setting, BRCA1-IRIS was detected by ChIP at a specific segment of the ErbB2 promoter. Also induced were abnormal mammary acinus formation and certain aspects of a neoplastic phenotype, including an epithelial to mesenchymal transition, membrane invasion, and hypermobility. Specific RNAi-driven depletion of endogenous, naturally overexpressed BRCA1-IRIS from certain breast cancer cell lines led to decreased expression of Her2 and cortactin and to marked suppression of anchorage-independent growth but no major effect on colony formation on a plastic surface. Taken together, our results indicate that, although it is the product of an established tumor-suppressing locus, BRCA1-IRIS is not infrequently overproduced in breast cancers and, when sufficiently overproduced, it can function like a transforming protein.

Reference

ElShamy, W.M., and Livingston, D.M. (2004). Identification of BRCA1-IRIS, a BRCA1 locus product. Nat. Cell Biol. 6, 954-967.

* contributed equally to this work

ESSENTIAL KINASES FOR HUMAN METASTATIC CELLS - PERTAINING TO C-MET SIGNALING.

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Kinases play key roles in the regulation of many cellular processes and are postulated to participate in the development of metastatic capability. To test the role of kinases in tumor progression, we compared matched pairs of non-metastatic and metastatic tumor cells, a commonly used model, to study the cellular changes that accompany metastasis. As such, we performed arrayed shRNA screens to determine how kinase requirements for survival and proliferation varied between pairs of non-metastatic and metastatic cell lines. These screens revealed patterns of kinase requirements among 4 pairs of cell lines. Of particular interest, we identified a set of kinases that were essential predominately for metastatic cells, including c-Met. In vivo metastatic experiments are underway to evaluate which kinases can promote metastasis when ectopically expressed in otherwise non-metastatic cells. Since inhibition of these kinases phenocopied c-Met inhibition, we also sought to test if any of these kinases are functionally linked to c-Met signaling. Indeed, several of the identified kinases, including PRKCZ, became essential when immortalized melanocytes were made tumorigenic and metastatic by introduction of activated c-Met. Biochemical and genetic rescue experiments are currently ongoing to further examine the links between these genes and c-Met. These studies offer new insights into the cellular signaling pathways operating in metastatic cancer cells and also suggest potential targets to treat metastatic cancers.

CDK2 AND CDK4 REGULATE THE SWITCH FROM PROLIFERATION TO DIFFERENTIATION IN NEURAL STEM CELLS.

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In stem cells, the switch from proliferation to differentiation is a highly coordinated event where cell cycle regulators play indispensible roles. Self-renewing neural stem cells typically remodel their cell cycle structure such that the length of G1 is extended as they gradually adopt more committed cell fates. Recent studies have assigned novel functions to components of the cell cycle machinery in the control of neurogenesis that are independent of their role in regulating cell cycle progression. This represents an effective way to concurrently initiate multiple processes so as to achieve a common desired effect. In view of the importance of Cdk2 and Cdk4 in G1/S transition, we sought to determine if they possess additional abilities to modify critical components required for stem cell maintenance such that when their activities are down-regulated during differentiation, the modification, and thus stem cell identity, is gradually lost. Cdk2 and Cdk4 double knockout neural stem cells were isolated from the telencephalon of E13.5 embryos and expanded in vitro as neurospheres. As expected, the neurospheres that lack Cdk2 and Cdk4 had a significantly lengthened G1 phase and differentiated into the neurons and astrocytes without problems. However, the DKO were more prone to spontaneous neuronal differentiation. More importantly, we found that a transcription factor necessary for the maintenance of the undifferentiated state in stem cells was directly modified in the absence of Cdk2 and Cdk4. We therefore hypothesize that Cdk2 and/or Cdk4 regulate the decision of stem cells to self-renew or to differentiate.

TRANSIENT EXPOSURE OF HCT116 TO MLN4924 INDUCES RE-REPLICATION, APOPTOSIS AND CELLULAR SENESCENCE

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MLN4924, an anti-cancer drug currently in clinical trials, is effective at inhibiting the proliferation of various human cancer cell lines. MLN4924 inhibits the NEDD8 pathway and consequently increases the abundance of substrates of cullin-RING E3 ubiquitin ligases whose activity is dependent on NEDD8 modification. Here we show that the stabilization of Cdt1 in S phase is the key step in the induction of DNA re-replication by MLN4924. Significantly, short-exposure of the human colon cancer HCT116 cells to MLN4924 is sufficient to induce DNA re-replication and activate both apoptosis and senescence pathways. MLN4924-induced cell senescence is inhibited, but not prevented, in cells lacking the tumor suppressor p53 or its downstream effecter the CDK inhibitor $p21^{Waf1}$. Despite this, $p53^{-/-}$ and $p21^{-/-}$ HCT116 cells are more susceptible to growth inhibition by MLN4924 than wild-type HCT116 cells, suggesting that apoptosis, and not senescence, is more important for decreasing cell numbers by MLN4924 and showing the utility of the investigational drug on p53- and p53+ cancer cells.

EPHA2-VAV3-RAC1 SIGNALING MEDIATES MIGRATORY AND INVASIVE BEHAVIOR OF PROSTATE CANCER CELLS

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Vav3 is a Rho GTPase guanine nucleotide exchange factor. Using tissue array immunostaining, we found that Vav3 expression was significantly increased during the progression of prostate cancer. The purpose of our study is to investigate the role of Vav3 in the prostate cancer metastasis. We showed that Vav3 expression was increased in androgen independent prostate cancer cells (LNCaP C4-2, DU145, and PC3) when compared with androgen dependent prostate cancer cells (LNCaP). Cells with increased Vav3 expression showed higher migration and invasion ability. Overexpression of Vav3 in LNCaP cells increased its migration and invasion ability, mainly through Rac1 activation. Vav3 was tyrosine phosphorylated upon activation of EphA2 by its cognate ligand, ephrinA1-Fc, suggesting ephrinA as a potential stimulus for Vav3 activation in LNCaP and PC3 cells. Knocking down Vav3 expression in PC3 cells leads to decreased Rac1 activity, as well as migration and invasion ability of the cells. Furthermore, mRNA levels in some MMPs, especially MMP3, 7, and 13, were reduced when reducing Vav3 expression in PC3 cells. Overall, our data provide evidence suggesting the involvement of Vav3 in migration and invasion of prostate cancer cells, via EphA2-Vav3-Rac1 signaling axis. The role of Vav3 in metastasis of prostate cancer is being tested in nude mice model using orthotropic implantation.

ACTIVATION OF CREB BY PI3 KINASE AND P38 MAPK IS ESSENTIAL FOR ELEVATED EXPRESSION OF TGFB2

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TGFβ2 is highly expressed in a variety of different cancer cell lines. Using Z12 cells, a mutant of 293 cells with overexpression of TGFβ2, we found that the cAMP-responsive element (CRE) sequence in the promoter of the TGFβ2 gene is crucial for its increased expression. Furthermore, constitutive phosphorylation of cAMP-responsive element binding protein (CREB) is increased in these cells. Treating Z12 cells with either the PI3 kinase inhibitor LY294002 or the p38 MAPK inhibitor SB203580 significantly inhibited both the phosphorylation of CREB and the expression of TGFβ2. In addition, treating Z12 or cancer cell lines with either of these two inhibitors significantly decreased their secretion of TGFβ2. These data suggest that activated PI3 kinase and p38 MAPK play an important role in the high expression of TGFβ2 in cancer cells by stimulating the phosphorylation of CREB, which activates the CRE element in the promoter of the TGFβ2 gene. We have identified an important link between PI3 kinase, p38 MAPK and TGFβ2, providing an additional rationale for using inhibitors of these kinases as therapeutic drugs in cancer.

PRECLINICAL AND MOLECULAR INSIGHTS INTO CANCER PROGRESSION USING GENETICALLY-ENGINEERED MOUSE MODELS OF PROSTATE AND BLADDER CANCER

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Our laboratories have been investigating novel mechanisms of cancer progression, as well as new therapeutic approaches for early intervention and treatment of advanced disease by integrating analyses of human clinical data with molecular and functional studies of genetically engineered mutant (GEM) mice. In our analyses of prostate cancer, we have generated GEM mice that recapitulate the entire spectrum of disease progression from pre-invasive lesions, termed PIN, to castration-resistant and metastatic disease, which are the lethal forms of prostate cancer. By comparative analyses of large-scale gene expression profiling data for mouse and human prostate cancer, we have been assembling molecular networks, called interactomes, to elucidate conserved cancer pathways and new druggable targets, and we have been evaluating the potential therapeutic relevance of these targets by pursuing preclinical studies in the GEM mice. For example, we have found that combinatorial inhibition of the Akt/mTOR and MAP kinase two signaling pathways, which are often co-activated in human prostate cancer, inhibits castration resistant prostate tumor growth, improves survival and inhibits metastases in preclinical studies in GEM mice. We are now translating these findings from GEM mice to human clinical trials.
In our analyses of bladder cancer, we have generated GEM mice that develop pre-invasive phenotypes, termed CIS, which progress to invasive bladder cancer. Analyses of these GEM mice have revealed a critical role for ARF in bladder tumorigenesis, as well as the therapeutic benefit of inhibiting mTOR signaling for both prevention and treatment of invasive bladder tumors. In summary, our investigations of prostate and bladder cancer in GEM mice have revealed promising new molecular targets and new therapuetic approaches for the treatment of human cancer.

SELECTIVE ACTIVATION OF P53-MEDIATED TUMOR SUPPRESSION IN HIGH-GRADE TUMORS

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Inactivation of the p53 pathway is a common feature of human tumorigenesis and has fostered the attractive notion that restoring p53 function in established tumors would constitute an effective and tumor-specific therapeutic strategy. Indeed, p53 restoration was recently shown to trigger dramatic tumor regression in distinct (oncogene overexpresion-driven) murine tumor models. However, recent data suggest that low-levels of oncogenic stress may be sufficient to initiate tumorigenesis but unable to induce a p53-mediated response, emphasizing the need for a better functional characterization of this potential therapeutic target during tumor evolution. To achieve this we carried out a comprehensive analysis of the timing and mechanisms of p53 activation during the evolution of non-small cell lung carcinoma (NSCLC). NSCLC is the leading cause of cancer-related death worldwide, with an overall 5-year survival rate of only 10-15%. Kras activation and p53 mutations are frequently found NSCLC. To define the nature and timing of p53 activation during NSCLC evolution we crossed the LoxStopLox-KrasG12D (KR) mouse model to our switchable p53 knock-in (KI) model and restored p53 function during distinct stages of NSCLC development in KR;p53KI/KI animals. Unexpectedly, p53 restoration failed to induce tumor clearance but effectively targeted high- (but not low-) grade lung tumors, leading to a significant decrease in the frequency of high-grade lesions. This tumor stage specific activation of p53 resulted from selective induction of its activator p19ARF in high-grade tumors. Furthermore, we show that the p19ARF/p53 axis is activated in response to increased oncogenic stress, as malignant progression is accompanied by an increase in phosphorylated-ERK levels and Kras copy number imbalance. Taken together, our data show that p53 restoration is able to cull the most malignant cells within a tumor, but allows for less aggressive cell populations driven by low-level oncogenic signals to survive. These data have major therapeutic implications as they indicate that at least in this tumor type, p53 restoration would be a means of tumor containment rather than eradication. The potential mechanisms of p53 activation in other tumor types will also be discussed.

A RAPID AND SCALABLE SYSTEM FOR MODELING LUNG ADENOCARCINOMA USING RNAI TRANSGENIC MICE

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RNA interference is a powerful tool for controlling gene expression in mammalian systems; as such, it has become an effective alternative to conventional knockout approaches. RNAi has proven to be an efficient method to inhibit tumor suppressor gene function and yield insight into the important players in cancer biology. Still, the promise of regulatable RNAi transgenic mice has yet to be realized because the reproducible generation of these animals remains a significant technical limitation. By combining optimized fluorescence-coupled mir30-based shRNA technology with high efficiency ES cell targeting, we developed a flexible and scalable pipeline for the rapid and reliable production of doxycycline-regulated shRNA transgenic mice. These RNAi mice contain single copy DOX-regulatable shRNAs downstream of the endogenous *Collagen Type 1* locus, allowing for spatial, temporal and reversible gene expression in mice. Using this platform, we generated novel DOX-regulated shRNA transgenic lines targeting the bioluminescence reporter *Luciferase* and endogenous tumor suppressor genes, including *Trp53, INK4a* and *ARF*, each showing strong doxycycline-dependent knockdown of its target protein, without disrupting processing of endogenous miRNAs. To study the role of these TSGs in the maintenance of $Kras^{G12D}$ driven lung adenocarcinomas, we crossed these to produce mice bearing an shRNA, CCSP-rtTA (clara cell specific promoter - reverse tet-transactivator), *LSL-*$Kras^{G12D}$ and *LSL-Luciferase* alleles. However, owing to the slow rate and high expense of producing quadruple transgenic mice, we later devised a strategy for "speedy" mouse model production. This approach entailed re-derivation of embryonic stem cells harboring the relevant alleles and subsequent generation of "mosaic" models produced by blastocyst injection. Using these RNAi mouse models, we show that *INK4a/ARF* or *Trp53* downregulation by RNAi cooperates with $Kras^{G12D}$ to accelerate lung tumorigenesis and recapitulate the phenotypes of knockout models. Additionally, we investigate whether *INK4a/ARF* or *Trp53* knockdown is required for tumor maintenance. Together, this work built a platform that greatly accelerates the rate at which one can study genetic interactions and tumor maintenance genes and also identify and validate new drug targets *in vivo*. This approach can be applied to build many other complex cancer models and thus may have significant implications for guiding future therapies.

PRE-CLINICAL MOUSE MODELS TO INVESTIGATE THE MOLECULAR MECHANISMS OF SMALL CELL LUNG CARCINOMA DEVELOPMENT.

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Small cell lung carcinoma (SCLC) is a neuroendocrine subtype of lung cancer with a 5-year survival rate of less than 10%. We have developed mouse models for human SCLC based on the observations that the *RB* and *p53* tumor suppressor genes are mutated in more than 90% of human SCLC tumors and on emerging evidence that loss of *p130* function correlates with poor prognosis. *Rb/p53* and *Rb/p53/p130* mutant mouse lung tumors closely resemble human SCLC at the cellular and molecular levels, providing *in vivo* models to investigate the mechanisms underlying the development and the maintenance of SCLC.

Hedgehog (Hh) signaling plays a key role during normal lung development and response to injury but normally has low activity in the adult lung epithelium. Preliminary evidence in a small number of human samples and tumor cell lines raises the possibility that SCLC tumors may rely on the Hh signaling pathway for their growth. However, the significance of these observations *in vivo* has not been tested, and whether Hh signaling plays a cell autonomous or a non-cell autonomous role is still controversial.

To investigate a potential functional role for Hh signaling in SCLC *in vivo*, we crossed *Rb/p53* and *Rb/p53/p130* mutant mice to *Ptch1*$^{lacZ/+}$ mice. Using this reporter strain and monitoring the activity of target genes of the Hh pathway, we found that Hh signaling was active in all the SCLCs that developed in these mutant mice. Experiments with cell lines derived from mouse tumors also indicated that the activation of Hh signaling in these cells was autocrine or juxtacrine. Constitutive activation of the Hh pathway using a mutant allele of *Smo* was sufficient to enhance SCLC development *in vivo*. Conversely, inhibition of the Hh pathway reduced the survival of SCLC cells in culture and of primary tumors *in vivo*, indicating that activation of the Hh pathway is also necessary for the expansion of these tumor cells. Thus, the Hh pathway is active in SCLC cells and plays an intrinsic role in the initiation and the maintenance of SCLC.

These observations in a pre-clinical mouse model identify Hh signaling as a therapeutic target against SCLC in patients.

RAPTOR IS REQUIRED FOR INTESTINAL REGENERATION AND TRANSFORMATION BUT NOT INTESTINAL HOMEOSTASIS

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The Apc (Adenomatous Polyposis Coli) tumour suppressor gene is mutated in approximately 80% of colorectal cancers. Its major tumour suppressor function is thought to be through the negative regulation of ß-catenin and downstream Wnt target genes such as c-Myc.

We have previously shown that the conditional deletion of Apc within the mouse intestine leads to a crypt progenitor-cell like phenotype, with intestinal enterocytes proliferating independently of position and failing to differentiate or migrate. These phenotypes are all associated with deregulated Wnt signalling and can be rescued by deletion of a single Wnt target gene c-Myc.

To assess c-Myc regulated Wnt target genes, we have examined the transcriptome of double Apc Myc knockouts and find that mTOR (mammalian target of rapamycin) appears to be a Wnt/Myc regulated target gene. To inhibit mTOR, we have conditionally deleted raptor (required for the MTORC1 complex) from the mouse intestine. Surprisingly intestines lacking raptor show no problems in homeostasis. However, intestinal regeneration is perturbed, a process which we have previously shown to be c-Myc dependent. Co-deletion of Apc and Raptor strongly suppresses the proliferation of Apc deficient cells and raptor deletion strongly suppresses tumorigenesis even in stem cell models of early intestinal cancer. Intriguingly, raptor deletion only marginally protected against tumorigenesis in models where both Apc and PTEN were deleted. Deletion of MTORC2 complex component Rictor had no impact on the phenotypes of Apc loss

Taken together this study highlights that MTORC1 is required for the proliferation of Apc deficient cells in vivo. In contrast to c-Myc, there are a number of inhibitors to MTORC1 which raises the possibility that these inhibitors may be able to prevent tumours arising in patients with high familial risk of colorectal cancer.

GENETIC INDUCIBLE MOSAIC ANALYSIS (GIMA): A NOVEL GENETIC METHOD FOR MODELING AND CHARACTERIZING SPORADIC TUMORIGENESIS IN THE MOUSE

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Current genetic mouse models of cancer do not accurately model sporadic tumors that comprise the vast majority of human cancers. Of crucial importance is to understand the role of dysregulated oncogenic pathways in individual tumor cells versus neighboring cells within the microenvironment, and how this influences tumor behavior from initiation to later stages of tumor progression. Furthermore, the molecular characterization of cancer cells versus microenvironment, at various tumor stages, is likely to reveal insights into cancer biology including the identification of putative therapeutic targets. Several critical issues that need to be addressed in modeling sporadic cancers using genetically engineered mice are: 1) controlling initial mutant cell number, 2) controlling timing of induction of mutations, and 3) being able to unambiguously identify mutant cells among the surrounding wild type microenvironment.
We have developed a novel genetic technique termed Genetic Inducible Mosaic Analysis (GIMA) to generate mosaic mice in which genes are conditionally mutated in single cells at endogenous loci and simultaneously marked with GFP under both temporal and cell type-specific control. This mosaic technique allows us to analyze and compare GFP+ mutant to GFP- wild type cells within a tissue or tumor *in vivo*. Specifically, we have generated a mouse strain (*R26-GIMA*) that activates an eGFPCre fusion protein following Flp-mediated recombination of the ubiquitously expressed *ROSA26* locus. To test the efficacy of our system, we combined the *R26-GIMA* allele with a transgene that broadly expresses an inducible Flp (*CAG-FlpeER*) and a floxed reporter allele (*R26R-lacZ*) to mimic a mutant allele. Efficient mosaic marking of cells (GFP+) was achieved within 24 hours of tamoxifen (Tm) administration and ~98% of GFP+ cells carried a mutant allele (LacZ+) by 72 hours after Tm administration with eventual 100% concordance. Furthermore, medulloblastomas were indeed induced using *CAG-FlpeER* and the *R26-GIMA* alleles to activate the Hedgehog pathway in relatively few cells in the cerebellum. Utilizing an optimized Flp (Flpo), we have generated *Math1-FlpoER* and *Nestin-FlpoER* mice to model specific subclasses of sporadic medulloblastoma thought to arise within granule cell or ventricular zone progenitor cells, respectively. Our GIMA approach allows for unambiguous lineage tracing of mutant cells from tumor initiation, thus enabling the isolation of pure populations of cancer cells versus tumor microenvironment cells for characterization of changes in gene expression and chromosome content at multiple stages of tumor progression. These studies lay the foundation for use of our novel GIMA approach for modeling and characterizing any sporadic cancer.

C-RAF, A BOTTLENECK FOR K-RAS ONCOGENIC SIGNALING IN NON SMALL CELL LUNG CANCER

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The K-Ras oncogene is mutated in about 25% of non-small cell lung cancer (NSCLC), one of the most common types of human cancer. Although Ras proteins are not druggable targets, they are known to signal through a cascade of kinases that, in principle, could be targeted with small molecule inhibitors. To determine which kinases are critical in mediating K-Ras oncogenic signaling in NSCLC, we have crossed our K-Ras$^{+/LSLG12Vgeo}$ strain, known to develop lung adenocarcinomas upon Cre-mediated recombination of the K-Ras^{LSLG12Vgeo} allele, with mice carrying either null or conditional mutations for each of the members of the Raf, Mek and Erk families of kinases.
Our results indicate that ablation of individual Mek or Erk kinases did not affect K-Ras^{V12}-induced lung tumorigenesis. However, combined elimination of either both Mek or both Erk in lung tissue, effectively impaired development of both adenomas and adenocarcinomas. These observations provide genetic evidence that the tumorigenic properties of K-Ras oncogene are solely mediated by the Mek/Erk kinase pathway, at least in lung tissue. Unfortunately, widespread depletion of both Mek or both Erk kinases in adult mice induces death of the animals in less than 4 weeks. Ablation of B-Raf had neither effect on tumor development nor Erk phosphorylation, suggesting that in the absence of B-Raf, c-Raf (and/or A-Raf) effectively mediated K-Ras oncogenic signaling. Surprisingly, K-Ras^{V12} induced few adenomas or adenocarcinomas in the absence of c-Raf. Indeed, these tumors expressed c-Raf, suggesting inefficient recombination of the c-Raf^{lox} alleles. These findings indicate that K-Ras oncogenic signaling is selectively mediated by c-Raf and that the absence of this kinase cannot be compensated by either B-Raf or A-Raf. Importantly, widespread depletion of c-Raf did not affect the overall health of the mice, suggesting that pharmacological inhibition of this kinase will be well tolerated. Therefore c-Raf could be a relevant therapeutic target to treat NSCLC induced by K-Ras oncogene. To further validate this concept, we are currently utilizing K-Ras$^{+/FSFG12V}$;c-Raf$^{lox/lox}$ mice to determine whether ablation of c-Raf (via Cre recombinase) in CT+ and PET+ tumors (induced by FLPase mediated recombination at the K-Ras locus) also results in efficient tumor inhibition.

ONCOGENIC BRAF^{V600E} ELICITS PAPILLARY THYROID CANCER IN THE MOUSE

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Mutational activation of *BRAF* is detected in ~7% of all human malignancies but with a particularly high frequency in papillary thyroid cancer (~40%). The most common mutation, T1799A, encodes BRAF^{V600E} the expression of which leads to sustained activation of the BRAF-MEK-ERK MAP kinase pathway. To understand the earliest effects of oncogenic BRAF^{V600E} we generated *BRaf^{CA}* mice carrying a Cre-activated allele of mouse *BRaf*. *BRaf^{CA}* expresses normal BRaf until recombined by Cre recombinase to express BRAF^{V600E} at normal levels of expression under the control of the chromosomal promoter.

Using *Thyroglobulin::CreER^{T2}* (*Thyr::CreER*) transgenic mice we initiated expression of BRAF^{V600E} in thyrocytes of adult mice. Within 1-2 weeks the mice developed a dramatically enlarged, goiterous thyroid that was readily detected using ultrasound. Concomitantly, the mice displayed a deficit in thyroid hormone production and/or release and a dramatic elevation in the levels of Thyroid Stimulating Hormone (TSH). Unlike the situation in lung epithelial cells or melanocytes where expression of oncogenic BRAF^{V600E} results solely in benign tumors that do not progress to cancer due to oncogenic-induced senescence, mice with thyrocyte expression of BRAF^{V600E} developed papillary thyroid cancer with 6-9 months without any deliberate manipulation of umor suppressor genes. However, we are crossing *Thyr::CreER; BRaf^{CA}* mice to mice carrying conditional alleles of *Trp53, Cdkn2a* or *Pten* to determine how tumor suppressor gene silencing influences BRAF^{V600E}-induced thyroid carcinogenesis in the mouse.

Thyr::CreER; BRaf^{CA} mice provide a convenient platform for pre-clinical evaluation of pharmacological agents that target signal transduction pathways in thyroid cancer. Consequently, we treated *Thyr::CreER; BRaf^{CA}* mice with either a MEK inhibitor (PD325901) or synthetic thyroid hormone or a combination of both. Whereas MEK inhibition had a dramatic effect on the size of the mouse thyroid, synthetic thyroid hormones were without effect. These data suggest that activated MEK is essential for the effects of BRAF^{V600E} in the thyroid but that endocrine stimulation of G protein signaling through the TSH receptor is not essential. These data further emphasize the utility of *BRaf^{CA}* mice for studying malignancies in which the RAF-MEK-ERK MAP kinase pathway is implicated in the initiation, progression or maintenance of disease.

GENETIC MOSAIC ANALYSIS REVEALS A CENTRAL ROLE OF OLIGODENDROCYTE PRECURSOR CELLS IN GLIOMAGENESIS

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Pinpointing the identity of tumor propagating cells (TPCs) in gliomas is critical for designing targeted therapies. Previous studies have postulated the neural stem cell (NSC) nature of TPCs based on their abilities to self-renew and to differentiate into multiple cell types in cell culture. However, the sole dependence of end-point analysis could be misleading since late-stage tumor cells could acquire aberrant features during malignant transformation. An ideal approach to fully understand TPCs would be to analyze mutant cells throughout the tumorigenic process, with a greater focus on stages prior to the malignant transformation. To overcome the difficulty in identifying pre-transformed mutant cells free of identifiable pathological features, we used a mouse genetic mosaic model termed MADM to generate sporadic GFP-labeled mutant cells in an otherwise colorless normal mouse, enabling mutant cell visualization upon their generation. By inducing co-LOH of p53 and NF1 from embryonic NSCs, MADM model effectively induced glioma formation with almost full penetrance within 4-6 months. Genomic fingerprinting confirmed the close relevance of this model to the proneural subtype of human GBMs based on recent TCGA classification. Taking advantage of the unambiguous GFP labeling of mutant cells, we analyzed the extent of cell expansion in each and every CNS lineages in 2-month-old mice, prior to any signs of malignancy. Surprisingly, although mutations were induced in NSCs, there was no detectable over-proliferation of mutant NSCs compared to WT ones. Among all neuroglial lineages, the oligodendrocyte precursor cell (OPC) lineage was the only one showing drastic over-expansion. Upon tumor formation, marker staining and microarray analysis again revealed that actively proliferating tumor cells share many OPC features. Interestingly, purified OPC-like tumor cells could self-renew in culture, differentiate into multiple cell types upon induction, and initiate secondary tumors by orthograft assay. In conclusion, MADM enabled us to study TPC identity as a developmental process. Our findings suggest that stem cell features of TPCs may not necessarily be an indication of their NSC nature, rather these features could be acquired by mutant OPCs during malignant transformation.

MOUSE MODELS TO INVESTIGATE MENOPAUSAL INFLUENCES ON EPITHELIAL OVARIAN CANCER RISKS

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Epidemiological data indicates that the risk of ovarian cancer is highest among peri- or post- menopausal women, while increased parity and the use of oral contraceptive are preventive. These data suggest that reproductive factors are associated with the risk of ovarian cancer, however, the molecular mechanisms underlying such a link remains obscure. We have established ovarian tumor mouse models that incorporate both the impact of menopausal physiology and genetic mutations using the germ cell-deficient Wv (White spotting variant) mice and targeted mutations of p53 and p27. The Wv mice harbor a point mutation at c-Kit, which reduces the tyrosine kinase activity to about 1%, resulting in a premature loss of ovarian germ cells and follicles. By 8 weeks of age, the ovarian follicles are depleted and ovarian surface epithelial cells proliferate and invaginate in the, and by 3 months of age benign ovarian epithelial tumors replace the entire ovary in virtually all the female Wv/Wv mice. Thus, the unique tumor prone phenotype in the ovaries of Wv mice may represent an exaggerated model of menopausal physiology on ovarian cancer risk. We have refined Wv/Wv ovarian tumor model by deleting tumor suppressor genes p53 or p27kip1 in ovarian surface epithelial cells. We found that both Wv/Wv:p27+/- and p27 -/- mice develop neoplastic ovarian epithelial tumors, which consist of papillary structures lined by hyperchromatic neoplastic cells. Cytokeratin 8 staining was positive in the Wv/P27 tumors indicating the epithelial origin of these tumors. When p53 was unilaterally deleted in the ovarian surface epithelial cells of the Wv/Wv:$p53^{flox/flox}$ mice by Adenoviral cre injection, malignant conversion of the tumors developed. Metaplastic differentiation and infiltration into the surrounding tissues were seen in the p53 deficient Wv tumor lesions. Thus, we conclude that oncogenic mutations can convert the benign epithelial lesions presented in follicle-depleted ovaries to neoplastic tumors. The results indicate that ovarian aging in postmenopausal physiology and oncogenic mutations cooperate in ovarian cancer development.

MUTANT P53 DRIVES AN INVASIVE PROGRAM THAT INVOLVES MULTIPLE RTKS

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The p53 tumour suppressor protein restrains malignant progression through a number of mechanisms, and most cancers show loss of the normal functions of p53. In many cancers this is due to a mutation in the p53 gene that leads to the expression of a mutant p53 protein. These tumour associated mutant p53s not only lose wild type p53 activity but can also acquire the ability to promote cell motility and migration, and so contribute to the development of metastases. We have found that tumour associated mutant p53s can promote invasion and loss of directionality when cells migrate in vitro. These activities are independent of the loss of wild type p53 function, and reflect activation of integrin and EGFR trafficking that depends on Rab-coupling protein (RCP) and which results in constitutive activation of EGFR/integrin signalling. Simultaneous loss of p53 and p63 recapitulates the phenotype of mutant p53, suggesting that this function of mutant p53 reflects, at least in part, the inhibition of the p53 family member p63. Using both stably expressing mutant p53 cells and cells that endogenously express mutant p53, we also observed a potent induction of mutant p53-dependent invasion of cells towards HGF, the ligand of the c-MET receptor. As with EGFR dependent invasion, c-MET dependent invasion requires engagement of α5β1 integrin and RCP, and correlates with inhibition of p63. HGF is also known as scatter factor and causes dispersion of colony forming cell lines by weakening cell-cell interactions via c-MET signalling. We have been able to show a role for mutant p53 in driving the dispersion of cells and a relocalization and/or loss of specific cell-cell junction proteins. Interestingly, a c-MET inhibitor could abrogate these effects of mutant p53. Together these data suggest that mutant p53 can drive a complex invasive program that involves multiple RTKs and open the possibility that blocking α5β1 integrin and/or the RTKs will have therapeutic benefit in mutant p53 expressing cancers.

RB REGULATES TUMOR-CELL PLASTICITY

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Mutation of the *RB-1* tumor suppressor occurs in one third of all human tumors and is particularly associated with retinoblastoma and osteosarcoma. We have modeled osteosarcoma in the mouse using a targeted conditional approach in which *Rb* and/or *p53* are deleted in pre-osteoblasts. We find that *Rb* loss synergizes strongly with *p53*-inactivation: it greatly accelerates tumor development and it expands the tumor spectrum from osteosarcoma in the *p53* single mutants to multiple soft tissue sarcomas in the *Rb*;*p53* DKO. This led us to hypothesize that *Rb* loss promotes cellular plasticity. Thus, to directly address the influence of *Rb* status in mesenchymal tumorigenesis we used inducible systems to control pRb's expression. Our data show that toggling between *Rb* loss or *Rb* re-activation is sufficient to switch the fate commitment of osteosarcoma tumor cells *in vitro* through direct regulation of master regulators of mesenchymal differentiation. In addition, we find that reactivation of *Rb* in tumors generated from *Rb*;*p53* DKO cells is sufficient to halt tumor progression *in vivo*. Taken together, our data suggest that *Rb* loss promotes tumorigenesis, at least in part, by modulating the differentiation potential of committed pre-osteoblasts.

LOSS OF THE *DROSOPHILA* RETINOBLASTOMA FAMILY PROTEIN PREDISPOSES CELLS TO DEDIFFERENTIATION

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Functional inactivation of the Retinoblastoma (pRB) pathway is an early and obligatory event in tumorigenesis. This is widely attributed to the need of tumor cells to bypass the ability of pRB to promote cell cycle exit. While support of this is well documented, pRB is involved in other cellular processes that are less understood. Here we demonstrate that independently of cell cycle exit control, pRB, in cooperation with the Hippo tumor suppressor pathway, functions to maintain the terminally differentiated state. Using the *Drosophila* eye as a model of tumorigenesis, we found that mutations in the Hippo signaling pathway, *wts* or *hpo*, trigger widespread dedifferentiation of *rbf* mutant cells. Initially *rbf wts* or *rbf hpo* double mutant photoreceptors are morphologically indistinguishable from their wild type counterparts as they properly differentiate, express mature neuronal markers, and develop axonal projections. However, double mutant cells stochastically lose their neuronal identity as they undergo dedifferentiation and become uncommitted eye specific cells. Surprisingly, this dedifferentiation is fully independent of cell cycle exit defects and occurs even when inappropriate proliferation is blocked by a *de2f1* mutation. The implication of these findings is that pRB functions to maintain an irreversible state of terminal differentiation. Thus, our results reveal the novel involvement of the pRB pathway in postmitotic cells and suggest that terminally differentiated *Rb* mutant cells are intrinsically prone to dedifferentiation, can be converted to progenitor cells, and thus contribute to cancer advancement.

P18INK4C LOSS LEADS TO TUMOR PROGRESSION WITHIN CYCLIN D1-DRIVEN SENESCENT PINEAL HYPERPLASIA IN-VIVO IRRESPECTIVE OF CDK2 DOWNREGULATION

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Cellular senescence is a tumor suppressor mechanism that is activated in response to oncogenic signals and other tumor-promoting insults. Tumor progression in premalignant senescent lesions is thought to arise when cells either primarily bypass, or secondarily revert from, cellular senescence. Progression from a premalignant, senescent lesion to an invasive tumor is presumed to occur in certain human cancers, since senescent lesions are found in proximity to malignant lesions in mouse models and human pre-invasive lesions, such as nevi, lung adenomas, or colorectal adenomas.
We now show evidence *in-vivo* that tumors can evolve within premalignant senescent lesions using a mouse model of Cyclin D1-driven pineoblastoma. While absence of p53 leads to primary evasion of senescence, loss of the Cdk-inhibitor p18Ink4c leads to tumor progression within a premalignant, largely senescent lesion. Although Cdk4-dependent Rb phosphorylation was decreased suggesting compensation for p18Ink4c in the senescent cells, tumor progression occurred in all *Cyclin D1, p18-/-* mice by 7 months of age. P15Ink4b was not expressed in *Cyclin D1, p18-/-* tumors, making this a possible mechanism for escape from senescence.
Notably, *Cyclin D1,p18-/-* tumors, which differed from *Cyclin D1, p53-/-* tumors in that they were preceded by a senescent state, also differed on a molecular level. Specifically, decreased expression of Cdk2 correlated with p53-dependent senescence and was abrogated in the absence of p53. On the contrary, Cdk2 downregulation still occurred in the absence of p18Ink4c as the cells senesced, and low levels did not prevent tumor progression. These findings suggest that prospects for use of Cdk2-inhibitors as senescence-promoting therapy may depend on the molecular characteristics of the tumor, and the mechanism by which bypass of senescence occurs.

COMBINING ATR SUPPRESSION WITH ONCOGENIC RAS EXPRESSION SYNERGISTICALLY INCREASES GENOMIC INSTABILITY, CAUSING SYNTHETIC LETHALITY OR ELEVATED TUMORIGENESIS IN A DOSAGE-DEPENDENT MANNER

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Previous studies indicate that oncogenic stress activates the ATR-Chk1 pathway. Here, we demonstrate that ATR-Chk1 pathway engagement is essential for limiting genomic instability following oncogenic Ras transformation. ATR pathway inhibition in combination with oncogenic Ras expression synergistically increased genomic instability, as quantified by chromatid breaks, sister chromatid exchanges and H2AX phosphorylation. This level of instability was significantly greater than that observed following ATR suppression in untransformed control cells. In addition, consistent with a deficiency in long-term genome maintenance, hypomorphic ATR pathway reduction to 10-25% of normal levels was synthetic lethal with oncogenic Ras expression in cultured cells. Notably, elevated genomic instability and synthetic lethality following suppression of ATR were not due to accelerated cycling rates in Ras-transformed cells, indicating that these synergistic effects were generated on a per-cell-cycle basis. In contrast to the synthetic lethal effects of hypomorphic ATR suppression, subtle reduction of ATR expression (haploinsufficiency) in combination with endogenous levels of K-Ras^{G12D} expression elevated the incidence of lung adenocarcinoma, spindle cell sarcoma and thymic lymphoma in p53 heterozygous mice. K-Ras^{G12D}-induced tumorigenesis in ATR$^{+/-}$p53$^{+/-}$ mice was associated with intrachromosomal deletions and loss of wild-type p53. These findings indicate that synergistic increases in genomic instability following ATR reduction in oncogenic Ras-transformed cells can produce two distinct biological outcomes: synthetic lethality upon significant suppression of ATR expression and tumor promotion in the context of ATR haploinsufficiency. The roles of the ATR pathway as a barrier to malignant progression and as a potential target for cancer treatment will be discussed.

CHRONIC CISPLATIN TREATMENT PROMOTES ENHANCED DAMAGE REPAIR AND TUMOR PROGRESSION IN A MOUSE MODEL OF LUNG CANCER

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Chemotherapy resistance is a major obstacle in cancer treatment, yet the mechanisms of response to specific therapies have been largely unexplored in vivo. By employing genetic, genomic, and imaging approaches, we have examined the dynamics of response to a mainstay chemotherapeutic, cisplatin, in multiple mouse models of human non-small cell lung cancer (NSCLC). We show that lung tumors initially respond to cisplatin by sensing DNA damage, undergoing cell cycle arrest and inducing apoptosis—leading to a significant reduction in tumor burden. Importantly, we demonstrate that this response does not depend on the tumor suppressor p53 or its transcriptional target p21. Prolonged cisplatin treatment promotes the emergence of resistant tumors with enhanced repair capacity that are cross-resistant to platinum analogs, exhibit advanced histopathology, and possess an increased frequency of genomic alterations. Cisplatin-resistant tumors express elevated levels of multiple DNA damage repair and cell cycle arrest-related genes, including p53-inducible protein with a death domain (Pidd). We demonstrate a novel role for PIDD as a regulator of chemotherapy response in human lung tumor cells. Preliminary data suggest that PIDD-induced chemo-resistance is due to its ability to promote cell cycle arrest. Our current focus is aimed at elucidating the signaling mechanisms that dictate this response.

53BP1 INHIBITS HOMOLOGOUS RECOMBINATION IN BRCA1-DEFICIENT CELLS BY BLOCKING RESECTION OF DNA BREAKS

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Defective DNA repair by homologous recombination (HR) is thought to be a major contributor to tumorigenesis in individuals carrying Brca1 mutations. Here, we show that DNA breaks in Brca1-deficient cells are aberrantly joined into complex chromosome rearrangements by a process dependent on the nonhomologous end-joining (NHEJ) factors 53BP1 and DNA ligase 4. Loss of 53BP1 alleviates hypersensitivity of Brca1 mutant cells to PARP inhibition and restores error-free repair by HR. Mechanistically, 53BP1 deletion promotes ATM-dependent processing of broken DNA ends to produce recombinogenic single-stranded DNA competent for HR. In contrast, Lig4 deficiency does not rescue the HR defect in Brca1 mutant cells but prevents the joining of chromatid breaks into chromosome rearrangements. Our results illustrate that HR and NHEJ compete to process DNA breaks that arise during DNA replication and that shifting the balance between these pathways can be exploited to selectively protect or kill cells harboring Brca1 mutations.

CONTRASTING PHYSIOLOGIC ROLES FOR *ARF* AND *INK4A* DURING MALE GERM CELL DEVELOPMENT

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When induced by sustained and elevated levels of mitogenic signals, production of the $p19^{Arf}$ tumor suppressor inhibits the Mdm2 E3 ubiquitin ligase to activate p53 in response to oncogenic stress. Although the accepted paradigm for Arf action involves p53 engagement, $p19^{Arf}$ interacts with protein targets other than Mdm2 and can play p53-independent roles in tumor suppression. For example, by inhibiting the Senp3 SUMO2/3 protease, $p19^{Arf}$ can induce the sumoylation of many proteins, including nucleophosmin (NPM, B23), Miz1, and others to affect cellular processes such as ribosomal biogenesis and RNA transcription in a p53-independent manner.

Arf is transiently expressed in mitotically dividing spermatogonia, but its expression is extinguished in the primary meiotic spermatocytes that arise from them. Lineage tracing experiments using an *Arf-Cre* "knock-in" mouse crossed to Rosa26 indicator strains that express LacZ or YFP in response to Cre recombinase demonstrated that expression of $p19^{Arf}$ in spermatogonia neither prevents their mitotic division nor their ability to generate meiotic spermatocytes and, ultimately, mature sperm. In direct contrast, *Arf* inactivation in spermatogonia results in p53-dependent apoptosis of meiotic spermatocytes, thereby contributing to significantly reduced spermatogenesis throughout the life of *Arf*-null mice. Analysis of $Arf^{Cre/Flox}$ mice revealed that the defect in spermatogenesis is germ cell-autonomous. The spermatocytes of *Arf*-null animals exhibit decreased levels of SUMO2/3-conjugates accompanied by increased γH2AX and TUNEL staining. Together, these findings indicate that transient $p19^{Arf}$ expression, which is normally restricted to spermatogonial progenitors, triggers a salutary program that protects their meiotic progeny from unrepaired DNA damage and p53-mediated apoptosis. Surprisingly, *Ink4a* inactivation leads to an increased number of spermatocytes and output of mature sperm. The effects of *Arf* inactivation are therefore offset in double knock-out *Ink4a-Arf*-null mice, which exhibit normal testis weights and sperm counts. These findings are without precedent in defining *in vivo* roles for Ink4a and Arf proteins in regulating normal tissue homeostasis outside the context of classical tumor suppression.

TGFβ SIGNALING DIRECTLY INDUCES *ARF* PROMOTER REMODELING BY SMAD-DEPENDENT AND -INDEPENDENT MECHANISMS

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The *Arf* tumor suppressor gene product, $p19^{Arf}$, is induced in response to oncogenic signals to block cancer development and progression. We previously showed that *Arf* expression is diminished in the absence of Tgfβ2 at several sites in the developing mouse. We used complementary *in vivo* and cell culture models to further explore the relationship between Tgfβ and *Arf* and to define the molecular mechanisms by which the latter is induced. First, we show that conditional inactivation of *Arf* in cells arising from a Wnt1-expressing lineage leads to pericyte hyperplasia around vessels in the primary vitreous. Conditional inactivaton of *TbrII* in this same lineage leads to a similar phenotype, suggesting that both Tgfβ receptor II and $p19^{Arf}$ act in the same cell to prevent proliferation of pericytes in the developing eye. To further demonstrate that Tgfβ resides upstream of *Arf*, we showed that its capacity to arrest cell proliferation *in vivo* depended on $p19^{Arf}$. Tgfβ1, 2, and 3 (but not the BMP-2, another member of the Tgfβ superfamily) induced $p19^{Arf}$ expression in wild type mouse embryo fibroblasts (MEFs), and they enhanced *Arf* promoter activity in $Arf^{lacZ/lacZ}$ MEFs. Chemical inhibitors of Smad-dependent and -independent pathways showed that SB431542, a Tgfβ type I receptor inhibitor, and SB203580, a p38 MAPK inhibitor, impeded Tgfβ2 induction of *Arf*. Genetic studies confirmed the findings: transient knock-down of p38 MAPK, Smad2 or Smad3 blunted Tgfβ2 effects, as did Cre recombinase-treatment of $Tbr\ II^{flox/flox}$ MEFs to delete Tgfβ receptor II. Chromatin immunoprecipitation revealed that Tgfβ rapidly induced Smad2/3 binding and histone H3 acetylation at genomic DNA proximal to *Arf* exon 1β. This was followed by increased RNA Polymerase II binding and progressively increased primary *Arf* transcripts from 24 through 72 hours, indicating that the induction was due to increased transcription. In summary, our results reveal the signal transduction cascade and molecular events at the *Arf* promoter that underlie its transcriptional induction by Tgfβ.

NEW MOLECULAR INSIGHTS INTO A KEY ROLE FOR TCTP IN TUMORIGENESIS AND TUMOR REVERSION

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We and others have provided evidence that aggressive tumors can quit their malignant state through a process called tumor reversion (1). By comparing the gene expression profiles of these revertant cells to their parental tumor cells, Translationally Controlled Tumor Protein (TCTP) was identified as the most significantly down-regulated gene in the revertant cells. This gene is conserved in all species examined and functions as a pro-survival factor. Here we show that TCTP and p53 are mutually repressive. Whereas p53 down-regulates TCTP at the transcriptional level, TCTP promotes MDM2-mediated degradation of p53 by competing with NUMB. This inhibition, of NUMB by TCTP, most likely affects the composition of the p53/MDM2/NUMB tri-complex (2). To assess the clinical relevance of our findings, we screened a cohort of 516 breast cancers for TCTP expression by immunohistochemistry. High TCTP expression is significantly associated with poorly differentiated, aggressive G3 tumors as opposed to low (G1) and intermediate (G2) grade tumors. High TCTP levels inversely correlate with a defective p53 status. Importantly, the elevated expression of TCTP also predicts poor prognosis in breast cancer patients ($p<0.0005$). TCTP is also highly expressed in normal mammary stem cells as opposed to differentiating progenitors, suggesting a role for TCTP in the maintenance of an undifferentiated state, a function that might be relevant to the pathogenesis of poorly differentiated tumors. This observation is consistent with a previously recognized role for TCTP in stemness via its ability to regulate the expression of oct4 and nanog. In conclusion, these results provide further support of a role for TCTP in tumorigenesis and new insights into its molecular mechanisms of action. The elevated expression of TCTP in stem cells and G3 tumors will be discussed in the framework of disease specificity, tumor progression and reversion.

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SLEEPING BEAUTY INSERTIONAL MUTAGENESIS REVEALS MULTIPLE NETWORKS OF GENES CO-OPERATING WITH *APC*-DEFICIENCY TO DRIVE INTESTINAL MUTAGENESIS

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The stepwise model of Vogelstein and Fearon represents the paradigm for colorectal cancer progression. However, recent studies have indicated that the molecular events involved are far more complex and suggest that a large repertoire of genes may contribute to intestinal mutagenesis (Wood *et al.*, *Science* **318**, 1108-13 (2007)). To identify alternative candidate genes and pathways implicated in the development of colon cancer we have performed a comprehensive forward genetic screen using insertional mutagenesis in mice carrying conditional (Apc^{floxed}) and constitutive (Apc^{Min}) mutations of *Apc*, representing models of sporadic colorectal cancer and familial adenomatous polyposis coli (FAP) respectively. We harvested 446 lesions for splinkerette-PCR and 454 sequencing, and parallel histopathological analysis. Using a Gaussian kernel convolution algorithm optimized over two scale spaces of 30kb and 120kb we identified 997 common insertion sites (CIS), equating to 958 potential cancer driver genes. This was compared to results generated by Monte Carlo simulations, a second statistical framework, to produce a concordance between the two methods of 75%. From cross-species oncogenomics 318 of these genes are also mutated in human colorectal carcinogenesis including 37 Wnt targets, of which 20 were found to be modifiers of Wnt signaling. Furthermore, 290 genes were identified in 148 significant canonical pathways using Ingenuity Pathway Analysis. Some 97 genes in 38 groupings co-occur with higher than predicted frequency in mini-networks. We also identified genes specifically associated with a subset of tumors showing aberrant Paneth cell differentiation, a hallmark of deregulated Wnt signaling, and genes associated with germline versus sporadic loss of *Apc*. In summary, this study expands the potential gene signature for colorectal cancer.

DICER1 IS REQUIRED FOR RETINOBLASTOMA FORMATION

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A large body of evidence indicates that alterations in the expression of miRNAs contribute to cancer pathologies. DICER1, which encodes an enzyme playing a critical role in miRNA biogenesis, is the target of mutations or haploid deletions in human cancers. Surprisingly, however, homozygous deletions or loss-of-function mutations in DICER1 are not found in human tumors.
Consistent with these observations, we have recently shown that Dicer1 functions as a haploinsufficient tumor suppressor in a preclinical mouse model of retinoblastoma. We now show that complete ablation of Dicer1 in retinal progenitor cells prevents the formation of retinoblastoma in mice that harbour inactivating mutations targeting both the Rb and p53 tumor suppressor pathways. Importantly, loss of Dicer1 in retinal progenitor cells does not affect survival (nor retinogenesis) in the absence of Rb; instead, it induces their apoptotic death upon concomitant inactivation of p53. miRNA profiling of Rb/p53-deficient mouse tumors, as well as human retinoblastoma samples, identified specific microRNAs as key pro-oncogenic agents in retinoblastoma. High-resolution Array-CGH revealed that focal amplification of or a gain of the genomic region where these microRNAs reside occurs in a subset of human retinoblastoma. Functional inactivation of these miRNAs resulted in the death of human retinoblastoma cell lines.
Our data therefore identify Dicer1 as the first synthetic lethal partner of tumor suppressor p53 and specific microRNAs as key therapeutics targets for the treatment of retinoblastoma.

PDE4D AND PROSTATE CANCER: FROM MOUSE GENETIC SCREENING TO CANDIDATE THERAPEUTICS

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We have been conducting a two-species screen for prostate cancer oncogenes that are suitable for use as new prostate cancer drug targets. The initial screen utilized transposon-based somatic mutagenesis in mice to induce prostate tumors and molecularly tag candidate oncogenes. This approach identified several candidate prostate cancer genes including known oncogenes such as *Nras* and *Braf* as well as new candidate oncogenes including *Pde4d*. A secondary screen used human prostate tissue microarrays (TMAs) to validate the relevance of new candidate oncogenes for understanding human prostate cancer. Automated quantitative analysis of PDE4D protein expression on the human TMAs confirmed its over-expression in human prostate cancer patients. The *Pde4d* mRNA is alternatively spliced to encode a family of related PDE4D enzymes. These enzymes share a common catalytic domain that gives all of the enzymes cAMP-specific 3',5'-cyclic phosphodiesterase activity. Protein domains outside the catalytic domain allow for differential protein regulation and sub-cellular localization of different PDE4D isoforms. Several small molecules have been identified that are highly selective inhibitors of PDE4D activity including cilomilast and NVP-ABE171. Testing of these small molecules has shown that they inhibit the growth of prostate cancer cell lines in vitro, and growth-inhibition was associated with down-regulation of hedgehog pathway signaling. Daily oral administration of PDE4D inhibitors also dramatically inhibited the growth of prostate cancer xenografts with no adverse affects on the xenograft hosts. These data indicate that PDE4D inhibitors are exciting new candidate drugs for prostate cancer.

PODOPLANIN ASSOCIATES WITH CD44 TO PROMOTE DIRECTIONAL CELL MIGRATION

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Podoplanin is a transmembrane glycoprotein up-regulated in human carcinomas. Its expression in tumour cells is linked to increased cell migration and invasiveness. Since podoplanin has no obvious enzymatic motif within its structure, the identification of podoplanin-binding proteins is crucial to fully understand its specific role in cancer. We report that CD44, the major hyaluronan (HA) receptor, is a novel partner for podoplanin. Expression of the CD44 standard isoform (CD44s) is co-ordinately up-regulated together with that of podoplanin during progression to highly aggressive spindle cell carcinomas in a mouse skin model of carcinogenesis, and during epithelial-mesenchymal transition (EMT). Furthermore, podoplanin binds to CD44s at cell surface protrusions in migrating cells and CD44 is required for podoplanin-enhanced cell migration and directionality in epithelial cells. Our results suggest a role for CD44-podoplanin interaction in driving tumour cell migration during malignancy.

THE ROLE OF DYSTROGLYCAN IN PROSTATE CANCER.

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Cell adhesion to the basement membrane is critical in rendering cell functionality, polarity and tissue specificity. Dystroglycan (DG) is a versatile signal transducing molecule that links cellular cytoskeleton to the basement membrane; it is postranslationally processed into two subunits termed as α-DG and β-DG. In majority of human cancers there is loss or weak expression of β-DG. Prostate cancer is now the most common cancer in men in the UK. It accounts for 24 out of every 100 cancers diagnosed in men. The loss of β-DG is associated with progression of prostate cancer (Cross et al., 2008). However, the mechanisms that regulate the functionality of β-DG in prostate cancer have not been thoroughly investigated. In the present study, we investigated the role of β-DG in the progression of prostate cancer using benign and malignant prostate cell lines. We have demonstrated using various antibodies a change in the sub-cellular localization of β-DG. β-DG is localized to the cell periphery and cell-cell junctions when probed with an antibody recognizing the c-terminus of β-DG, but was detected exclusively in the nucleus on probing with an antibody that recognizes phosphorylated (pY892) DG. Post-translational modification of β-DG is postulated to be involved in the sub-fragmentation of β-DG. On transient over expression in cancer cell lines the cytoplasmic domain of β-DG fused to GFP (cβ-DG-GFP) is localized to the nucleus while the construct with a mutated nuclear localization domain (cβ-DG Δ NLS-GFP) was targeted to the cytoplasm. Prostate cancer cells respond to androgen stimulus mediated by intracellular androgen receptor (AR). As expected AR protein was detected in the nuclear fraction of cells treated with dihydrotestosterone. Interestingly β-DG was also detected in the nuclear fraction. A microarray analysis comparing prostate cell lines transfected with the cβ-DG-GFP and cβ-DG Δ NLS-GFP constructs will be performed to study the significance of β-DG processing in cancer. Using immunoprecipitation experiments we are currently investigating the probable interaction between AR and the nuclear localization of β-DG. We have also developed a 3D culture model of benign and malignant human prostate cells; this will be a useful tool to evaluate the contribution of β-DG towards maintaining prostate tissue polarity. Our results suggest key mechanisms involving the regulation of β-DG in prostate cancer cells.

Reference:

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SYNERGISTIC INDUCTION OF APOPTOSIS BY THE HDACI, PANOBINOSTAT, COMBINED WITH RHTRAIL OR BORTEZOMIB, IN MULTIPLE MYELOMA

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The HDACi panobinostat belongs to a class of chemotherapeutics with demonstrated anticancer activities in laboratory studies and within the clinic. Here we investigated the ability of panobinostat to induce apoptosis in human multiple myeloma (MM) cell lines, alone and in combination with agents that target the extrinsic apoptosis pathway (TRAIL) or proteosome inhibition (bortezomib). We have employed the Vk*MYC model of MM to determine the effects of panobinostat and combination therapies in an in vivo setting. Human MM cell lines were treated with panobinostat, TRAIL or bortezomib alone or in combination. Cell viability and cell cycle were assessed by FACS. Histone acetylation was assessed by western blot. C57BL/6 mice bearing transplanted Vk*MYC MM were treated with panobinostat (25mg/kg, d1-4; 15mg/kg 5d/wk, 3wk), MD5-1 (mouse α-DR5 Ab, 50ug/mouse, d1, 4, 8, 12), bortezomib (0.5mg/kg, every 4d) or vehicle (D5W ± UC81B9). Combination therapies were panobinostat (10mg/kg) + MD5-1 or bortezomib. M-protein was assessed weekly in all mice. Treatment of MM cell lines with panobinostat resulted in time- and dose-dependent induction of apoptosis with G1/G2-M phase arrest and histone H3 acetylation. Combined panobinostat with TRAIL or bortezomib synergised to mediate robust and rapid apoptosis in the human MM cells. Serum M-protein in mice with Vk*MYC MM cells was significantly reduced after treatment with panobinostat, but not MD5-1 or bortezomib, alone compared with control mice ($p<0.01$). Overall, panobinostat induced a significant survival advantage compared with vehicle (median=36.5 vs. 16 d). Combinations of panobinostat with bortezomib synergised to reduce M-protein levels to a greater extent than either agent alone. Combination therapies with MD5-1 are ongoing. These results suggest that panobinostat has single agent activity against MM cells *in vitro* and provides significant benefit to mice with syngeneic MM tumours. Moreover, therapies consisting of HDACi with TRAIL or bortezomib may provide a rational approach to the treatment of MM. Future studies will investigate the molecular events underpinning the effects mediated by combined panobinostat with MD5-1 or bortezomib in the Vk*MYC MM model.

MICRORNA–TUMOR SUPPRESSOR NETWORKS ON T-CELL LYMPHOBLASTIC LEUKEMIA

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MicroRNAs have emerged as important new cancer genes and individual microRNAs have been shown to behave as oncogenes or tumors suppressors in many hematological cancers. However, a comprehensive analysis of the contribution of several microRNAs in one cancer has been lacking. Here we combine an analysis of microRNA expression changes in T-ALL with an unbiased microRNA library screen to systematically identify microRNA activities that contribute to T-ALL. Following validation in a murine T-ALL model and in xenografted human T-ALL, we confirm several known oncogenic (e.g. miR-19) and tumor suppressive (e.g. miR-451) microRNAs along with new candidates. Strikingly, it emerges that these microRNAs act on overlapping sets of tumor suppressor genes including Ikaros, Pten, Phf6, and Bim. Hence, a systematic analysis of microRNA functions in one cancer reveals a surprisingly clear pattern of the microRNA-tumor suppressor interactions involved in T-cell leukemia.

INDUCIBLE, REVERSIBLE ESSENTIAL GENE KNOCKDOWN BY TRANSGENIC RNA INTERFERENCE

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RNA interference (RNAi) technology enables systematic loss-of-function genetics in mammalian systems. Previous work from our laboratory has shown that regulated expression of transgenic shRNAs targeting tumor suppressor genes can recapitulate null phenotypes in adult mice. To determine whether the same technology could be applied to study essential genes, we aimed to inducibly and reversibly target cell proliferation. Initially, an *in vitro* screen with an shRNA library targeting known and putative DNA replication factors was conducted to identify gene targets whose loss inhibited cell proliferation. Multiple shRNAs against Replication protein A3 (Rpa3) were identified. We then targeted embryonic stem (ES) cells with an inducible Rpa3 shRNA cassette at the collagen A1 locus. Doxycycline-driven shRNA expression inhibited cell proliferation in ES cells and mouse embryonic fibroblasts derived from transgenic animals.

When Rpa3 shRNA expression was induced in adult mice, they quickly lost weight and became moribund within two weeks. Histological analysis revealed a complete destruction of the intestinal epithelium upon Rpa3 knockdown. Other highly proliferative organs were also atrophied, especially the spleen. Proliferating cells were absent in the liver and kidneys, which maintained normal architecture but were reduced in size. Remarkably, if shRNA expression was extinguished even after dramatic weight loss, transgenic mice could regain weight and survive. Thus, we can model a transient blockade in cell proliferation that mimics the effects of conventional chemotherapy. Looking ahead, the ability to target anti-proliferative genes in our system will be an important tool to model inhibition of rational cancer drug targets. In general, inducible, reversible essential gene inactivation in adult mice brings mammalian genetics a step closer to other model organisms where temperature-sensitive mutations are used to study lethal phenotypes.

IN VIVO RNAI SCREENING IN HEMATOPOIETIC MALIGNANCIES

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Mouse models have dramatically improved our understanding of cancer development and tumor biology. However, these models have shown limited efficacy as tractable systems for unbiased genetic experimentation. We have previously adapted a transplantable mouse model of Burkitt's lymphoma to *in vivo* screening applications, and have now extended this technology to investigate modulators of central nervous system metastasis in hematopoietic malignancies. Metastasis to the brain represents a significant clinical challenge in certain leukemias and lymphomas. Patients who relapse in the CNS have an extremely poor prognosis, and there are very few stratifiers to effectively identify patients who are at high risk for CNS involvement. To look for novel determinants of leukemia cell metastasis to the CNS, we performed an RNAi-based screen in a mouse model of acute lymphoblastic leukemia (ALL). This screen has yielded a number of genes, including the pro-inflammatory cytokine IL-1β, that are important modulators of CNS infiltration. Together, these screens have identified critical determinants of tumor cell behavior in vivo, highlighting the utility of performing shRNA screens within a relevant physiological context.

UNDERSTANDING THE ROLES OF BCCIP IN PREVENTION OF CHROMOSOME INSTABILITY AND MITOSIS

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Polyploidy and aneuploidy are major sources of chromosome instability frequently observed in cancer cells. Published studies from the Shen laboratory have shown, that severe knock down, BRCA2 and CDKN1A (Cip/p21) Interacting Protein (BCCIP) results in reduced homologous recombination (HR) mediated DNA repair, polyploidy, aneuploidy, formation of multiple spindles and abnormal centrosome amplification during mitosis. In addition, down regulation of BCCIP exhibits an increase in the percentage of cells joined by intercellular bridges, suggesting mitotic and/or cytokinesis defect. Although these data clearly suggest that BCCIP plays a role in preventing in chromosome instability and mitotic errors, the mechanisms by which BCCIP regulates these processes have not been determined.

In this study, we used serial time lapse microscopy followed by frame by frame analysis of BCCIP deficient HT1080 cells. We found that BCCIP deficient cells take a significantly longer time to complete mitosis than BCCIP proficient cells ($p=0.001$). This suggests that mitosis may be extended in BCCIP knockdown cells. In addition to the extended mitosis in BCCIP knockdown cells, it was also observed that some BCCIP knockdown cells initiated mitosis but aborted prior to the formation of a visible cytoplasm bridge at cytokinesis, suggesting a possibility of endomitosis. In some instances the cells aborted mitosis and re-entered interphase, to undergo asymmetric division and produced three daughter cells. Further analyses by using BCCIP deficient HT1080 stable cell lines expressing GFP tagged H2B, revealed that BCCIP deficient cells also required more time for chromosome segregation as well as exhibited defects in metaphase plate formation. Approximately 50% of BCCIP deficient cells showed delay in completion of chromosome segregation, compared to control cells.

BCCIP has been known to function in the regulation of homologous recombination (HR) mediated DNA repair as well as regulation of BRCA2 function. Our new data suggest that the functions of BCCIP in HR may be necessary in preventing chromosome damage such as caused by replication stress, which may subsequently affect mitosis. However, the novel BCCIP functions during G2/M checkpoint and/or mitosis as well as cytokinesis may further contribute to prevention of mitotic errors and chromosome instability.

LINT, A NOVEL DL(3)MBT (*DROSOPHILA* LETHAL 3 MALIGNANT BRAIN TUMOR) COMPLEX, WITH A ROLE IN STABLE REPRESSION OF GERMLINE-SPECIFIC GENES

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The MBT (Malignant Brain Tumor) domain is a "chromatin reader", a module that specifically recognizes mono- and di-methylated lysines within histone tails and has been suggested to compact nucleosomes to form higher order chromatin structures. Interestingly, MBT domain containing proteins were previously shown to have critical functions in developmental processes, maintenance of transcriptional repression and tumor suppression. Data from both *Drosophila melanogaster* and human suggest that MBT domain proteins are part of multisubunit protein complexes. In human, the functional analysis of MBT proteins is complicated by the existence of at least eight closely related paralogues. In contrast, Drosophila possesses three MBT proteins and therefore is a helpful model organism to better understand and unravel the functional roles of MBT proteins. Besides dL(3)mbt (*Drosophila* Lethal 3 malignant brain tumor), the founding member of the family of MBT domain proteins, there are the two Polycomb group proteins, called Scm and Sfmbt. The latter two act in concert with transcriptional repressor complexes, to counteract Trx proteins and stably repress hox gene expression. While the polycomb group proteins are rather extensively studied, little is known about the molecular functions of dL(3)mbt. Previous studies found dL(3)mbt to be a substoichiometric subunit of the Myb-MuvB/dREAM complex and in this context to be involved in the repression of a certain subset of dE2F-regulated genes. Moreover, *in vitro* studies have implicated dL(3)mbt in the process of chromatin maturation.

Using anti-Flag affinity purification and subsequent peptide mass fingerprinting, we biochemically isolated and identified LINT (L(3)mbt interacting), a novel complex containing dL(3)mbt, the uncharacterized protein dLint-1 (exhibiting a PHD finger like domain), histone demethylase and histone deacetylase activity. Microarray expression analysis upon dL(3)mbt or dLint-1 knockdown in Kc cells revealed that LINT is critical for the maintenance of stable repression of genes with testis- or ovary-specific expression patterns. Further investigation by chromatin immunoprecipitation (ChIP) demonstrates that LINT binds directly to promoter regions of target genes, which display reduced nucleosome occupancy. The loss of LINT from the promoter upon dL(3)mbt knockdown, which in turn leads to the derepression of target genes, is accompanied by changes in specific histone modifications. Current experiments aim to gain deeper insight into the mechanism of gene repression by the novel dL(3)mbt containing LINT complex.

ROLE OF YAL066W LOCUS IN MAINTENANCE OF GENOME STABILITY

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The results of a recent genetic screen to identify dosage-sensitive Saccharomyces cerevisiae genes affecting chromosome stability revealed that heterozygous deletion of YAL066w results in a highly reproducible 100 fold increase in spontaneous chromosome instability. The putative ORF of YAL066w encodes a 102 amino acid peptide but the protein product of this gene has never been detected. Moreover, most transcriptome studies failed to demonstrate a polyadenylated transcript derived from this locus. We thus reasoned that YAL066w might encode a non-coding RNA. To support this hypothesis, we generated several nonsense mutations in YAL066w and determined the chromosome instability rate of the resulting strains. While c.77T>A, c.131T>A, c.246T>A nonsense mutations have no effect on genome stability, c.186C>G heterozygous mutation strain shows increased genome instability although a C>T change at the same base has no phenotype. This result demonstrates that nonsense mutations of YAL066w, unlike typical protein coding genes, have very low effect on YAL066w gene function, indicating it is possible that YAL066w might encode a non-coding RNA (ncRNA) which functions in maintaining chromosome stability. However, both reverse transcriptase-PCR and primer extension experiments optimized for short RNA transcripts have failed to detect transcripts from this locus. Further investigation of whether non-coding RNA transcripts are encoded in YAL066w locus or if inter-chromosomal and/or intra-chromosomal interactions with YAL066w might account for the possible mediation of chromosome instability are underway.

MUTUAL REGULATION CONNECTS C-MYC AND SIRT1

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SIRT1 is an NAD+-dependent deacetylase which inhibits pro-apoptotic factors including p53. Here we show that activation of the c-MYC oncogene mediates an increase in SIRT1 protein level and activity, which is necessary for suppression of apoptosis and senescence in human cells immortalized by c-MYC. A previously unknown c-MYC target gene facilitated the c-MYC induced SIRT1 activation. Additionally, c-MYC sequestered the SIRT1-inhibitor DBC1 (deleted in breast cancer 1) from SIRT1. Inactivation of SIRT1 by RNA interference, chemical inhibitors or ectopic expression of DBC1 sensitized c-MYC-expressing cells to apoptosis. Furthermore, SIRT1 interacted with, and deacetylated c-MYC resulting in increased c-MYC transcriptional activity. Collectively, the mutual regulation of c-MYC and SIRT1 constitutes a positive feedback loop that promotes cell proliferation and survival. Deregulation of this circuitry presumably contributes to cancer but may also provide an attractive target for therapy.

EXPLORING THE ROLE OF PTEN IN TUMOR MAINTENANCE USING CONDITIONAL RNA INTERFERENCE

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The PI3K-Akt-mTOR (PAmT) pathway is mutationally activated in a broad range of human cancers and is the target of various drugs in clinical trials. The PTEN gene encodes a lipid phosphatase that acts as a negative regulator of the PAmT pathway, and is lost or inactivated in many tumor types. To genetically dissect the importance of the PAmT pathway in tumor maintenance, we generated a transgenic RNAi-based mouse model that allows conditional PTEN inactivation in a time- and tissue-specific manner. We have targeted the 3´UTR of the col1A1 locus in ES cells with an expression cassette consisting of the tet-responsive TRE-promoter followed by EGFP and a PTEN shRNA embedded into the miR30 backbone. This system enables single-copy tet-regulated expression of the PTEN shRNA which can be easily tracked by EGFP monitoring. To explore the effects of PTEN knockdown in the lymphoid system, we crossed PTEN shRNA transgenic mice to a lymphoid-specific transactivator mouse line. These mice initially showed increased numbers of CD4/CD8 double-positive (DP) T-cells, and subsequently developed T-cell lymphoma/T-ALL at a similar latency to PTEN KO animals. The diseased mice presented with an enlarged thymus, spleen and peripheral lymph nodes, consisting of EGFP+, CD4/CD8 DP T-cells, which also infiltrated other organs like liver and kidney. Western blot of tumor tissues demonstrated knockdown of PTEN to almost undetectable levels, and CGH analysis revealed recurrent genetic abnormalities, including those involving c-myc. To study the effects of PTEN reactivation in these tumors, we serially transplanted malignant cells and treated part of the mice with doxycycline (dox). Suppression of the PTEN shRNA by dox reactivated PTEN expression and led to a substantial survival advantage, although all mice eventually succumbed to cancer. Thus, PTEN restoration appears to delay, but not abolish, tumor progression. The biological and molecular basis for these observations are currently under investigation. Understanding how tumor cells respond to genetic inhibition of the PAmT pathway may help guide the clinical use of small molecules that inhibit its components.

TREATMENT-INDUCED SENESCENCE PRESERVES STEM CELL PROPERTIES OF Eμ-Myc DRIVEN LYMPHOMAS

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A connection between "stemness" and senescence has been recently suggested, where senescence-relevant pathways limit the efficacy of reprogramming somatic cells to pluripotency. Senescence and "stemness" thus use the same regulatory networks, but in opposite ways, mutually controlling each other. Viewing "stemness" as the critical capacity determining long-term faith of cancer cells, we studied the interference of the senescence machinery with stem cell characteristics.
We analysed the expression of stem cell-associated gene sets in untreated vs. adriamycin-exposed, therapy-induced senescent (TIS) Eμ-myc lymphomas (apoptosis-protected by Bcl2 overexpression). The clonogenicity and proliferative capacity of cells were tested upon disrupting senescence by acute deactivation of senescence-specific regulators.
Results show that TIS in Eμ-Myc lymphoma cells leads to significant changes in the expression of two alternative stem cell-defining genetic signatures, the embryonic stem cell-like (ESCL) and an adult tissue stem cell (ATSC) signature. The ESCL signature, indicative of aggressive tumor behavior, was reduced in TIS. However, the ATSC profile was highly upregulated, along with several transcripts associated with invasive neoplastic features, like Twist1/2, Snail1/2, Cdh2 or Mmp2. Even with ESCL profile reduced in senescent cells, the levels of reprogramming factors such as Klf4 and Sox2 remain high. Upon releasing the senescence barrier by disabling downstream senescence-specific pathways, cells resumed to proliferate with increased clonogenic potential and reverted to the initial ESCL profile. A similar oscillation between two stemness-maintaining profiles is observed in hematopoietic stem cells alternating between proliferation and quiescence. Analogous to quiescence as an acknowledged stemness-preserving condition, our results indicate that cells in a formally terminal cell-cycle arrest preserve or even enrich the components of a self-renewal machinery.
These results argue for a bi-directional relation between senescence and "stemness" rather than a mere mutual-restraining. While leading to a robust cell-cycle arrest, induction of TIS simultaneously promotes aggressive cancer features, like self-renewal, invasion and motility, suggesting that neoplastic properties are kept in check by similar molecular events as cellular senescence. Disrupting this broadly operating failsafe mechanism, for instance by inactivating mutations, will unleash a spectrum of detrimental growth capabilities.

A DISTINCTIVE DNA DAMAGE RESPONSE IN HUMAN HEMATOPOIETIC STEM CELLS REVEALS AN APOPTOSIS INDEPENDENT ROLE FOR P53 IN SELF-RENEWAL

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Highly regenerative tissues such as blood must possess effective DNA damage responses (DDR) that balance long-term regeneration with protection from leukemogenesis. Hematopoietic stem cells (HSC) sustain life-long blood production, yet their response to DNA damage remains largely unexplored. We report that human HSC exhibit delayed DNA double strand break rejoining, persistent γH2AX foci, and enhanced p53 and ASPP1-dependent apoptosis following γ-radiation compared to progenitors. p53 inactivation or Bcl-2 overexpression reduced radiation-induced apoptosis and preserved in vivo repopulating HSC function. Despite similar protection from irradiation-induced apoptosis, only Bcl-2 overexpressing HSC showed higher self-renewal capacity, establishing that intact p53 positively regulates self-renewal independently from apoptosis. The reduced self-renewal of HSC with inactivated p53 was associated with increased spontaneous γH2AX foci in secondary transplants of HSC. Our data reveal distinct physiological functions of p53 that together ensure optimal HSC function: apoptosis regulation and prevention of γH2AX foci accumulation upon HSC self-renewal.

INVESTIGATING UHRF1 AS AN ONCOGENE IN HEPATOCELLULAR CARCINOMA

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Background: The cell cycle regulator UHRF1 mediates gene silencing by epigenetic mechanisms, including DNA methylation. UHRF1 is over expressed in many human cancers and its depletion from cancer cells leads to apoptosis or sensitization to DNA damage. In zebrafish, *uhrf1* is essential for hepatocyte proliferation during liver development and liver regeneration following injury. Therefore, increased expression of UHRF1 may cause aberrant cell proliferation.

Aim: To test the hypothesis that UHRF1 participates in hepatocellular carcinoma (HCC) we are (i) investigating UHRF1 expression in human HCC patients and (ii) examining the effects of liver-specific UHRF1 over expression in transgenic zebrafish.

Methods & Results: UHRF1 mRNA expression was examined in tumor samples from 78 patients with hepatitis C. There is a significant positive correlation of UHRF1 expression with disease progression. While UHRF1 expression was not significantly changed in dysplastic nodules, there was 5-fold upregulation in early HCC and 22-fold upregulation in very advanced HCC. UHRF1 protein expression mirrors this pattern. Additionally, the HCC samples with the highest UHRF1 levels fall into a subclass of tumors characterized by increased proliferation and chromosomal instability. To explore the role of UHRF1 as a candidate oncogene in vivo, we created transgenic zebrafish which over express human UHRF1 in hepatocytes (*Tg(fabp10: UHRF1-GFP*)). Over expression of an oncogene in non-transformed cells can induce cellular senescence, resulting in negative selection of oncogene expressing cells. We found that cells over expressing UHRF1 in the developing liver are out competed by non-expressing cells. In addition, when UHRF1 is highly expressed in all hepatocytes in stable *Tg(fabp10: UHRF1-GFP)* fish, although embryogenesis appears normal, these fish had a mean survival of less than one month. Preliminary histological analysis of the livers from *Tg(fabp10: UHRF1-GFP)* fish reveal nuclear crowding, degeneration of hepatocytes, nuclear atypia, and destruction of the hepatic architecture.

Conclusions: Tumors from patients with advanced HCC demonstrate significantly higher expression of UHRF1 mRNA and protein. Forced high expression of UHRF1 in the zebrafish liver of *Tg(fabp10: UHRF1-GFP)* fish resulted in decreased competitive advantage of over expressing cells and reduced survival along with abnormal livers of stable transgenics. Ongoing work is focused on delineating the precise cellular abnormalities that occur in hepatocytes that over express UHRF1.

MIR99A, A MICRORNA THAT IS REPRESSED DURING PROSTATE CANCER PROGRESSION, CAN MODULATE THE CELLULAR RESPONSE TO DNA DAMAGE BY REGULATING CDC25A SYNTHESIS.

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Exposure of cancer cells to ionizing radiation or DNA damaging chemotherapy activates checkpoint mechanisms where Cdc25a is degraded following phosphorylation by Chk1, which leads to an increase in phosphorylation of CDK2 tyrosine-15 by Wee1 kinase and a corresponding decrease in DNA synthesis. In a screen for microRNAs that are decreased during prostate cancer progression, we found miR99a to be significantly downregulated during progression. Additionally, expression of miR99a was lower in breast cancer cell lines that had acquired mutations in p53. Cdc25a was predicted as a target of miR99a by multiple algorithms. The main function reported for Cdc25a is that its degradation following DNA damage inhibits S-phase progression as part of the cell's adaptive response. When an exogenous mimetic of miR99a was introduced into C4-2 prostate cancer cells, Cdc25a transcript shifted from being predominantly associated with polyribosomes to monoribosomes, indicating an inhibition of its translation. In the presence of exogenous miR99a, Cdc25a levels declined in C4-2 cells following DNA damage by ionizing radiation. Additionally, exogenous miR99a expression suppressed DNA synthesis following irradiation of prostate cancer cell lines. These data suggest that miR99a is important for the cellular response to DNA damage by regulating the rate of Cdc25a synthesis, which becomes apparent following the destabilization of Cdc25a protein post-irradiation. Loss of miR99a during prostate cancer progression or in breast cancer cells mutated in p53 thus decreases the cellular response to DNA damage. The results imply that progression associate changes in microRNAs could make cancers less sensitive to chemo- or radiotherapy.

REGULATION OF CELL CYCLE CHECKPOINT PATHWAYS BY 14-3-3Γ

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Background:
14-3-3 proteins are phospho-serine/phospho-threonine binding proteins that interact with a diverse array of binding partners and regulate the core phospho-regulatory signaling pathways of eukaryotic cells. 14-3-3 binds to the ligands containing mainly two different phosphorylated consensus motifs known as mode-I [RSXpSXP] or mode-II [RX(Y/F)XpSXP]. We have shown that two of the 14-3-3 isoforms, 14-3-3ε and 14-3-3γ, form a specific complex with the mitotic phosphatase cdc25C, and inhibit its function in vivo.

Materials and Methods:
To understand the role of 14-3-3ε and 14-3-3γ in cell cycle checkpoint function and cellular transformation, levels of these two isoforms have been down regulated by vector driven sh-RNA and lentiviral vectors. 14-3-3γ knockdown clones show abrogation of G2/M checkpoint function which could be restored by the sh-RNA-resistant-14-3-3γ. Down regulation of 14-3-3γ also shows higher rate of premature chromatin condensation, higher mitotic index and higher frequency of multi-centriole formation during mitosis. To study the role of 14-3-3γ further, a novel proteomic screen was designed to identify specific binding partners for 14-3-3γ. Ligands for 14-3-3γ were identified by comparing the phosphorylation level of the binding partners from knockdown and vector-control cells. GST-pulldown, western blot, MALDI-TOF and immuno-precipitation analyses were performed to identify and validate the binding partners. Using this screening module, several ligands for 14-3-3γ such as IRS1, c-Cbl, Kinesin1 (KIF5B), KLC-2, hsp7C, Zinc finger protein 224, GCP2 have been identified. Binding partners identified so far indicate the involvement of 14-3-3γ in insulin signal transduction pathway and regulation of centrosome duplication.

Results and Conclusions:
In brief, the work done so far shows that 14-3-3γ is required to maintain (i) the incomplete S phase and (ii) G2/M checkpoint function. Ligands of 14-3-3γ obtained from this screen help to understand the role of 14-3-3γ in cellular transformation via insulin signal transduction pathways and regulation of centrosome duplication. It has been demonstrated by other studies that deregulation of 14-3-3γ is associated with colorectal,lung and breast cancer. Therefore understanding the role of 14-3-3γ in cell cycle checkpoint function and cellular transformation will be important for future therapeutic applications.

TARGETING DRUG-INDUCED SURVIVAL PATHWAYS - RATIONAL DEVELOPMENT OF COMBINATORIAL THERAPIES.

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Alterations in the PI3-kinase pathway are common in breast, endometrial, and ovarian cancer and are thought to serve as major drivers of proliferation and survival. Multiple compounds targeting PI3-kinase pathway have been generated. We have examined the sensitivity of ovarian tumor cell lines to PI3K/mTOR inhibitors in 3D tumor spheroid assays. Interestingly, tumor cells that are not attached to matrix are generally highly sensitive to NVP-BEZ235, whereas those in contact with matrix are highly resistant. In order to understand the mechanism of this resistance and to identify candidate targets to abrogate the matrix resistance, we performed reverse phase protein array of cells treated with NVP-BEZ235. While NVP-BEZ235 inhibited known downstream targets of PI3K/mTOR, there was significant upregulation of multiple proteins known to regulate cell survival including IGF1R, pEGFR, p90RSK and Bcl-2. This response raised the question whether combined inhibition of PI3K/mTOR and any of the induced proteins would increase cell death in the matrix-attached cells. Interestingly, inhibition of Bcl-2 by ABT737 caused widespread apoptosis in the 3D structures, but only in combination with NVP-BEZ235. In addition, matrix-resistance was abrogated by combined treatment with NVP-BEZ235 and inhibition of EGFR or knockdown of p90RSK or IGF1R.

These results indicate that inhibition of PI3K/mTOR leads to induction of protective compensating pathways and that suppression of these pathways can significantly enhance sensitivity to PI3K/mTOR targeted therapies. In addition, these studies suggest a rational approach to the development of combination therapies -- that is, through identification of drug-induced survival pathways and targeting these pathways in combination. Our current efforts center on investigating the mechanisms of PI3K/mTOR inhibition resistance, and identification of other therapeutically relevant drug combinations.

FUNCTIONAL EXPRESSION OF FRS2/3 IN PROSTATE CANCER

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Enhanced FGF signaling pathway is commonly implicated in the initiation and progression of prostate cancer. FGF receptor substrates such as FRS2 and FRS3 are known key adaptor proteins that interact with the FGF receptor to mediate FGF-FGFR downstream signalling. In this study, we set out to investigate the functional expression of FRS2 and FRS3 in prostate cancer.

The expression levels of FRS2 and FRS3 were characterised by real-time PCR and western blotting in a number of prostate cancer cell lines. Laser capture microdissection techniques were used in clinical samples obtained from prostate gland biopsies to differentiate specimens according to Gleason grade (3, 4 or 5). Protein expression was studied by immunohistochemistry in a large tissue microarray (TMA). An in vitro model was designed to assess the functional effect of FRS2 and FRS3 manipulation in prostate cancer.

Heterogeneous FRS2 and FRS3 expression was noted in both malignant and benign cells in vitro and in our clinical samples. Interestingly, we observed a preferential over-expression of FRS3 compared to FRS2 in a subset of high grade cancers. To further examine the functional effect of an increased FRS3 expression, stable FRS3 over-expressing DU145 prostate cancer cell lines were produced. We found that FRS3 over-expression results in an enhanced colony formation ($p=0.001$), cell proliferation ($p=0.005$) and migration ($p<0.05$) compared to control vector cells. Furthermore, over-expression of FRS3 was able to compensate for targeted knockdown of FRS2. We next tested the therapeutic principle of targeting FRS molecules in prostate cancer cells using dual silencing of FRS2 and FRS3. In these experiments co-suppression of FRS2/3 resulted in a significant inhibition of pERK activation and a concomitant reduction in cell proliferation ($p<0.05$), migration and invasion ($p<0.05$) regardless of the ligand stimulus. Finally, synchronous knockdown of FRS2/3 with concomitant exposure to cytotoxic irradiation resulted in a significant reduction in cell survival compared to cytotoxic irradiation alone ($p<0.05$).

Our results confirm the functional redundancy of the FRS adaptor proteins in FGF mediated growth factor signalling pathway in prostate cancer. Furthermore, our results suggest a potentially important role for the FRS3 homologue in a subset of aggressive prostate cancers. Thus strategic inhibition of FRS2/3 may be a useful therapeutic target to disrupt global aberrant FGF signalling in prostate cancer.

RAC1 DRIVES HYPERPROLIFERATION FOLLOWING APC LOSS IN THE SMALL INTESTINE.

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Inactivation of the gene encoding the adenomatous polyposis coli (APC) tumour suppressor protein is recognized as the key early event in the development of colorectal cancers. APC loss leads to nuclear localization of beta-catenin and constitutive activity of the beta-catenin-Tcf4 transcription complex. Direct targets of this complex include the c-Myc proto-oncogene and cyclin D2. Acute loss of APC in the small intestine leads to hyperproliferation within the intestinal crypt, increased levels of apoptosis, and perturbed differentiation and migration. Recent work has shown that co-deletion of c-Myc rescues these abnormalities indicating that c-Myc is a key mediator of the phenotypic effects of APC loss (Sansom et al., 2007).

Microarray analysis from mice deficient for APC or both APC and c-Myc identified the guanine nucleotide exchange factor T-cell lymphoma invasion and metastasis 1 (TIAM1) as a potential c-Myc target gene (Sansom et al., 2007). TIAM1 activates small rho GTPase proteins such as Rac1, Cdc42 and RhoA. Rho GTPases control a variety of cellular processes including cell migration, differentiation and cell-cycle progression. In this study we identify a requirement for Rac1 in promoting crypt hyperproliferation following deletion of APC. We find that following APC loss in the small intestine TIAM1 is upregulated and this coincides with an increase in levels of GTP bound active Rac. Furthermore, co-deletion of Rac1 and APC strongly perturbes the hyperproliferation phenotype associated with APC loss. This correlates with a decrease in the activity of several cyclin proteins indicating that Rac1 may be required for efficient G1/S phase cell cycle progression in vivo. We also find that the TIAM-Rac inhibitor NSC23766 strongly attenuates the hyperproliferative phenotype associated with APC loss. These data indicate that TIAM1-Rac1 operate downstream of c-Myc, are required for hyperproliferation following loss of APC and that Rac maybe a useful therapeutic target in colorectal cancer. Future work will attempt to determine the mechanisms by which Rac1 drives APC loss induced hyperproliferation and whether its inhibition can perturb growth of APC deficient tumours.

THE ONCOPROTEIN GLI1 INDUCES A PROGRAM OF REGRESSION THAT CONFERS A BASAL-LIKE AND ANDROGEN-INDEPENDENT PHENOTYPE UPON LNCAP PROSTATE CANCER CELLS

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The GLI transcription factors have been implicated in the development and progression of prostate cancer although the mechanism(s) by which this occurs and how this may relate to androgen receptor (AR) function remains unclear. We observed that GLI1 mRNA expression and GLI reporter activity were both higher in normal (PNT-2) and tumourigenic (DU145 and PC-3) androgen-independent cells compared to androgen-dependent LNCaP cancer cells. We, therefore, investigated the hypothesis that: 'GLI1 confers androgen-independence upon epithelial prostate cells'. Ectopic GLI1 induced a dramatic change in LNCaP cell morphology that correlated with increased expression of the basal/stem-like markers CD44, β1-integrin, ΔNp63 and Bmi-1, as well as decreased expression of the luminal markers AR and PSA. Accordingly, LNCaP-GLI1 cells were viable in the presence of the AR inhibitor bicalutamide. Global gene expression profiling revealed that LNCaP-GLI1 cells are more similar to DU145 and PC-3 cells than to LNCaP cells and identified an EMT signature (low E-cadherin, high vimentin) that was verified at the protein level. Indeed, LNCaP-GLI1 cells were highly invasive through a Matrigel substrate and they also had a higher proliferative potential when seeded at a low density. Intriguingly, however, unlike LNCaP cells neither LN-GLI1 nor DU145 cells formed colonies in soft agar or prostaspheres in suspension indicating that tumours may arise from luminal-like cells. In summary, these data provide further evidence that GLI1 promotes prostate tumour cell migration and, more significantly, that it may help confer resistance to androgen ablation therapy through the induction of a basal-like phenotype which is characteristic of some advanced tumours.

UNDERSTANDING THE EFFECTS OF CYTOTOXIC CHEMOTHERAPEUTICS ON THE INNATE IMMUNE SYSTEM.

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Infiltration of myeloid-derived inflammatory cells to tumors is associated with tumor progression because different types of myeloid cells can suppress the anti-tumor immune response and produce factors that promote tumor growth, angiogenesis, or metastatic spread.

Chemotherapeutic drugs, such as doxorubicin, frequently suppress immune cells, particularly myeloid cells, in the bone marrow; yet, their effects on myeloid cells within tumors are relatively unknown. We hypothesized that in tumors, chemotherapy alters the balance between different types of myeloid-derived immune cells, thereby changing the tumor microenvironment.

Using the polyoma middle T antigen (PyMT) mouse model of breast cancer, we identified changes in myeloid cell populations following doxorubicin treatment. To directly visualize the effects of chemotherapeutic treatment on tumor-infiltrating myeloid cells, intravital imaging was performed. PyMT mice were cross bred with those expressing enhanced cyan fluorescent protein (ECFP) in cancer cells and enhanced green fluorescent protein (EGFP) in the myeloid cell population. This enabled us to use spinning disk confocal microscopy to follow individual cells in tumors of live mice for up 40 hours. Intravital microscopy showed that myeloid cells were recruited to areas with cancer cells that were killed by doxorubicin. Immunohistochemistry identified the major infiltrating myeloid cell population as neutrophils/immature monocytes. Cytokine profiling by antibody arrays showed an upregulation of the chemokines CCL2 and CCL12 following doxorubicin treatment, suggesting a role for these molecules in the recruitment of the neutrophils/immature monocytes. Ongoing studies include flow cytometric analyses of changes in immune cell populations within the tumor following chemotherapy, and dual treatments of doxorubicin and CCL2/CCL12 antagonists to determine the importance of these chemokines in myeloid cell recruitment and host response to chemotherapy.

In conclusion, after treatment with the chemotherapeutic drug doxorubicin, the balance between different tumor infiltrating myeloid cells are pushed towards neutrophils and immature monocytes due to increased recruitment of these cells to the treated tumor. Given the many different roles of different myeloid cells on tumor progression, these findings may have clinical implication.

RAPIDLY FATAL MYELOPROLIFERATIVE DISORDERS IN MICE LACKING CBL AND CBL-B IN HEMATOPOIETIC STEM CELLS

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Cbl family E3 ubiquitin ligases are negative regulators of tyrosine kinase signaling. Recent work has revealed a critical role of Cbl in the maintenance of hematopoietic stem cell (HSC) homeostasis and mutations in CBL have been identified in myeloid malignancies. Here we show that, in contrast to Cbl- or Cbl-b- single-deficient mice, concurrent loss of Cbl and Cbl-b in HSC compartment leads to an early-onset lethal myeloproliferative disease in mice. Importantly, expression of mutant Cbl was not required for pathogenesis. Cbl, Cbl-b double-deficient bone marrow cells are hypersensitive to cytokines, and show altered biochemical response to thrombopoietin. Thus, Cbl and Cbl-b play redundant but essential roles in HSC regulation whose breakdown leads to a hematological malignancy that phenocopies crucial aspects of mutant Cbl-driven human myeloid malignancies.

DIFFERENTIAL REQUIREMENTS FOR THE CATALYTIC AND STOICHIOMETRIC ACTIVITIES OF CDK4 AND CDK6.

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The cyclin D-cdk4/6 complex has two distinct functions. Its catalytic role involves its ability to act as serine/threonine kinase, responsible for phosphorylation of substrates required for cell cycle transitions, while its sequestration function involves its ability to act as a reservoir for p27Kip1. This association sequesters p27 from cyclin E-cdk2 complexes, allowing them to remain active. We exploited the ability of TGF-β to induce p15Ink4b in order to ascertain whether the catalytic and sequestration roles of cdk4 and cdk6 were required equally during proliferation. p15 binding destroys the cyclin D/cdk4 complex, negating both the catalytic and sequestration functions and results in G1 arrest. Expression of wild type, catalytically inactive or p15-resistant cdk4 or cdk6 mutants rendered Mv1Lu cells resistant to TGF-β-mediated growth arrest. Cells expressing a catalytically inactive, p15-resistant double cdk6, but not cdk4, mutant were also resistant to TGF-β-mediated arrest, proliferating in the absence of all cdk4/6 kinase activity. Expression of this cdk6 mutant acted as a sink for p27, allowing cdk2 to remain active, and suggested that the sequestration function was sufficient in continuously proliferating cells. Interestingly, the homologous cdk4 mutant was unable to overcome TGF-β-mediated arrest. When contact arrested cells were released from quiescence in the presence of exogenous cdk4, the G0-G1 transition was accelerated in a kinase-dependent manner. In contrast, overexpression of both catalytic and non-catalytic cdk6 alleles accelerated G0 exit, suggesting that cdk6 may play a different role in the G0-G1 transition. Gel filtration analysis of cdk4 and cdk6 pools in proliferating and arrested cells revealed the presence of a previously unidentified cyclin D-cdk6 dimer. Phosphoamino acid analysis demonstrated that cdk4 and cdk6 were differentially modified in proliferating cells. Taken together, the gel filtration data and phosphoamino acid data may explain the differential activity of these kinases to promote proliferation of cells both in cycle and upon reentry from the quiescent state.

HMGB1 REGULATES A SPECIFIC TRANSCRIPTIONAL PROGRAM AND IS REQUIRED FOR TUMOR GROWTH AND METASTASIS

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High mobility group box 1 (HMGB1) is a small DNA-binding protein with roles in DNA repair and transcription. It is thought to bind DNA through its HMG-box motifs without sequence specificity and aid in distorting the DNA structure to allow access for other proteins. In addition to functions in the nucleus, HMGB1 acts as a cytokine when released from cells. Extracellular HMGB1 binds to the Receptor for Advanced Glycation End Products (RAGE) or TLR2/4 and causes downstream p42/44 MAP kinase and NFκB pathway activation. HMGB1 is overexpressed in various cancers and is correlated with poor prognosis, higher tumor grade and metastasis. Although many correlations between HMGB1 expression and cancer exist, its specific functions are unclear. We have used a stable lentiviral knockdown system in MDA-MB-231 breast cancer cells to further study the role of HMGB1 expression in cancer. HMGB1 depletion causes a change in cell morphology, decreases cell scattering and reduces F-actin foci in vitro. In a xenograft model HMGB1 knockdown tumors grow at a significantly slower rate than control tumors and show reduced angiogenesis and increased necrosis. Despite decreased growth in vivo, there is no difference in doubling time in vitro. Knockdown cells also produce about 50% fewer metastases in a colonization/metastasis assay in mice. HMGB1 depletion does not reduce p42/44 MAPK pathway activity, likely due to preexisting activating mutations in this pathway in MDA-MB-231 cells. Using a microarray, we have found 44 genes significantly down-regulated by HMGB1 shRNA and 167 genes that are up-regulated. Notably, HMGB1 knockdown decreases pro-angiogenic VEGF expression and increases the anti-angiogenic semaphorins expression, consistent with our observations of decreased angiogenesis in the absence of HMGB1. The metastasis-related protein CD24 is also down-regulated with HMGB1 knockdown. CD24 is a ligand for the endothelial cell surface protein P-selectin and can promote tumor cell adhesion and metastasis, which is in line with our finding that HMGB1 is required for colonization of tumor cells from the circulatory system. We have used our HMGB1-dependent gene signature and compared it to primary tumor array data. A set of 18 genes, including the semaphorins, is significantly correlated with HMGB1 expression in primary breast tumors, suggesting HMGB1 regulates a specific gene expression program in breast cancer.
This work shows a role for HMGB1 in tumor growth, angiogenesis and metastasis that are independent of MAPK pathway activation and defines a set of genes that are regulated by HMGB1 expression.

IDENTIFYING MEDULLOBLASTOMA AND RHABDOMYOSARCOMA SUSCEPTIBILITY GENES IN *PATCHED1* MUTANT MICE USING SLEEPING BEAUTY TRANSPOSON MUTAGENESIS SCREEN.

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Mutations in the human homologue of the *Drosophila Patched1* (*Ptc1*) gene are responsible for basal cell nevus (BCN) syndrome, and are commonly found in sporadic basal cell carcinoma (BCC), medulloblastoma (MB) and rhabdomyosarcoma (RMS). MBs are highly malignant tumors originating in the cerebellum and represent the most common pediatric brain neoplasia. RMSs are tumors of the striated skeletal muscle and are the most common pediatric soft-tissue sarcoma. Mice heterozygous for an inactivating mutation in *Ptc1* (*Ptc1*$^{+/-}$) spontaneously develop MB and RMS, and develop BCC following radiation exposure. The susceptibility of *Ptc1*$^{+/-}$ to MB or RMS is highly dependent on the genetic background of the mice. C57BL/6 (B6) *Ptc1*$^{+/-}$ mice are susceptible to MB while BALB/c (BALB) *Ptc1*$^{+/-}$ mice are susceptible to RMS.
We are using Sleeping Beauty (SB), a member of the Tc1/mariner superfamily of transposable elements isolated from salmon fish genome, for our forward genetic screen to discover cancer genes. This is a bipartite system that consists of an inducible SB transposase gene under the control of Cre-recombinase and multiple copies of a mutagenic SB transposon that is engineered to either deregulate the expression of proto-oncogenes or inactivate the expression of tumor suppressor genes. To sensitize the system for MB and RMS, we combined the SB mutagenesis system with the *Ptc1* mutation. We find that SB decreases the latency for the development of MB and RMS, increases the penetrance of the disease and the incidence of RMS in *Ptc1*$^{+/-}$ mice that is of a predominately B6 background. We are currently in the process of cloning SB integration sites to identify genes/pathways critical for the initiation and propagation of MB and RMS. By combining the SB mutagenesis system with *Ptc1* mutation, we have a powerful new model for dissecting the cancer genome of MB and RMS.

ANTAGONIST ROLES OF β-ARRESTINS IN THE MDM2-MEDIATED DEGRADATION OF G PROTEIN-COUPLED RECEPTOR KINASE 2

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G protein-coupled receptor kinase 2 (GRK2) plays a central role in the regulation of G-protein-coupled receptors (GPCR) by triggering receptor desensitization and GPCR internalization. However, this protein is also able to phosphorylate non-GPCR substrates and associate with a variety of signaling molecules, thus emerging as a relevant node in signaling pathways with unforeseen influences in basic cellular processes such as cell cycle progression or cell motility. Consistently, the expression and function of GRK2 is tightly regulated and its protein levels are often altered in pathological conditions such as inflammatory diseases and certain tumors. Related to this, our group has shown that GRK2 is rapidly degraded by the proteasome pathway in a c-Src and MAPK phosphorylation-dependent manner and that Mdm2, an E3-ubiquitin ligase involved in tumorigenesis, plays a key role in its ubiquitination and turnover. Upon GPCR challenge, the adaptor proteins β-arrestins facilitates the Mdm2 and GRK2 association, while over-stimulation of the PI3K/Akt axis prevents the Mdm2-mediated degradation of GRK2 and leads to its accumulation in several transformed cell lines, contributing to alter their migratory properties and proliferation. Interestingly, changes in the expression level of β- arrestins and in their scaffolding ability have also been described in different tumor cells and signaling contexts what could affect the stability of GRK2. Therefore, we have addressed the impact of β-arrestins as Mdm2 co-factors in the modulation of GRK2 turnover in response to different challenges. Our results indicate that in the absence of GPCR activation, β-arrestins do not perform an adaptor role for GRK2/Mdm2 association, but rather compete with GRK2 for direct Mdm2 binding. On the other hand, β-arrestin modifications as those promoted by receptor tyrosine kinase activation interfere with the GPCR-triggered recruitment of c-Src and MAPK required for phosphorylation of GRK2, thus favoring kinase stabilization. Our data suggest that disruption of the scaffolding functions of β-arrestins in Mdm2-dependent GRK2 degradation, kinase levels may lead to kinase upregulation and contribute to tumorigenesis in particular settings.

MODELING ACUTE LYMPHOBLASTIC LEUKEMIA AND ITS CHEMO-IMMUNE TREATMENT IN HUMANIZED MICE

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Traditional chemotherapy of hematologic malignancies is increasingly being supplemented with the use of monoclonal antibodies that target specific epitopes on cancer cells.
Here we generated a platform to model chemo-immune treatment of human B-cell malignancies, using the humanized mouse model. Human hematopoietic stem cells (HSCs) were isolated from cord blood, expanded in vitro, and transduced with a lentivirus in which the B-cell-specific human CD19 promoter controls the expression of cMyc and Bcl-2. Ex vivo infected HSCs were injected into sublethally irradiated NOD-SCID-IL2R$\gamma^{-/-}$ (NSG) mice. Two months following this reconstitution, mice develop an acute lymphoblastic leukemia, where malignant human pro-B cells infiltrate numerous host organs and lead to rapid weight loss, neurological symptoms and eventual death. Transplanting leukemic cells into non-irradiated (secondary) NSG mice induced a phenotypically identical disease. Notably, leukemic cells express CD52, the epitope targeted by the monoclonal antibody Alemtuzumab. Secondary NSG mice were transplanted with human pro-B ALL; 2-3 weeks later they were treated with 1-3 i.v. injections of Alemtuzumab. Treated mice showed a significant decrease in leukemic cell frequency in the spleen, liver, and peripheral blood, but not in the bone marrow or the brain. Killing of leukemic cells required Alemtuzumab to possess an intact Fc region, but was independent of complement fixation. Depleting macrophages *in vivo* prior to Alemtuzumab treatment abrogated the therapeutic response, strongly suggesting that macrophages mediate the anti-leukemic effect of Alemtuzumab, by binding and destroying antibody-coated leukemic cells. In order to overcome resistance to Alemtuzumab in the bone marrow, we initiated leukemia treatment using Cyclophosphamide, mimicking chemoimmunotherapy in clinical practice. Combining Cyclophosphamide with Alemtuzumab led to a nearly complete clearance of human leukemic cells from both the brain and the bone marrow.
Here we introduce a humanized mouse model of pro-B ALL, where leukemic cells arise *de novo* from lentivirus-infected human HSCs. This model provides the opportunity to test *in vivo* the efficacy of human-specific monoclonal antibodies against human leukemia cells. Using this model we elucidated the mechanism of its action and developed a successful synergistic chemo-immune therapy. Future experiments will address mechanisms of treatment resistance in the bone marrow using *in vivo* RNAi-screening technology.

A NOVEL FUNCTION FOR THE RETINOBLASTOMA PROTEIN IN EPITHELIAL CELL MIGRATION

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The retinoblastoma protein (pRB) is thought to act as a tumor suppressor mostly through its ability to restrain cell division. Here we report a previously unappreciated role for pRB in epithelial cell migration. We have discovered that Rb null chimeric embryos have impaired eyelid closure, a defect commonly associated with mutations in genes affecting cell motility. Indeed, by generating Rb null keratinocytes, we have found that Rb mutant cells show reduced migratory capacity, both as single cells and wounded monolayers. This defect is further highlighted by the inability of Rb-deficient skin to effectively repair wounds. What is the basis of this altered motility in Rb deficient epithelial cells? Our data suggest that deregulation of the EGF signaling pathway is the underlying cause of the tissue closure defects in Rb mutant mice and of the altered motility in Rb deficient epithelial cells. These findings open the door to the possibility that the mechanisms by which Rb acts as a tumor suppressor may extend to processes beyond the cell cycle. As cell migration represents a fundamental facet of tumorigenesis, the discovery that Rb appears to play a role in coordinating cell dissemination may offer new insight into its role in cancer.

DUAL ROLES OF BRCA2 C-TERMINAL REGION FOR TUMOR SUPPRESSOR FUNCTION OF BRCA2.

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Germ-line mutations that inactivate BRCA2 predispose to early-onset cancer. In previous studies, BRCA2 was shown to control the HR (homologous recombination) through regulating Rad51 oligomerization. The partaking in the HR makes BRCA2 absolutely required for error-free DNA repair, but other functions of BRCA2 are largely unknown. Recently, we have found that the C-terminus of BRCA2 (B2-9 fragment) interacts with a spindle assembly checkpoint protein, BubR1. This suggests for a role of BRCA2 in the regulation of accurate chromosome segregation. Moreover, the B2-9 fragment contains a motif that is required for Rad51 oligomerization. Thus, we suspected that ectopic expression of B2-9 fragment may induce an unbalanced HR and spindle assembly checkpoint function. Therefore, we generated a transgenic mouse ubiquitously expressing the C-terminus of Brca2. Indeed, the embryonic fiblroblasts from the B2-9 transgenic mice showed defects in both spindle assembly checkpoint and DNA damage response, suggestive of functional disruption of BRCA2. Furthermore, these mice developed various type of tumor including skin squamous cell carcinoma, adenocarcinoma, teratoma-like mass and leukemia at 71~109 weeks after birth. Taken together, our study reveals the dual function of Brca2 C-terminus in regulating HR and the mitotic checkpoint.

HIGH THROUGHPUT PRODUCTION OF ES CELL LINES EXPRESSING INDUCIBLE MICRORNAS AND SENSORS FOR THE PRODUCTION OF TRANSGENIC MICE.

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MicroRNAs are critical regulators that influence numerous cellular and developmental processes through their ability to control gene expression. Nearly one thousand human and more than 600 mouse microRNA sequences have been described, many of which are mis-regulated in diseases, including cancer. Despite much investigation, their roles and targets are largely unknown. Generation of animal models in which microRNA levels can be regulated *in vivo* will significantly expedite our understanding of microRNA biology. We therefore have launched an effort to generate a collection of ES cells expressing tet-regulatable microRNAs and sensors using an established Flp/*Frt* recombinase-mediated cassette exchange system that utilizes our own custom-designed Flp-in targeting vector. All mouse microRNAs known to date, and their corresponding sensors (microRNA complementary sites) have been inserted downstream of GFP in the "tet-on"-Flp-in targeting vectors, which allows for regulatable expression of microRNAs and sensors in ES cells. The microRNAs will be expressed in two different contexts; 1) in its natural context and 2) embedded in the miR30 backbone. The latter is potentially advantageous in that it bypasses the natural inhibitory pathways of microRNA biogenesis. These ES cell lines can be used to generate transgenic mice expressing regulatable microRNAs and sensors to study the role of microRNAs in cancer. This collection of ES cell lines will be made available to the community via the NCI Mouse Models of Human Cancer Consortium mouse repository. We anticipate that this resource will serve as a powerful tool for the research community at large.

TUMOR SUPPRESSOR FUNCTION OF LKB1 IS LINKED TO THE CONTROL OF POLARIZED EPITHELIAL ARCHITECTURE

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Cellular organization into epithelial architecture maintains structural integrity and homeostasis by suppressing cell proliferation and apoptosis. We have previously shown that differentiated epithelial organization of MCF10A 3D acini blocks the ability of c-Myc to re-initiate cell cycle progression and induce transformation. This epithelial architecture imposed restraint to the oncogenic c-Myc is dismantled upon silencing of LKB1, the mammalian homologue of polarity protein PAR4 (Partanen et al., PNAS 2007). Here we have explored the role of LKB1 in formation and maintenance of epithelial architecture in vivo using mice, in which Lkb1 alleles can be conditionally deleted in the luminal epithelial cells of mammary gland. We show that loss of Lkb1 induces gross alterations in the epithelial architecture of 3D structures and in the mammary gland, manifested by loss of cell polarity, defects in the basement membrane structure and spontaneous hyperbranching. Loss-of Lkb1, when combined with mammary specific overexpression of c-Myc, led to dramatic acceleration of c-Myc induced mammary tumorigenesis. These mice exhibit increased tumor multiplicity and volume, and in addition the tumors show histopathological features distinct from Myc-induced mammary tumors. Furthermore, the cooperation of Lkb1 loss and c-Myc activation resulted in strikingly transformed 3D acini, which allows studies on molecular mechanisms underlying the synergy. In conclusion, our data show evidence that Lkb1 has a crucial role in formation of epithelial architecture and apicobasal polarity and that loss of Lkb1 significantly cooperates with activated c-Myc oncogene in tumorigenesis.

TUMOR SUPPRESSOR CHD5 REGULATES GENE EXPRESSION BY RECOGNITION OF UNMODIFIED H3K4

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Deletions in the short arm of human chromosome 1 (*1p36*) are frequently observed in a variety of human cancers. Our laboratory identified *Chromodomain Helicase DNA-binding domain 5* (*CHD5*) as a novel tumor suppressor mapping to 1p36. Recent studies show that CHD5 is hypermethylated or mutated in several forms of human cancer. As a member of the CHD family of SWI/SNF-like ATP dependent-chromatin remodeling proteins, CHD5 has been proposed to regulate chromatin dynamics.

Eukaryotic transcription is dynamically regulated by different posttranslational modifications of histone tails, which are identified by distinct sets of protein complexes. Here we show that Chd5 recognizes unmethylated H3K4 through its predicted tandem plant homeodomains (PHDs). We identified specific amino acids within this domain that are essential for mediating H3K4me0 binding. Mutation of these residues abolishes Chd5 function. Overexpressing or reducing the level of Chd5 identifies its role in both activating and repressing target gene expression. Mutations in PHD fingers that abrogate H3 binding fail to modulate Chd5-target gene expression. These findings reveal a critical role of histone interaction by the tandem PHD modules in mediating Chd5's cellular function. This study further expands our view on CHD5's tumor suppressive mechanism that may help to design novel anti-cancer strategies.

UNRAVELING NOVEL P53-INDEPENDENT MECHANISMS LEADING TO HIGH PENETRANCE LYMPHOMAGENESIS IN EM-V-CYCLIN TRANSGENIC MICE

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Kaposi`s sarcoma herpesvirus (KSHV) is a human tumor virus and the causative agent of three human malignancies: Kaposi sarcoma (KS), primary effusion lymphoma (PEL), and multicentric Castleman's disease. KSHV latent protein v-cyclin is a cellular cyclin D2 homolog reported to promote G1-S transition in the cell cycle, induce DNA damage and have oncogenic potential. To assess the oncogenic potential of v-cyclin in vivo we have targeted v-cyclin expression to the B and T lymphocyte compartments using the Eμ-promoter/enhancer. Expression of v-cyclin in the CD1/ICR genetic background leads to markedly accelerated tumorigenesis, as the mice develop undifferentiated T-cell lymphomas even as early as 1 month of age with a disease penetrance over 90%. The onset of tumorigenesis is p53-independent, as the mice retain wildtype *TP53* and *INK4a*. Intriguingly, the pre-tumorigenic thymi and spleens had a decreased cellularity in the v-cyclin transgenic mice, although the number of proliferating cells was similar or even higher as in the non-transgenic littermates, and apoptosis was not increased. Preliminary studies on the differentiation status of the T lymphocytes suggest alterations in the v-cyclin transgenic mice over the non-transgenic littermates. We are currently addressing the mechanisms and pathways leading to this high-penetrance tumorigenesis and altered differentiation of the T lymphocytes by genome-wide gene expression analysis combined with functional assays.

EPIGENETIC ALTERATIONS OF SELECTED HISTONE H3 MODIFICATIONS/EXPRESSION OF THEIR MODIFIERS IS ASSOCIATED WITH EPITHELIAL PLASTICITY DURING EPITHELIAL-MESENCHYMAL TRANSITION (EMT)

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During cancer progression epigenetic events, like alteration of histone modification markers, co-exist with genetic events and affect cell properties line cell migration and invasion. We analysed the role of global histone modifications, how this modifications may be affected by pathways activated by oncogenes and their association with epithelial plasticity.
We generated oncogene-transformed colon cell lines by RASV12, BRAFV600E oncoproteins. Notably, the phenotype of the H-RASV12 oncoprotein-transformed cells (Caco-H) is associated with Epithelial-Mesenchymal Transition (EMT) characteristics (3). We have shown that E-cadherin is regulated by TAF12 transcription factors (4).
A global histone modification analysis revealed a general de-regulation of histone modification markers, in particular H3K27me3 by H-RAS. The dependence of histone modification marker by MER-ERK signalling pathways (5). Variations of methyl- and acetyl-transferase enzymes as EZH2, JMJD3, PCAF GNC5 and HDACs are associated with appearance of aggressive tumour properties. Evidence will be presented for the role of particular histone methyl- and acetyltransferases in cell processes associated to Epithelial-Mesenchymal Transition (EMT) . In order to elucidate the mechanisms, ChIP analysis has been used to identify their target genes in EMT. Interestingly, Cyclin D1 and E-cadherin genes demonstrate inverse histone repression patterns on their promoter, associated to their inverse expression levels.
This study provides details on a) Cyclin D1 and E-cadherin promoters are regulated by histone modifications in a RAS-dependent manner. b) EMT associated E-cadherin expression correlates with existence of H3 histone methylation markers on the promoter c) global histone modification changes and/or their histone modifiers can be proven reliable tumour markers.

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DIFFERENTIAL EFFECT OF BRAF AND RAS ONCOGENES THROUGH PI3K AND MEK PATHWAYS ON RHO-FAMILY REGULATION OF CELL MIGRATION AND INVASION PROPERTIES

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During colorectal cancer progression BRAF and KRAS oncogenes are often mutated in about 15% and 35% respectively, but the two mutants rarely coexist in the same tumour. Unlike BRAF and KRAS, mutations in RHO genes are extremely rare in tumours, but they contribute with distinct roles in invasion and metastasis. The aim of this study is to compare and define the effect of BRAFV600E, KRASG12V and HRASG12V oncoproteins in cell migration and invasion pathways mediated by specific members of the Rho GTPase family. For that, RAS and BRAF oncogenes were either stably transfected or silenced in colon cancer cells (1, 2, 3) and analysis of Rho GTPase pathways was performed.
Evidence presented here indicate that BRAFV600E provides colon cancer cells with high migration and invasion properties in vitro, through activation of RhoA. BRAFV600E regulation of RhoA activity is mediated by MEK-ERK pathway. Cross-talk analysis revealed that RhoA may have an antagonistic function with Cdc42 in Caco-BR cells. KRASG12V works towards enhancing the ability of colon adenocarcinoma cells Caco-K to migrate and invade through filopodia formation and PI3K-dependent Cdc42 activation. Interestingly, in Caco-K cells Cdc42 mediates expression of Rac1, as shown by siRNA approach. HRASG12V induces a well differentiated mesenchymal morphology, through the Epithelial to Mesenchymal Transition (EMT) process, associated with Rac1 activity. RhoA activity is suppressed in Caco-H cells through activation of PI3K pathway. Moreover, BRAF and KRAS oncogenes cooperate with TGFβ-1 pathway to provide cells with additional transforming properties. This study discriminates oncogene-specific cell migration and invasion pathways mediated by Rho GTPases and by their regulatory GAPs , GEFs and GDIs in colon cancer cells and reveals potential new oncogene-specific characteristics for targeted therapeutics.

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ONCOGENES OF RAS PATHWAY CAN SENSITISE COLORECTAL TUMOURS TO TRAIL INDUCED APOPTOSIS: FROM CELL AND ANIMAL MODELS TO THE CLINIC

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Most data on the therapeutic potential and expression of TRAIL in colorectal cancer have shown increased sensitisation of tumour cells as compared to premalignant or physiological cells or tissues. Thus, particular oncogenes like MYC and RAS can be sensitisers for TRAIL induced apoptosis. We have previously shown that RAS oncogenes can sensitise colon cells to TRAIL induced apoptosis (1). We have presented evidence in cell models that this effect is usually mediated by TRAIL receptor DR4 and DR5 overexpression and/or redistribution (2).
In this study, colorectal cell lines bearing RAS and BRAF mutant oncogenes or colorectal clinical samples were either treated with recombinant TRAIL or analysed for the presence of RAS and BRAF oncogenic mutations and DR4, DR5 expression. We present evidence that BRAF oncogenes can sensitise colon cancer cells to TRAIL induced apoptosis via TRAIL receptor DR5. The underline mechanisms for this sensitization involve MAPK and PI3K pathways, depending on the genetic alteration profile of the individual tumour . We have also shown that DR5 is the most frequently upregulated DR in clinical samples of colon cancer. Furthermore, the presence of K-RAS and BRAF mutations in the tumour may directly or indirectly enhance DR expression (3). Mutations on K-RAS and BRAF oncogenes have been shown in many studies to be associated with resistance to EGFR targeted therapeutics and combinations. TRAIL-based therapeutics, other as mono- or combination therapy could provide a promising alternative for K-RAS/BRAF bearing colorectal tumours. The implications of these findings on personalized therapeutics will be discussed.

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TARGETING BONE MARROW MEDIATED TUMOR ANGIOGENESIS AS A NEXT GENERATION CANCER THERAPY.

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Targeting the process of tumor vascularization is a key aspect of modern anticancer therapy. However, the cellular and molecular mechanisms by which tumors enroll vasculature remains relatively undefined. Furthermore, the role of host inflammatory cells, and bone marrow derived (BMD) cells in this process remains controversial. BMD endothelial progenitor cells (EPCs) have been identified by our lab and others as being critical for the angiogenic switch[1,2]. However, because different markers are used to track these cells in the bone marrow, blood and tumour-stroma, controversy exists as to whether the same cell is truly being followed *in vivo*. We have developed a series of lentiviral based gene reporters which can be used to dynamically track EPCs *in vivo*; and shown that EPCs form a distinct lineage which can be tracked from the BM to their incorporation as part of tumour vasculature. Follow-up work using transgenic mice has confirmed that the Inhibitor of DNA Binding 1 (Id1) is a marker of EPCs; and plays an important role in their function[2]. Finally, these same tools have been used to identify the molecular mechanisms which might underlie tumour-EPC biology *in vivo*; including the role and identity of putative EPC-linked and endothelial microRNAs.

THE SPY1/RINGO FAMILY PLAYS A KEY ROLE IN THE REGULATION OF MAMMARY GROWTH AND TUMORIGENESIS

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Spy1A is a unique cell cycle activator known to mediate cell cycle progression and override the DNA damage response. This work focused on determining the role of this protein on postnatal mammary gland morphogenesis and neoplasia. We have demonstrated that Spy1A levels are tightly regulated during mammary gland development and that ectopic expression stimulates precocious development and results in disrupted morphology of the gland. Importantly, we demonstrate that overexpression of Spy1A accelerates tumorigenesis in vivo and that elevated protein levels are implicated in a number of human cancers, including that of the breast. We have begun to dissect the molecular mediators regulating Spy1 expression and Spy1-mediated effects during mammary development and tumorigenesis. Collectively our work is the first to determine that the Spy1/RINGO family of proteins may play an essential role in regulating both normal and abnormal growth processes in the breast.

DEVELOPMENT AND USE OF CHIMERIC LUNG CANCER MODELS IN CANCER DRUG DISCOVERY

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Mouse models of cancer have led to important insights, aided mechanistic understanding of tumorigenesis as well as guided therapeutic development. To facilitate experimental use of genetically engineered mouse models while recapitulating the stochastic nature of human cancer, we developed a mouse tumor model strategy involving stepwise genetic manipulation of embryonic stem (ES) cells and chimera formation to enable tumor induction in tissues containing both normal and engineered cells. An allelic series of lung cancer models containing HER2, KRAS, or EGFR oncogenes demonstrated that resultant
adenocarcinomas arising within normal lung tissue exhibited features of advanced malignancies.

An experimental therapeutic trial performed on the EGFRL858R and KRASG12V chimeric models with an EGFR inhibitor accurately reflected clinical observations with the ERBB-driven tumors responding well to treatment and the KRAS-driven tumors exhibiting resistance. On the molecular level, transcriptome and immunohistochemical analyses revealed that these tumors featured differences in downstream pathway activation: namely, PI3K pathway activation was unique to ERBB-driven tumors while KRAS- driven tumors showed activation of JNK pathways. The latter suggests a novel therapeutic point of intervention in this important and therapeutically non-responsive tumor category. In addition, we tested the response of KRAS- and EGFR-driven lung tumors to tivozanib, a potent, selective VEGFR inhibitor, currently in pivotal trials in Kidney cancer and in earlier stage studies in NSCLC. Uniform regression of these tumors was observed resulting in a significant survival benefit to the tumor bearing mice in both models. With these combined findings, we have demonstrated that our chimeric models not only provide insight into key pathway activation mechanisms of cancer biology, but can also aid in targeted drug efficacy studies, potentially leading to improved patient therapies.

A FLEXIBLE TECHNOLOGY FOR RAPID POOL BASED SHRNA SCREENING IDENTIFIES MODULATORS OF *IN VIVO* THERAPEUTIC RESPONSE

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High throughput chemical screens identify daunting numbers of cytotoxic compounds. However, a major bottleneck in drug development is the systematic description of how these cytotoxic compounds exert their effects *in vitro* and *in vivo*. While high throughput microarray and pool-based RNA interference-based approaches can be used to examine drug mechanism, their scale limits broad dissemination. Specifically, there is an unmet need for a measurement system that is capable of examining the function of an entire gene family in a broad array of physiologic contexts and drug conditions. We present a flexible Luminex based assay with which we systematically interrogate how the entire BCL-2 family modulates therapeutic response *in vitro* and *in vivo*. Using this approach, we perform the first *in vivo* screen for modulators of therapeutic response. Notably, we find that the *in vivo* suppression of components of the extrinsic death pathway can promote resistance to front-line genotoxic chemotherapy.

THE HUMAN DEK ONCOGENE STIMULATES EMT, INVASION AND STEMNESS IN BREAST CANCER CELLS

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Breast cancer is a major cause of cancer-related deaths in American women; therefore, the characterization of novel breast-cancer related molecules for the discovery of new markers and drug targets remains essential. The human DEK gene encodes a chromatin-binding protein with specific roles in the regulation of DNA topology. Using classical transformation assays and knockout mice, we have recently identified DEK as a bona fide oncogene in keratinocytes, but the role of DEK in breast cancer is unknown. We demonstrate here that DEK is highly expressed in breast cancer cell lines compared to immortalized cells and normal tissue. DEK over-expression in non-tumorigenic MCF10A mammary epithelial cells resulted in increased growth rates and motility with evidence of an epithelial-to-mesenchymal transition (EMT). Conversely, DEK knockdown in tumorigenic MCF7 and MDA-MB-468 breast cancer cells resulted in decreased cell proliferation, survival and migration. The use of DEK-proficient and -deficient breast cancer cells in an orthotopic xenograft model further suggested that DEK contributes to tumor formation and metastatic growth *in vivo*. Molecular analyses for proteins commonly deregulated in breast cancers revealed that DEK positively regulated ΔNp63 expression and β-catenin signaling, pathways which also are important for the growth of normal and cancer stem cells. To determine whether DEK might regulate cancer stem cell formation or maintenance, we subjected DEK knockdown and overexpressing breast cancer cells to mammosphere assays. The results suggest that DEK increases mammosphere formation. Together, our data indicate that DEK expression stimulates the growth, motility and stem cell character of breast cancer cells *in vitro* and *in vivo*.

TARGETING STRESS RESPONSE TO ROS FOR SELECTIVE KILLING OF CANCER CELLS

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Inducing cancer cell death with DNA-damaging chemotherapeutic agents is the most widely used strategy in cancer therapy. However, DNA-damaging drugs kill both normal and cancerous cells, which severely limits current treatment regimens. A high-throughput, cell-based screen for small molecules that activate transcription of a p53-regulated target gene in cancer cells uncovered a remarkable naturally occurring small molecule, piperlongumine (PL). PL selectively induces apoptosis in cancer cells and tumor explants regardless of p53 status while leaving normal cells and tissues unaffected. A quantitative proteomic method for target identification and mechanistic studies revealed that PL induces reactive oxygen species (ROS) in cancer cells but not normal cells by inhibiting proteins involved in ROS metabolism. In addition, PL protected normal cells from chemo-drug-mediated DNA damage response. In vivo experiments demonstrate potent anti-tumor activities of PL at low concentrations with no apparent adverse effects on normal organ and tissue function. PL-induced selective killing of cancer cells supports the theory that cancers, including their cancer stem cells in the case of ROS, have increased dependence on stress-response pathways. By targeting ROS, PL constitutes a novel strategy for cancer therapy that preferentially eradicates cancer cells.

P63 IS REQUIRED FOR MAINTENANCE OF SQUAMOUS CELL CARCINOMA *IN VIVO*

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Squamous Cell Carcinoma (SCC) is a common form of human cancer that can arise in various epithelial tissues such as the lungs, skin, and head and neck region. Morbidity and mortality rates for SCC are high compared to many other cancers, and relatively little progress has been made in treatment due to a lack of molecular understanding of the disease. The p53-related protein p63 is essential for the formation of various epithelial tissues, such as the skin, and has many reported roles including suppression of apoptosis, maintenance of stem cell regenerative potential, regulation of differentiation, and maintenance of cellular adhesion. In SCC, p63 is over-expressed and has found to be the target of genomic amplification suggesting an important role in this disease. Previous *in vitro* studies have shown that p63 is required for survival of SCC cells in culture, however, cell lines lack the complex microenvironment found in human cancer, and thus the i*n vivo* relevance of these results is unclear. To determine the role of p63 *in vivo*, we have generated a murine model of SCC, which faithfully recapitulates many of the molecular and pathological features of human SCC including invasion and metastasis. To test the role of p63 in tumor maintenance we crossed this model to a conditional p63-null allele ($p63^{flox}$) and a tamoxifen-inducible Cre transgene, *K14-CreER*. We have found that genetic excision of both copies of *p63* in established SCC tumors results in rapid and dramatic regression of tumors. In addition, even loss of a single copy of the *p63* gene results in a reduced rate of growth, suggesting that SCC tumors are exquisitely sensitive to levels of p63 protein. These results support the idea that the p63 pathway is critical for the survival of SCC tumors *in vivo*, and targeting this pathway in SCC may result in more effective therapy for this highly refractory tumor type.

INHIBITION OF PRO-SURVIVAL PROTEINS IN MLL FUSION DRIVEN ACUTE MYELOID LEUKEMIA

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The pro-survival Bcl2 family members Bcl2 and Mcl1 are currently being evaluated as drug targets in many types of cancers. Overexpression of either Bcl2 or Mcl1 is commonly found in acute myeloid leukemia (AML) and mediates resistance to therapeutics. We previously developed mouse models of human leukemia and showed that those expressing MLL fusion proteins, like their human counterparts, were refractory to conventional chemotherapy. In order to determine the effect of Bcl2 and Mcl1 inhibition on established MLL/AF9 driven AML, we developed a system for inducible *in vivo* RNAi in mosaic mouse models. Tet-regulatable shRNAs targeting Mcl1, Bcl2, or neutral control genes were transduced into TetOn-competent MLL/AF9-AML cells, which were subsequently transplanted into recipient mice. Following disease onset, mice were treated with doxycycline to turn on shRNA expression. RNAi-mediated suppression of either Mcl1 or Bcl2 led to rapid depletion of leukemic blasts *in vivo*. Histology indicated clearance of leukemia cells from infiltrated tissues, leading to a substantial survival benefit, though mice eventually succumbed to the disease. To determine whether these effects were leukemia specific, we tested the same shRNAs for effects on normal hematopoiesis. Remarkably, suppression of Mcl1 completely abolished reconstitution of the hematopoietic compartment, while Bcl2 suppression was well tolerated in all lineages. Nonetheless, leukemia bearing mice did not respond to the available inhibitors of Bcl2 (ABT-737, Obatoclax), indicating either that they are not sufficiently potent or that RNAi-mediated inhibition affects distinct activities of Bcl2 that are not targeted by these agents. In summary, we demonstrate that MLL/AF9 driven AML is sensitive to RNAi-mediated inhibition of either Bcl2 or Mcl1. While Mcl1 inhibition is likely toxic in normal hematopoietic cells, Bcl2 may be an effective target in MLL rearranged AML, although more effective inhibitors are needed.

COMPARATIVE COMPOUND SCREENING OF TUMOR SPHERES AND ADHERENT CULTURES FROM MELANOMA BRAIN METASTASES

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Malignant Melanoma is a highly aggressive and therapy-resistant cancer with a high affinity to disseminate cells into the brain. Patients who suffer from CNS metastasis are confronted with a poor prognosis and conventional therapy brings only little benefit (e.g. surgery, radio- and chemotherapy result in <13% 1-year survival rate). It remains yet unclear what cellular mechanisms or what cellular identities can be made responsible for the remarkable therapy resistance. Recent studies exposed tumor cell heterogeneity and the identification of tumor initiating subpopulations as crucial elements for the development of new therapeutic approaches. It was shown that primary cell cultures maintained under “stem cell conditions” could recapitulate cellular heterogeneity in vitro and in vivo. These conditions generally imply the use of defined serum-free media and a continuous supply of growth factors for the maintenance of cells in vitro. It would be reasonable to assume that these methods are ideally suited for the application of high throughput drug screening assays. Here, we present data acquired from a primary compound screen using the Killer Collection® library that encompasses 160 cellular and biochemical toxins. Three samples of melanoma brain metastases were investigated in adhesive vs. anchorage-independent settings. We found an intriguing dissemination of identified hits in our experiments that could not be explained based on sample variance alone. Our data rather suggest that the influences of cell-cell and cell-matrix interactions on the readout of primary drug screening assays are currently underestimated.

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SURVIVAL PATHWAYS OF HIGH-RISK NEUROBLASTOMA IDENTIFIED BY FUNCTIONAL GENOMICS.

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Neuroblastoma (NB) is the second most common extracranial pediatric tumor and the most common pediatric solid tumor. High-risk NB is a molecularly heterogeneous disease with amplification of the MYCN oncogene detected in about one third of high-risk cases. Despite intensive therapy, survival of patients with high-risk disease is less than 40%. A parallel high throughput siRNA screen was performed in high-risk neuroblastoma cell lines with and without MYCN amplification to identify critical survival pathways and therapeutic targets for high-risk NB. The screen included a set of 43 genes previously implicated in neuroblastoma pathogenesis and a collection of siRNA targeting the entire human kinome (~750 genes). Network pathway analysis was utilized to integrate the gene "Hits" from the screen with available microarray data (Oncogenomics website link: http://pob.abcc.ncifcrf.gov/cgi-bin/JK). The screen highlighted differential sensitivities between MYCN amplified and non-amplified lines to inhibition of key players of WNT and NGF signaling, as well as to inhibition of mitotic kinases, such as STK6 (Aurora A), NEK2, NEK4 and WEE1. However, additional genes, not previously implicated in NB were also revealed. A complete list of "hits" will be presented and linked to both known and novel NB pathways, which could constitute potential therapeutic targets. This study identified druggable genes and pathways required for proliferation of high risk NB cells in vitro. In vivo validation of these genes through stable RNAi or using available small molecule inhibitors, will provide evidence for future therapeutic target development. Furthermore, our study indicates the importance of combining functional genomics with gene expression data to select within the patient population who could benefit the most from specific biologic therapies.

AN *IN VIVO* RNAI SCREEN TO INVESTIGATE MECHANISMS OF CHEMOTHERAPEUTIC RESPONSE IN B-CELL LYMPHOMA.

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Chemotherapy resistance is a major obstacle in the treatment of cancer. Investigating the mechanisms of how tumors evade or succumb to chemotherapeutics can help researchers design more effective therapies and improve customized regimens for patients with specific tumor genotypes. One method of identifying genes that modulate chemotherapy response is by using loss of function genetic approaches. For example, RNA interference (RNAi) screens have been performed in cell culture to identify pathways that impact the response of cancer cells to chemotherapeutics. While these cell culture (or *in vitro*) screens can be informative, they fail to model important aspects of tumor biology due to the absence of many relevant factors found in animals. For this reason, we used an *in vivo* screening approach to identify modulators of response to vincristine (VCR), a frontline chemotherapeutic indicated in the treatment of several types of cancer. By performing *in vivo* and *in vitro* screens in parallel, we were able to identify genes that mediate chemotherapeutic response specifically in the *in vivo* setting.

AEBP1 MEDIATES STROMAL-EPITHELIAL CROSSTALK IN THE REGULATION OF MAMMARY GLAND DEVELOPMENT AND TUMORIGENESIS

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A complex interplay between epithelial and stromal compartments of mammary tissue is critical in the control of mammary gland development. Disruption of the mammary epithelium and stroma communication leads to aberrant mammary gland development and can induce mammary tumorigenesis. Macrophages are an important stromal component and NF-κB has been implicated in these processes. Here we show rescuing the mammary gland defect by transplanting wild-type bone marrow into $AEBP1^{-/-}$ mice that exhibit reduced NF-κB activity, whereas transplanting $AEBP1^{TG}$ bone marrow into non-transgenic mice result in alveolar hyperplasia with up-regulation of NF-κB activity and increased TNFα expression in mammary gland as displayed by $AEBP1^{TG}$ mice that over-express macrophage AEBP1. We further show that stromal macrophage-supplied TNFα is critical for promoting survival signal, Akt activation, and NF-κB activation in mammary epithelium. Our results presented here show that macrophage AEBP1 is a critical stromal factor that mediates stromal-epithelial crosstalk in signaling physiological apoptosis and proliferation of mammary epithelium through its positive regulation of NF-κB in macrophages that is constitutively activated in many tumors.

A POOLED SHRNA SCREEN FOR MODIFIERS OF ERLOTINIB RESISTANCE IN NON-SMALL LUNG CANCER.

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Targeted therapeutics are the next wave of chemotherapy drugs. On the forefront of designed therapies, the orally available, small molecule tyrosine kinase inhibitors (TKIS) such as erlotinib (OSI Pharmaceuticals, Roche) have already been FDA approved and have been in use in clinical use for years. Unfortunately only a small percentage of patients respond to erlotinib, and of those who respond, resistance often arises quickly, sometimes on the order of just a few months. While we have begun to understand the role of mutations in EGFR, which may underlie susceptibility to erlotinib, we still know very little about the downstream signaling changes that facilitate differential sensitivity. To further investigate downstream signaling and to identify ways in which we can increase susceptibility, we performed an shRNA screen in PC9, a non-small cell lung cancer cell line, known to harbor an EGFR amplification and an EGFR mutation. This cell line is exquisitely sensitive to erlotinib, with an IC20 of ~20nM. The shRNA library contained roughly 10,000 shRNAs, covering approximately 3,000 genes, or ~10% of the genome. 23 candidates were selected for follow up analysis, two of which validate to date. The top candidates cause a downward shift in the IC20 of cells stably infected with shRNAs. When compared to parental cell lines, shRNA expressing cells are at a competitive disadvantage, dropping out of the population when treated with erlotinib. Work on identifying downstream alterations affecting sensitivity is still ongoing in lab. We are also working to expand these findings to multiple cell lines.

RAS/RAF/ERK SIGNALLING AND SCHWANN CELL DEDIFFERENTIATION

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Schwann cells, the glia of the peripheral nervous system, retain the capacity for self-renewal throughout the lifespan of an organism. Following damage to peripheral nerves mature, specialised Schwann cells dedifferentiate and re-enter the cell cycle giving rise to a pool of progenitor like cells with the potential to re-differentiate. This dynamic behaviour is critical to the success of peripheral nervous system repair but must be tightly regulated to avoid both tissue degeneration and aberrant proliferation. Work from our laboratory has demonstrated a role for the Ras/Raf/ERK signaling pathway in controlling Schwann cell plasticity. The molecular mechanism controlling this switch in cell state is however, unknown. Here we show that Raf/ERK signaling drives Schwann cell dedifferentiation via the direct and potent repression of myelin gene promoters. This effect is dominant over the activity of the pro-differentiative transcription factor, Krox-20 and results in the rapid downregulation of myelin-associated genes. The effects of Ras signalling on Schwann cell behaviour is of particular interest since hyperactive Ras is known to be causal to the formation of Schwann cell-derived tumours in patients with the cancer predisposition syndrome Neurofibromatosis Type I. Identification of the molecular mechanisms controlling Schwann cell dedifferentiation could therefore provide important insights into the development of neurofibromas and may provide novel therapeutic targets for the treatment of this disease.

MYC'S REGULATION OF MRNA STABILITY IS CRITICAL FOR TUMORIGENESIS

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Myc oncoproteins are master transcription factors that coordinate cell metabolism, growth and division, and that trigger apoptosis during tumorigenesis. Myc binds to target genes harboring preferred E-boxes to induce their expression, but this response explains only a fraction of the biological responses controlled by Myc. Regulation of mRNA stability controls the expression of many genes, and one mechanism involves AU-binding proteins (AUBPs), which generally bind to AU-rich elements (AREs) found within the 3' untranslated regions (3' UTRs) of ~10% of all mammalian mRNAs. ARE-containing mRNAs have short half-lives and contribute to a variety of biological processes including cell growth, the immune response, differentiation, and apoptosis. In human Burkitt lymphoma (BL) and Eμ-*Myc* transgenic mice, a model of BL, expression profiling revealed that Myc regulates hundreds of ARE-containing genes. Notably, the expression of several AUBPs is also altered by Myc, including the RNA-destabilizing AUBP Tristetraprolin (TTP), which is directly suppressed by Myc. To test the contribution of the Myc-to-TTP pathway in lymphomagenesis we generated Eμ-*TTP* transgenic mice, and crossed these to Eμ-*Myc* transgenics. Strikingly, restoration of TTP expression in Myc-expressing B cells significantly delays lymphoma development; thus, TTP is a novel tumor suppressor. Finally, analyses of these mice revealed that the Myc-to-TTP pathway regulates a very select cast of ARE-containing genes having known roles in proliferation, apoptosis and transformation. These findings support a model whereby Myc targets ARE-containing genes that control cell fate and transformation via the agency of AUBPs, which are revealed as a new cast of tumor suppressors and oncogenes.

THE DNA DAMAGE RESPONSE CONTRIBUTES TO P53-DEPENDENT SENESCENCE IN RESPONSE TO CYCLIN D1 TRANSGENIC EXPRESSION *IN-VIVO*

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Transgenic expression of Cyclin D1 in the mouse pineal gland results in pineal hyperplasia, limited by p53-dependent senescence. We set out to dissect the contribution of the p53 pathway to the initiation and maintenance of senescence in this *in-vivo* model.
We found that p53 was activated at 10 days of age in response to Cyclin D1 in the mouse pineal cells, at which time proliferation was still ongoing. Investigation of signaling upstream of p53 showed that p19Arf was dispensable. However, the DNA damage response (DDR) was activated at 10 days of age, temporally correlating with activation of the p53 tumor suppressor pathway. At that time, the cells acquired phospho-H2AX foci, and showed expression of phosphorylated Chk1 and p53. In the absence of *p53*, the DDR was activated but ineffective, resulting in continued cell proliferation and tumor formation.
Despite activation of the DDR and p53 pathway, apoptosis was only slightly increased at 10 days of age; however cellular proliferation slowly ceased over the next 2 weeks, and senescence set in over a period of 3-4 weeks, with chromatin condensation in senescence-associated heterochromatin foci (SAHF). Interestingly, the DNA damage foci resolved by time of senescence, suggesting either completed damage repair, or hiding of damaged genomic sequences within the SAHF.
To dissect the pathways linking the DDR pathway to senescence in this model, we have succeeded in growing primary cultures of murine pineal cells explanted at a pre-senescent stage. We found that the senescence response to Cyclin D1 in these neuronal cells can be recapitulated in culture, and that the DDR is activated in cultured cells as well. This *in-vitro* system will now allow us to investigate mechanistic details of the p53-dependent senescence response to Cyclin D1 signaling, including the specific roles played by members of the DDR pathway.

CDK4 IS UNIQUE IN ALLOWING NORMAL CENTROSOME DUPLICATION, AND IS REDUNDANT IN MEDIATING CENTROSOME AMPLIFICATION IN *P53*-NULL MEFS.

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The centrosome maintains euploidy by directing the formation of a bipolar mitotic spindle, leading to the equal segregation of sister chromatids during mitosis. To maintain a normal content of one or two centrosomes, the centrosome duplication cycle must be tightly coordinated with the cell cycle. Aberrant centrosome duplication results in centrosome amplification (CA), attachments of spindle fibers to misaligned chromosomes (merotelic attachments), aneuploidy and chromosome instability (CIN). CA and aneuploidy are proposed to contribute to the genesis and progression of most cancers, since most human cancers display elevated frequencies of those abnormal phenotypes. Nonetheless, the cell cycle molecules directly mediating CA in cancers are unknown. Even though biochemical evidence showed that Cyclin E/A/Cdk2 coordinates the centrosome duplication cycle with the cell cycle, a genetic approach showed that *Cyclins E1*$^{-/-}$*E2*$^{-/-}$ or *Cdk2*$^{-/-}$ mouse embryonic fibroblasts (MEFs) harbored normal centrosomes. This finding suggested that another G_1 Cdk -perhaps Cdk4- individually or cooperatively regulates the centrosome cycle. We showed that while ablation of *Cdk2* or *Cdk4* results in minor defects in entry into S phase, only *Cdk4*$^{-/-}$ MEFs display major defects in the centrosome duplication cycle. On the other hand, de-regulated cyclinE/A/Cdk2 or cyclinD/Cdk4 activities correlate with CA and CIN. p53 has been postulated to signal centrosome amplification through $p21^{CIP1}$, a regulator of Cdk2 activity. However, this was based on correlative, and not genetic evidence. We find that Cdk2 or Cdk4 mediate CA and CIN in *p53*-null cells. Ablated *Cdk2* or *Cdk4* prevents centrosome amplification by restricting excessive licensing and centriole duplication. Ablated *Cdk2* or *Cdk4* restricts excessive licensing of the centrosome cycle by preventing hyper-phosphorylation of NPM, a major negative regulator of the centrosome cycle. Indeed, introduction of an NPM mutant lacking Cdk2 and Cdk4 phosphorylation sites also prevents CA and CIN in *p53*-null MEFs. Our findings demonstrate that Cdk2 and Cdk4 are unique in their ability to regulate centrosome duplication, but redundant in mediating CA and CIN. We propose a model where Cdk2 and Cdk4 converge in centrosome regulatory targets to signal CA.

SWI/SNF CHROMATIN REMODELING ENZYMES: EPIGENETIC MODULATORS IN MELANOMA INVASIVENESS AND SURVIVAL

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Metastatic melanoma is an extremely aggressive disease that is resistant to current therapy and has a poor prognosis. The progression of melanoma involves changes in gene expression that promote metastasis to distant organs. Transcriptional activation often requires epigenetic changes that convert repressive chromatin structure to a permissive conformation. Understanding the epigenetic mechanisms involved in the deregulation of gene expression during metastasis is important for developing an effective strategy to treat melanoma. SWI/SNF enzymes are multi-subunit complexes that include BRG1 or BRM ATPase subunit and alter chromatin structure and regulate gene expression. Several SWI/SNF subunits have been shown to be aberrantly expressed in a number of human cancers. We previously demonstrated that heterogeneous SWI/SNF complexes containing either BRG1 or BRM are epigenetic modulators that regulate important aspects of the melanoma phenotype and are required for melanoma tumorigenicity in vitro.

Our new studies indicate that stage IV melanoma tumors express significantly higher levels of BRG1 compared to normal melanocytes and melanoma in less advanced stages. To determine the role of BRG1 in melanoma metastasis, we over-expressed BRG1 in an established melanoma cell line that lacks BRG1 expression. We found that BRG1 dramatically altered expression of a number of adhesion proteins and extracellular matrix remodeling enzymes. BRG1 altered melanoma adhesion to different extracellular matrix components. Expression of BRG1 in melanoma cells that lack BRG1 increased invasive ability while down-regulation of BRG1 inhibited invasive ability in vitro. Activation of metalloproteinase (MMP) 2 expression greatly contributed to the BRG1 induced increase in melanoma invasiveness. We found that BRG1 is recruited to the MMP2 promoter and directly activates expression of this metastasis associated gene. The results suggest that high levels of BRG1 are required to promote the epigenetic changes required for melanoma metastasis.

BASAL-LIKE BREAST CELL LINES CONTAIN CELL SUBPOPULATIONS WITH STEM CELL FEATURES AND SHOW PROCLIVITY FOR EPITHELIAL-MESENCHYMAL-TRANSITION

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The normal mammary gland is composed of distinct cell populations that can be distinguished by the expression of the cell-surface markers EpCAM and CD49f: differentiated luminal cells (EpCAM+/CD49-), luminal progenitors (EpCAM+/CD49+), stromal cells (EpCAM-/CD49-) and basal myoepithelial/stem cells (EpCAMlow/CD49high). It has been hypothesised that breast basal-like tumours, which are characterised by the mixed expression of basal and luminal markers, may originate from the transformation of mammary stem cells that have undergone a block in their differentiation program.
Here we tested if normal and tumorigenic human basal-like cell lines contain cell subpopulations with stem cell features. First, we observed that basal-like, but not luminal, cell lines contain distinct subpopulations that resemble those observed in the normal mammary gland. After isolation by FACS sorting, these subpopulations were subjected to a variety of in vitro methods to assess their stem cell potential. We demonstrated that EpCAM+ cells and CD49high cells show stem–cell characteristics: high Aldefluor activity, formation of acini-like hollow structures in matrigel, generation of anchorage-independent mammospheres and the ability to regenerate all cell populations within the parental cells. In contrast, EpCAM-/CD49- cells show highly fibroblastic phenotype and form invasive cell strands in matrigel, suggesting that they have undergone an Epithelial-Mesenchymal-Transition (EMT). Surprisingly, these cells show very limited repopulation ability, low Aldefluor activity and reduced mammosphere formation, indicating that they have decreased stem-cell potential.
Moreover, microarray-based gene expression profiling in the different sorted populations identified a number of transcription factors potentially involved in maintaining the stem-cell phenotype and inducing fibroblastic conversion in basal-like cells.

In conclusion, our data indicates that basal-like cell lines contain a heterogeneous mixture of cell subpopulations, some of which show stem cell features. We suggest that these cells may be unable to fully differentiate into myoepithelial cells in vitro, but instead may have intrinsic proclivity for EMT.

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PARALLEL SIGNALING AND RESISTANCE TO TARGETED THERAPIES IN LYMPHOMA

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Targeted therapeutics have changed the treatment myeloproliferative diseases like CML, but in genetically more complex cancers their clinically success has been mixed. Inactivation of tumor suppressors including Arf, p53 or Pten have been invoked along with re-setting of signaling pathways through feedback mechanisms. Taking the example of lymphoma treatment with inhibitors of mTOR, we show that signaling redundancy inherent to these tumors significantly hampers the effectiveness of both targeted and conventional therapies. For example, the constitutively active Pim kinases are abundantly expressed in lymphomas, act through mechanisms that parallel the PI3K/Akt pathway, and affect the clinical outcome of lymphoma therapy. Using murine lymphoma models and xenografted human lymphomas we explore different avenues to overcome resistance caused by redundant signaling in lymphoma. These include combinations of targeted inhibitors affecting either upstream components of both pathways or blockade of key downstream effectors that are shared by overlapping signaling cascades. Genetically well-defined murine models provide an excellent system to test the combinatorial strategies in genetically complex cancers.

DNA BINDING COOPERATIVITY OF P53 MODULATES THE DECISION BETWEEN CELL CYCLE ARREST AND APOPTOSIS

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p53 limits the proliferation of precancerous cells by inducing cell-cycle arrest or apoptosis. How the decision between survival and death is made at the level of p53 binding to target promoters remains unclear. Using cancer cell lines, we show that the cooperative nature of DNA binding extends the binding spectrum of p53 to degenerate response elements in proapoptotic genes. Mutational inactivation of cooperativity therefore does not compromise the cell-cycle arrest response but strongly reduces binding of p53 to multiple proapoptotic gene promoters (BAX, PUMA, NOXA, CASP1). Vice versa, engineered mutants with increased cooperativity show enhanced binding to proapoptotic genes, which shifts the cellular response to cell death. Furthermore, the cooperativity of DNA binding determines the extent of apoptosis in response to DNA damage. Because mutations, which impair cooperativity, are genetically linked to cancer susceptibility in patients, DNA binding cooperativity contributes to p53's tumor suppressor activity.

AN ONCOGENOMICS BASED IN VIVO RNAI SCREEN LEADS TO THE IDENTIFICATION OF TUMOR SUPPRESSOR NETWORKS IN HUMAN LYMPHOMA.

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In vivo RNAi screens in mice have been proven valuable for filtering oncogenomic data to identify "driver" mutations relevant to human cancer. To identify novel tumor suppressor genes in human lymphoma, an shRNA library was designed to cover the mouse orthologues of 329 human genes that are recurrently deleted in human Non-Hodgkin lymphoma. The library, spanning 1429 shRNAs and sub-divided into 14 pools, was screened for the ability to promote lymphomagenesis in an in-vivo myc-driven lymphoma model. A candidate tumor suppressor was defined as a gene for which multiple shRNAs were found enriched in accelerated tumors and/or a single shRNA was found in multiple tumors. We validated the novel tumor suppressor genes by individual re-testing of multiple shRNAs for each candidate in vivo. Strikingly, some of our new tumor suppressor genes are genetically linked at 8p21-23, an area of frequent deletion in human lymphoma but also many epithelial tumor types. Examples include the BAR domain family factor Bin3 and the complement regulator CSMD1. Interestingly, both genes have previously been linked to cancer, as Bin3 +/- mice develop lymphoma and Bin1, a paralogue of Bin3 has been identified as a Myc interactor with tumor suppressor properties. Moreover, CSMD1 is a candidate tumor suppressor in other cancer types, particularly gastric carcinoma. We also identified multiple tumor suppressors targeting known signaling networks that have not been linked to tumor suppression before. Our results therefore provide insight into the organization of the cancer genome, and point towards new tumor suppressor networks relevant to human lymphoma and perhaps other cancer types.

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MECHANISTIC INSIGHTS INTO THE REGULATION OF C-MYC PROTEIN STABILITY AND ONCOGENIC ACTIVITY IN BREAST CANCER

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The c-Myc oncoprotein is highly expressed in most human tumors and its high expression has been linked to poor outcome. In this study we have investigated post-translational mechanisms that regulate both c-Myc expression and its oncogenic activity in breast cancer. Using breast cancer cell lines and paraffin sections from primary patient tumor samples we show that phosphorylation at the conserved Serine 62 (S62) residue is enhanced while phosphorylation at Threonine 58 (T58) is reduced in breast cancer relative to control, and this is associated with increased c-Myc protein stability. The signaling cascade that controls phosphorylation at T58 and S62 is coordinated by the scaffold protein Axin1. Analysis of Axin1 in breast cancer revealed both decreased *AXIN1* expression and a shift in the ratio of two naturally occurring *AXIN1* splice variants. We demonstrate that both contribute to increased c-Myc protein stability, altered phosphorylation at S62 and T58 and increased colony-forming ability in human breast cancer cells. In addition, we have developed unique c-*myc* knockin mice that conditionally express either c-MycWT, or the c-MycT58A or c-MycS62A phosphorylation mutant, from the constitutively active *ROSA26* locus in response to Cre recombinase to study the role of these phosphorylation sites *in vivo*. Using a mammary specific Cre model, we found that expression of c-MycT58A, which has a similar phosphorylation ratio to c-Myc in human breast cancer, had increased tumorigenic activity compared with deregulated expression of c-MycWT, and this was associated with increased genomic instability and suppressed apoptosis. Alternatively, c-MycS62A expression reduced mammary gland density relative to control glands, and this was associated with increased genomic instability and normal apoptotic function. Our work reveals that key phosphorylation events on c-Myc regulated by Axin1 control both c-Myc expression level and its specific oncogenic activity in the mammary gland. These discoveries provide mechanistic insight into the development of human breast cancer, which could lead to new therapeutic strategies.
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IDENTIFICATION OF p53 MODULATORS AND EFFECTORS USING HIGH THOUGHPUT APPROACHES

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The ability of the p53 transcriptional factor to elicit apoptosis, growth arrest, or DNA repair upon diverse stress conditions is the core of its tumor suppressor function. p53 acts not as a single polypeptide, but rather as a complex protein assembly, that remodels itself in a different way depending on a type of stimulus, leading to different biological outcomes. In essence, to understand p53 is to understand how its interaction with proteins and thus DNA, is controlled. In order to address these thrilling questions of p53 biology and to identify key p53 modulators and effectors contributing to alternative p53-mediated biological responses we integrated genome-wide expression profiling data, ChIP-seq and proteomics data. As a model we used MCF7 breast carcinoma cells treated with small molecules activating p53, RITA and nutin3a. Although both molecules target p53/MDM2 interaction, nutlin3a induces growth arrest in MCF7 cells, whereas RITA triggers apoptosis. Thus, RITA and nutlin3a represent excellent research tools to address the mechanisms of differential outcomes upon p53 activation. Notably, analysis of microarray data followed by functional validation suggest that transcriptional repression of crucial pro-proliferative and survival genes by p53, including c-Myc, Mcl-1, Bcl-2, survivin, PI3 kinase, and IGF-1R greatly contribute to the induction og apoptosis by p53. Our integrated analysis lead to the identification of a set of 52 key nodes and candidate cofactors of p53, as well as a set of novel p53 target genes. We analyzed the contribution of the selected 52 factors to the p53 biological response using shRNA-mediated depletion. Interestingly, functional analysis revealed that more than a half of the factors which can affect p53-mediated growth suppression are involved in the IGF-1 receptor signalling. In order to understand the molecular mechanisms of their impact, we analyzed gene expression profiles upon p53 activation in cell lines stably expressing shRNA for selected factors. Further characterization and functional analysis of factors/genes directing alternative biological responses by p53 will help to answer important questions of p53 biology, thus opening the way to novel therapeutic approaches.

ROLE OF BRCA1 IN REGULATING AN ONCOGENIC MICRO RNA

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Breast cancer is the most common malignancy in women. To date, inheritance of a mutant BRCA1 or BRCA2 gene is the best-established indicator of an increased risk of developing breast cancer, the most frequently diagnosed cancer in women. For BRCA1/2 mutation carriers the lifetime risk of developing breast cancer is up to 80%. Therefore, it is not surprising that individuals with a family history of these cancers are now opting to know if they have a mutation in one of these genes. One of the inherent drawbacks of sequencing-based approaches is the determination of the actual risk associated with any variant identified in the gene. This is evident by the fact that more than 800 variants of BRCA1 and 1100 variants of BRCA2 are listed as variants of unknown clinical significance in the breast cancer information core database. We have recently reported a mouse embryonic stem cell based assay to test the functional significance of variants of unknown clinical significance identified in BRCA1 and BRCA2. The assay is based on the ability of human BRCA1/2 to complement the loss of endogenous genes in mouse embryonic stem cells. The procedure involves generation of a desired mutation in BRCA1 or BRCA2 present in a bacterial artificial chromosome (BAC) and introduction of the BAC into ES cells engineered for the assay. Using this assay we have now characterized R1699Q, a low risk variant of BRCA1. Interestingly this variant affects ES cell survival but exhibits no defect in genomic stability or cell cycle regulation. We have used this variant to uncover the role of BRCA1 in regulation of an oncogenic micro RNA. We show that over expression of this micro RNA contributes to tumorigenesis. Our findings show how BRCA1 can act as a tumor suppressor by regulating an oncomir.

RECIPROCAL REGULATION OF HER-2 AND ANNEXIN A2 IN HER-2 NEGATIVE BREAST CANCER

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The biology and the signal transduction pathways that lead to the development of triple negative breast cancer (TNBC) is not understood. There are no established biomarkers for TNBC. In the present study, we evaluated the expression of Annexin A2 (AnxA2) and its role in Her-2 negative breast cancer progression. Immunohistochemical analysis of breast cancer tissue specimens demonstrated that AnxA2 staining might be a good indicator of cancer progression in Her-2 negative breast cancer ($p<0.002$). However in Her-2 positive breast cancer, AnxA2 expression is inversely correlated with Her-2 staining pattern ($p<0.008$). Our cell line analysis revealed that TNBC cells with basal or nil Her-2 levels, express high AnxA2 and the cells with Her-2 amplification demonstrate a very low level of AnxA2. We found increased membrane localization and secretion of AnxA2 in TNBC cells. AnxA2 induction after Her-2 downregulation and increased expression with EGFR in TNBC ascertains its role in various cancer associated functions. In this study, we validated the role of AnxA2 in EGFR downstream signaling in triple negative breast cancer cells. We made a novel observation that, presence of AnxA2 is very critical for the constitutive activation of the EGFR downstream signaling molecules like ERK1/2, AKT and STAT-3 and in further regulation of cancer cell metastasis, survival and apoptosis. The specific expression pattern of AnxA2 in TNBC cells, increased secretion compared to normal cells and its role in the regulation of EGFR downstream signaling makes AnxA2 as a potential tissue and serum biomarker and an excellent therapeutic target in TNBC.

CIGARETTE SMOKE-INDUCED NF-κB ACTIVATION IN LUNG EPITHELIAL CELLS IS MEDIATED PREDOMINANTLY BY C-REL

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Epidemiological studies show that cigarette smoke (CS) is a major risk factor for several life threatening diseases including lung cancer. A large body of evidence indicates that in majority of cancer cases transcription factor NF-κB is constitutively active in the nucleus. NF-κB is a dimeric complex formed from five subunits: RelA (p65), RelB, c-Rel, p50 (NF-κB1) and p52 (NF-κB2). The combinatorial diversity of subunit composition contributes to the regulation of distinct, but overlapping sets of genes. In unstimulated cells NF-κB remains in the cytosol in an inactive form associated with regulatory proteins called inhibitors of κB (I-κB), such as I-κBα, I-κBβ and I-κBξ. NF-κB is activated mainly through the phosphorylation of I-κB by the I-κB kinase (IKK) complex, which results in proteasome-mediated degradation of I-κB, followed by nuclear translocation of NF-κB dimers that turns on expression of target genes. Different I-κB molecules have distinct and overlapping specificities and they may also differ in their tissue distribution pattern. Thus I-κB molecules control the regulation of different genes in various tissues by inhibiting specific NF-κB subsets.

The effect of CS on NF-κB activity has been first reported in 1999. However the upstream events leading to this activation is unclear as there are conflicting reports regarding the role of I-κBα in this process. As NF-κB plays a pivotal role in generation and maintenance of malignancies, we have targeted our efforts to understand the detailed mechanism of CS-induced NF-κB activation. Our results showed that in alveolar epithelial A549 cells, CS-induced NF-κB activation is independent of I-κBα. Instead it involves the degradation of I-κBξ, which is highly expressed in lung. Contrary to a previously published report showing the involvement of p65 subunit, nuclear-cytosolic fractionation, immunofluorescence and si-RNA mediated gene silencing experiments demonstrate that CS-induced NF-κB activation is mediated predominantly, if not exclusively, by nuclear translocation of c-Rel. This observation was further bolstered by immunostaining result on lung sections obtained from CS-exposed guinea pigs. Further study revealed that the treatment of A549 cells with cigarette smoke extract (CSE) causes activation of IKK, which is required for I-κBξ phosphorylation and CS-induced NF-κB activation. Therefore, the current study provides a new axis of NF-κB activation in lung epithelial cells wherein I-κBξ and c-Rel play the central role. Role of c-Rel has long been implicated in different forms of malignancy. Thus the present study provides a possible mechanism of cancer growth in smoker's lung and also identifies c-Rel as a putative target of therapeutic interventions as c-Rel knockout mice do not display any significant phenotypic defects.

MOLECULAR BASIS OF AUTOPHAGY-MEDIATED RESISTANCE TO RADIATION AND APO2L/TRAIL THERAPY OF PROSTATE CANCER

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Ionizing radiation (IR) and Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) regulate cell death as well as survival signaling and have been used widely as therapeutics for prostate cancer (PCa). However, cellular resistance that is mostly cell type specific once developed hinders the effectiveness of therapy. We found PC3 cells to be more sensitive to TRAIL induced cell death compared to LNCaP-derived C42 cells. Both apoptotic and non-apoptotic cell death was observed indicating that other pathways, such as autophagy can also cause cytotoxicity. Following TRAIL and IR treatment of PC3 cells, microtubule associated protein-1 light chain LC3, a classical marker for autophagy, was recruited into autophagosomes following its cleavage (LC3 I) and lipid modification (LC3 II). In contrast, LC3 was not observed to be associated with the autophagosomes in C42 cells. Moreover, inhibition of autophagolysosome formation with chloroquine resulted in accumulation of LC3 II to a larger extent in C42 cells, suggesting that lipid modification of LC3 is intact and that LC3 turnover by autophagy might be faster in C42 cells. We further determined the rate of autophagic flux using the GFP-mCherry-LC3 fusion protein. Interestingly, C42 cells showed more lysosomal LC3 II whereas PC3 had predominantly autophagosomal LC3 II, indicating that the rate of autophagic outflux is higher in C42 cells upon TRAIL treatment. Electron micrographs also indicated that C42, unlike PC3 cells have efficient autophagy without accumulation of autophagosomes. We next examined the expression and sub-cellular distribution of other autophagy-related proteins. Membrane associated ATG5 is decreased in PC3 and C42 cells following TRAIL and IR treatment. The SQSTM1/p62 levels increased in poly-ubiquitinated aggregates in PC3 but not in C42 cells upon TRAIL treatment, further indicating slower autophagy in PC3 cells. Moreover, we observed differences in ATG5 and p62-associated native protein complexes among PC3 and C42 cells upon TRAIL treatment. Characterization of these complexes would provide new insights into autophagic signaling and TRAIL response. Our results suggest that in TRAIL or IR-sensitive PC3 cells accumulation of autophagosomes triggers cell death whereas a more efficient autophagic response in C42 cells might be the reason for their resistance against TRAIL or IR. Further defining the function of autophagy-regulatory genes will delineate their role in mediating cell death or survival outcomes in a cell context dependent manner, which may lead to the design of better therapies for PCa.

ENDOGENOUS MYC IS ESSENTIAL FOR MAINTENANCE OF THE PANCREATIC TUMOR MICROENVIRONMENT

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Tumor cells are completely dependent on their local inflammatory and angiogenic microenvironment, making the tumor microenvironment a potential target for cancer therapy. However, whether the tumor instructs or drives changes in the tumor microenvironment or vice versa remains unclear. This, plus the abiding degeneracy in extracellular signaling pathways, has confounded pharmacological targeting of the tumor microenvironment. To address the relationship between tumor and microenvironment, we combined the well characterized *RIP1-Tag2* mouse islet tumor model with a mouse in which endogenous Myc function can be reversibly inactivated in vivo. We demonstrated that transient inhibition of endogenous Myc, either systemically or only in the β cell compartment, triggers rapid collapse of tumor stromal vasculature, disappearance of inflammatory cells and hypoxia, precipitating tumor regression. Hence, endogenous Myc in tumor cells is causally and non-redundantly required to instruct and maintain the tumor microenvironment. Targeting Myc could therefore circumvent the robustness inherent in the tumor microenvironment.

THE ATM/CHK2/P53 PATHWAY CONTRIBUTES TO TUMOR SUPPRESSION AND IS REQUIRED FOR RADIATION RESPONSE IN GLIOMAS

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Glioblastoma mutliforme (GBM) is the most common and lethal tumor of the central nervous system in the adult. Despite decades of efforts, GBMs patients remain refractory to standard therapies and they have a very low survival rate. Understanding the mechanisms responsible for this poor therapy response is instrumental to develop new treatment modalities. Preservation of genomic integrity is essential for embryonic development and adult tissue homeostasis. Defects in the DNA-damage response (DDR) machinery, a network of protein complexes capable of detecting the DNA lesions and signaling to downstream effector pathways (cell cycle checkpoints, DNA repair, apoptosis, etc.), are linked to numerous pathological states including cancers. The DDR has been suggested to act as a barrier against tumor progression, where precancerous lesions need to inactivate p53 or other elements of the DDR to proceed to a more aggressive status.
A detailed analysis of the TCGA (The Cancer Genome Atlas) database revealed that many genes encoding component of the DDR are frequently altered in human glioblastomas, being CHEK2 loss one the most frequent events. To investigate the role of this pathway in gliomagenesis we took advantage of the RCAS/tv-a system, which allows postnatal gene transfer and expression in a cell-type-specific manner. We collected evidences that some of the essential molecules of the DDR, such as Chk2, ATM and p53 exert an important tumor suppressive role in PDGF induced gliomagenesis, and loss of any of those genes not only shorten tumor latency but also lead to a more aggressive phenotype, increasing the frequency of high-grade tumors (GBMs). We further analyzed the role of Chk2 in GBM radiation response both in vivo and in vitro and we show that Chk2 null gliomas present defects in both apoptotic response and cell-cycle checkpoints. Notably we discovered a clear dependence on Chk2 for G1/S checkpoint control in glioma tumor neurospheres and normal neuronal stem cells (NSCs), a situation that contrasts with what observed in other murine cell types. Our data suggest that regulation of both apoptosis and cell-cycle checkpoint could contribute to the tumor suppressor activity of the ATM/Chk2/p53 cascade in gliomas.

AN IN VITRO MODEL OF GENOMIC INSTABILITY UNDER HYPOXIA REVEALS SELECTION OF REGIONS SUBJECT TO *ALU* MEDIATED RECOMBINATION

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Hypoxia is an important feature of solid tumors and contributes extensively to the genetic heterogeneity and instability in the aggressive phenotype. Active repeat mediated recombination is one of the mechanisms which lead to genomic instability in cancer and cell stress. We have previously studied the phenomenon of active *alu* mediated recombination in two such cellular stress models. Glial cells treated with sequentially increasing doses of cisplatin and cells exposed to long term (6 week) hypoxia, showed increased transcription and mobilization of *alu* repeat sequences. In light of these observations, we were interested in developing hypoxia models which could identify active recombinogenic fragments which formed a part of genome content of cells which were selected for survival in hypoxic stress. Hypoxia models of short (24 hours onwards) and long term models (upto 9 weeks) were propagated and studied for consistent changes in the banding patterns using inter-*alu* PCR. Inter-*alu* PCR, with primers directed outwards from the *alu* sequence, amplifies fragments that represent regions flanked atleast at one end by *alu* repeats. Changes detected (as compared to normoxia controls) represent *alu* mediated recombination events. Most of the changes were random in the different inter-*alu* PCR profile. However, a few alterations appear to be reproducibly consistent under progressive durations of hypoxia, indicating selection of certain population of cells harboring a particular genome content. We identified one such consistently altered fragment :an exonic region of F-Box 27 (FBXO27) flanked by inverted *alu* repeats enhanced during the short term and also in the progressively long term hypoxia model. F Box proteins are a large family of proteins with at least one F-Box motif and form a part of the Skp, Cullin, F-box containing complex (or SCF complex) which is a multi-protein E3 ubiquitin ligase complex. The F Box protein directly interacts with S-phase kinase protein (SKP1) through these motifs. An increase in transcript levels of both FBXO27 and SKP1 in long term hypoxia suggest that cells with this alteration are significantly selected for and populated in chronic hypoxia. We are currently investigating the functional significance of our observations. Our results suggest that cells exposed to short and long term hypoxia are useful for identifying active repeat mediated alterations which are specifically selected for survival during stress.

ANALYSIS OF STEM-LIKE CELL FEATURES OF HUMAN SYNOVIAL SARCOMA

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(Background) A certain population of tumor cells which exhibits stem cell-like features should be therapeutic target. To date, a number of molecules were reported as markers for cancer stem-like cells in various types of tumors. However, most of them are epithelial malignancy referred as carcinoma, and for mesenchymal malignancy as sarcoma, none of the marker was established as cancer stem-like cells. In this study, we focused on the human synovial sarcoma (SS), which generally arises in young adults, caused by chimeric gene product SYT-SSX (Nagai, M., et al., PNAS, 98, 2001). Precise role for SYT and SSX is unknown, but we have reported that SYT is essential for mice embryonal development (Kumura, T., et al., Lab. Invest., 89, 2009).
(Methods and Results) First, we focused on the role of CD133, and found that CD133 mRNA was detectable in two human SS cases. CD133 positive fraction is isolated by using SS cell lines, SYO-1 and Fuji. The growth of CD133 positive cells was slower than CD133 negative cells in vitro and in vivo, and the phosphorylation levels of Akt were elevated in CD133 negative cells. As Akt inhibitor suppressed growth of CD133 negative cells, regulation of Akt maybe involved in the growth of these cells. Second, we established sphere culture condition of human SS cell lines and analyzed the feature of sphere formed cells which express Nanog, Oct3/4, and Sox2, and found that sphere cells grow slower than non-sphere cells in vivo. Gene expression profile of sphere cells were analyzed by micoarray and several transcription factors were up-regulated and tyrosine kinase receptors were down-regulated in sphere form of cells. Currently, we are focusing on the possibility that these molecules include markers which define and contribute to the feature of sarcoma stemness.

MEMBRANE-TYPE-3 MATRIX METALLOPROTEINASE (MT3-MMP) FUNCTIONS AS A MATRIX COMPOSITION –DEPENDENT EFFECTOR OF MT1-MMP ACTIVITY AND TUMOR CELL INVASION.

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Membrane-type matrix metalloproteinases MT1- and MT2-MMP promote tumor cell invasion through basement membranes and collagen type I-rich tissues, whereas the functions of MT3-MMP in tumor invasion have remained less clear. Interestingly, MT3-MMP has been reported to be overexpressed in human melanoma selectively in the most aggressive nodular type tumors. We demonstrate here that MT3-MMP inhibits MT1-MMP-driven melanoma cell sprouting and single cell invasion in three-dimensional collagen, thus resulting in restricted, yet MT1-MMP-dependent expansion of compact cell colonies. In WM852 cells that have been originally isolated from nodular human melanoma, endogenous MT3-MMP expression was associated with rapid fibrin invasion and unexpectedly limited MT1-MMP-driven collagen invasion, both of which were reversed by siRNA-mediated MT3-MMP gene silencing. Consistent with such reverse matrix composition-dependent MT3-MMP functions, MT3-MMP overexpression reduced collagen invasion in parallel with increased fibrin invasion of Bowes cells with superficially spreading melanoma origin. Rather than altering MT1-MMP transcription, catalytically active MT3-MMP interacted with MT1-MMP thus impairing the proinvasive MT1-MMP activity in collagen. While MT3-MMP, unlike MT1-MMP, is not universally overexpressed in primary melanoma, it was significantly upregulated in pathologic specimens of human melanoma metastasis to lymph nodes. MT1-MMP-MT3-MMP interactions thus seem to provide tumors with a novel mechanism to restrict local cell invasion but allow nodular tumor cell growth in collagen-rich tissues such as skin, while simultaneously promoting invasive spread into distant sites.

PHARMACOLOGIC INHIBITION OF MEK PROMOTES REGRESSION OF BOTH $KRAS^{G12D}$- AND $BRAF^{V600E}$-INDUCED LUNG TUMORS

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Lung cancer leads to the death of ~165,000 deaths/year in the USA. Mutational activation of *KRAS* or *BRAF* is detected in ~25% and 5% of lung cancers respectively. Hence, it is likely that the RAS-regulated RAF-MEK-ERK MAP kinase pathway may be an important target for treatment of a subset of lung cancers. Conditional mouse models for both $KRAS^{G12D}$- and $BRAF^{V600E}$-induced lung tumorigenesis have previously been described. Intranasal instillation of Ad-Cre into $KRas^{LSL}$ or $BRaf^{CA}$ results in the expression of $KRAS^{G12D}$ or $BRAF^{V600E}$ in the distal lung epithelium. Although early stages of $KRAS^{G12D}$- and $BRAF^{V600E}$-induced lung tumorigenesis appear to be almost identical, only mice expressing $KRAS^{G12D}$ develop malignant adenocarcinoma of the lung where mice expressing $BRAF^{V600E}$ develop benign lung tumors. However, when $BRAF^{V600E}$ is combined with silencing of either Trp53 or Cdkn2a expression malignant lung cancers develop. An important question is the role of the RAF-MEK-ERK MAP kinase pathway in the maintenance of $KRAS^{G12D}$-induced lung cancers. To address this we employed PD325901, a potent, specific and selective inhibitor of MEK1/2, in the $KRas^{LSL}$ model in conjunction with in vivo imaging using the LucRep transgene. As observed with $BRAF^{V600E}$-induced lung tumorigenesis, MEK inhibition entirely prevented the onset of $KRAS^{G12D}$-induced lung tumors. Moreover, MEK inhibition alone promoted dramatic regression of established $KRAS^{G12D}$-induced lung tumors as evidenced by in vivo imaging. These data indicate that single agent MEK inhibition suffices to promote regression of $KRAS^{G12D}$-induced lung tumors in mouse models and that such agents may be useful in the clinical management of both $KRAS^{G12D}$- and $BRAF^{V600E}$-induced lung cancers in the clinic.

DEFINING THE ROLE OF DEVELOPMENTAL STAGE IN SUSCEPTIBILITY TO TRANSFORMATION WITHIN THE B CELL LINEAGE

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Within all developmental lineages, cells are more or less susceptible to transformation and thus have intrinsically different propensities to become a cell of origin for cancer. Our understanding of the hematopoietic system, particularly B lymphopoiesis, provides a unique opportunity to test the vulnerability of developmental cell stages. The study of Myc-driven lymphomagenesis in murine models has revealed that a critical constraint to transformation is the p53-dependent apoptotic response that sustained Myc expression evokes. With the removal of this constraint, aggressive B cell lymphomas develop. This type of modeling, however, largely relies on expression of an oncogene at an early developmental timepoint (e.g. HSC). The stage at which an oncogene or tumor suppressor is creating the conditions necessary for transformation is thus unclear. We have devised a strategy to model Myc expression with ‘p53 loss’ at specific developmental points within the B cell lineage. From these studies we hope to establish if tumors arise regardless of the stage at which an oncogenic state is created. Additionally, we would like to exploit this system to study how cells respond to other putative tumor-initiating events when their expression is restricted to a specific developmental stage.

NOL6, A NOVEL NUCLEOLAR PROTEIN, ASSOCIATES WITH B23 AND P19^{ARF} AND CAN REGULATE RIBOSOMAL BIOGENESIS AND THE CELL CYCLE.

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Cell proliferation and growth rate is dependent on the regulation of the protein synthesis apparatus, the ribosomes. Nol6/Nrap (Nucleolar RNA Associated Protein) is a nucleolar protein which is highly conserved across eukaryotic organisms, however, its function is poorly characterised. Recently, Nol6 was found to have a significant role in ribosomal biogenesis. Our work shows that its expression markedly affected the levels of 45S rRNAs indicating its functional role in rRNA synthesis. Ribosome biogenesis is known to be regulated by nucleophosmin (B23/NPM) and its interacting partner, p19^{ARF} which are abnormally expressed in cancer cells. Here we show that Nol6, is highly expressed in tumor tissues and it exists in a complex with the nucleolar proteins B23 and p19^{ARF} in primary mouse embryonic fibroblast cells. Nol6 knock-down using siRNA significantly increased B23 protein levels and down-regulated the protein levels of p19^{ARF}. In addition, Nol6 has also been found to co-localise with SUMO and seems to be sumoylated. B23 and p19^{ARF} have previously been shown to regulate the progression of cell cycle. While exogenous Nol6 is rapidly degraded in normal cells, loss of Nol6 has been found to lead to cell apoptosis and de-stabilises the normal progression of the cell cycle. We hypothesise that loss of Nol6 leads to cell apoptosis by triggering the p53 pathway. In this work, we demonstrate for the very first time, the functional role of NOL6 within the nucleolus and the cell cycle and its association with B23 and p19^{ARF} and propose Nol6 to be a key regulator in maintaining their expressions within a non tumor cell.

FUNCTIONAL CHARACTERIZATION OF THE TRITHORAX PROTEIN ASH2 AND THE ASH2-MLL METHYLTRANSFERASE COMPLEX IN TUMOR FORMATION

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Epigenetic alterations, including DNA methylation and histone modifications, have been recognized as important hallmarks for tumor development. One group of complexes involved in regulating one particular histone modification is referred to as ASH2-MLL complexes. These have attracted considerable interest, both from a mechanistic and a clinical point of view. These complexes possess histone methyltransferase activity and control gene expression by trimethylating lysine 4 of histone H3, a histone mark that is associated with actively transcribed genes. Moreover several subunits of these complexes are associated with tumorigenesis, suggesting strongly that ASH2-MLL complexes are important regulators of cell physiology. Mixed-lineage leukemia (MLL) genes and proteins have been found deregulated and/or mutated in many cancers. These proteins are the catalytic subunits of ASH2-MLL complexes, i.e. MLL proteins are histone methyltransferases. Another subunit associated with at least some ASH2-MLL complexes is Menin, a tumor suppressor protein. Finally we identified human ASH2 as a interactions partner of the oncoprotein MYC. This finding, together with the observation that hASH2 interacts with MLL, led us to test whether hASH2 itself is involved in transformation. We observed that hASH2 cooperates with activated RAS to transform cells. This finding suggests that ASH2 functions as an oncoprotein. Although hASH2 expression at the mRNA level was generally not deregulated, hASH2 protein expression was increased in most human tumors and tumor cell lines. In addition, knockdown of hASH2 inhibited tumor cell proliferation. Taken together, these observations define hASH2 as a novel oncoprotein. To further validate this model we have generated transgenic mice that express ASH2 specifically in the liver. The tumor development in these animals also in combination with a MYC transgene will be discussed.

KAPOSI'S SARCOMA HERPESVIRUS MICRORNAS REGULATE APOPTOSIS BY TARGETING CASPASE 3

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Herpesviruses are known to encode micro (mi)RNAs and to use them to regulate the expression of both viral and cellular genes. The genome of Kaposi's sarcoma herpesvirus (KSHV) encodes a cluster of twelve miRNAs, which are abundantly expressed during both latency and lytic infection. Relatively few cellular targets of KSHV miRNAs are known. Here, we used a microarray expression profiling approach to analyze the transcriptome of both B lymphocytes and endothelial cells stably expressing KSHV miRNAs and monitor the changes induced by the presence of these miRNAs. We generated a list of potential cellular targets by looking for miRNA seed-match-containing transcripts that were significantly down regulated upon KHSV miRNAs expression. Interestingly, the overlap of putative targets identified in B lymphocytes and endothelial cells was minimal, suggesting a tissue-specific target-regulation by viral miRNAs. Among the putative targets, we identified caspase 3, a critical factor for the control of apoptosis, which we validated using luciferase reporter assays and western blotting. In functional assays we obtained further evidence that KSHV miRNAs indeed protect cells from apoptosis. Currently we are analysing this protective effect in primary effusion lymphoma (PEL) cell line, which is naturally carrying KSHV genome, and also in endothelial cell line stably expressing KSHV miRNAs.

AUTOCRINE WNT SIGNALING IS UPREGULATED AT HIGH FREQUENCY AND DRIVES PROLIFERATION OF MULTIPLE HUMAN SARCOMA SUBTYPES THROUGH A NOVEL TCF/β-CATENIN TARGET GENE, CDC25A

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Wnt/β-catenin signaling is important for normal tissue homeostasis and is also implicated in the genesis of several different types of carcinomas. Earlier, we had shown that high levels of Wnt signaling inhibit multi-lineage differentiation of human mesenchymal stem cells (hMSCs) in vitro and in vivo. Sarcomas are mesenchymal tumors that account for a majority of pediatric cancers. Recent studies indicate that at least certain sarcomas originate from hMSCs, which we have shown to exhibit low levels of Wnt signaling. Current work identifies that a high fraction of human sarcomas and sarcoma cell lines of distinct histological subtypes show upregulation of canonical Wnt pathway. Multiple molecular mechanisms including overexpression of Wnt ligands and LRP5/6 co-receptors and loss of different antagonists contribute to autocrine activation in most sarcoma cell lines. Importantly, downregulation of Wnt pathway induces growth arrest both in vitro and in vivo. Also, our study identifies CDC25A, a novel Wnt target gene, to be overexpressed in sarcomas. The high prevalence of Wnt pathway activation in human sarcomas described here and the ability to identify Wnt pathway activation in primary sarcoma tissues make it reasonable to test whether naturally occurring Wnt antagonists such as DKK1 or FRP or recently reported small molecule Wnt inhibitors may complement standard agents in the treatment of this most common childhood malignancy.

THE *HELICOBACTER PYLORI* VIRULENCE FACTOR CAGA ACTIVATES JNK SIGNALING IN A TRANSGENIC *DROSOPHILA* MODEL SYSTEM

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During *Helicobacter pylori* infection of the human stomach, the CagA effector protein is translocated into host cells where its presence is associated with gastric cancer formation. CagA has been shown to interact with a number of host cell proteins belonging to several conserved signaling pathways, and these interactions are thought to facilitate the initiation of carcinogenic events. Using a transgenic *Drosophila* model, we have shown that expression of CagA results in dramatic perturbations of the simple model epithelium created by the larval wing imaginal disc. In cultured cells, CagA has been shown to disrupt epithelial cell polarity via interaction with tight junction proteins. Loss of cell polarity is a potent activator of JNK signaling which can lead to apoptotic cell death or cooperate with oncogene activation to promote tumorigenesis. Therefore we postulated that CagA might activate JNK signaling in epithelial cells to give rise to downstream effects associated with *H. pylori* virulence. Our results demonstrate that expression of CagA in the developing wing causes a distinct pattern of apoptosis characterized by extrusion of apoptotic cells from the basal surface of the epithelium. This cell death phenotype is enhanced by coexpression with Basket (Bsk), the *Drosophila* homolog of JNK, and suppressed by coexpression with a dominant-negative form of Bsk. In addition, we have shown that CagA expression can enhance the growth and invasion of tumors generated by expression of activated Ras in a *Drosophila* model of metastasis. These data provide *in vivo* evidence that CagA causes JNK-dependent apoptosis in a simple polarized epithelium, and enhances tumorigenesis caused by oncogene activation. We are currently testing whether CagA activates JNK signaling via disruption of epithelial cell polarity, and if CagA cooperates with activated Ras to enhance tumorigenesis through JNK pathway activation.

SIGNALLING PATHWAYS UNDERLYING CELLULAR SENESCENCE IN HUMAN MAMMARY EPITHELIAL CELLS

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Cells from organisms with renewable tissues can permanently withdraw from the cell cycle in response to a variety of stimuli, including dysfunctional telomeres, DNA damage, physiological stress, and activation of certain oncogenes. This phenomenon, cellular senescence, is controlled by the p53 and RB tumour suppressor proteins, and constitutes a potent anti-tumour mechanism.
The underlying mechanism controlling cellular senescence and the signal transduction pathways involved are poorly defined and the critical downstream targets of the p53 and RB pathways in this process have not been identified. As most breast cancers originate in epithelial cells, we developed a conditionally immortalized human mammary epithelial line, 226 8/13, derived from human mammary luminal epithelial cells, in which we carried out a genome wide loss of function RNA interference screen to identify these targets. 226 8/13 cells constitutively express hTERT, the catalytic component of human telomerase, and a temperature sensitive non-DNA-binding mutant of Simian Virus 40 large T (LT) antigen. These cells grow at the permissive temperature, 34°C, but undergo a rapid irreversible arrest at the non-permissive temperature, 38°C, upon inactivation of the thermolabile LT antigen allowing activation of the pRB and p53 tumour suppressor pathways.
The Open Biosystems human GIPZ lentiviral shRNAmir library and pSM2 retroviral shRNAmir library were applied to the conditional system, and several genes whose inhibition leads to cells overcoming the conditional growth arrest were identified. These genes are now being investigated in the context of several breast cancer cell lines. Furthermore, we will test if knock-down of these genes can rescue from Ras-induced senescence in the context of primary human mammary epithelial cells (HMECs).

S-NITROSYLATION FROM GSNOR DEFICIENCY IMPAIRS DNA REPAIR AND PROMOTES HEPATOCARCINOGENESIS

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Background: Human hepatocellular carcinoma (HCC) is associated with elevated expression of inducible nitric oxide synthase (iNOS), but the role of nitric oxide (NO) in the pathogenesis of HCC remains unknown. Deactivation of NO bioactivity and control of protein *S*-nitrosylation critically depends on *S*-nitrosoglutathione reductase (GSNOR). The human GSNOR gene (ADH5) is located at approximately 4q23, a region in which chromosomal deletion occurs frequently both in cirrhotic and dysplastic hepatocytes and in HCC.
Objectives: We investigated whether GSNOR deficiency, through dysregulated *S*-nitrosylation, is a critical mechanism of hepatocarcinogenesis.
Methods: The abundance and activity of GSNOR were analyzed in pairs of HCC and associated noncancerous liver tissues from 24 HCC patients. Mice deficient in GSNOR or deficient in both GSNOR and iNOS were employed in the models of both spontaneous and carcinogen-induced HCC. Also studied in the HCC models were DNA repair proteins and related repair of specific DNA lesions.
Results: We found that both GSNOR enzymatic activity and amount of GSNOR protein were significantly decreased in ~50% of the patients with HCC, with GSNOR reduction of 80 to 90% in 20% of the patients. GSNOR-deficient mice were very susceptible to spontaneous and carcinogen-induced HCC. During inflammatory responses, the livers of GSNOR deficient mice exhibited substantial *S*-nitrosylation and proteasomal degradation of the key DNA repair protein O^6-alkylguanine-DNA alkyltransferase. As a result, repair of carcinogenic O^6-alkylguanines in GSNOR-deficient mice was significantly impaired. Predisposition to HCC, S-nitrosylation and depletion of alkylguanine-DNA alkyltransferase, and accumulation of O^6-alkylguanines were all abolished in mice deficient in both GSNOR and iNOS.
Conclusion: Our data suggest that GSNOR deficiency, through dysregulated S-nitrosylation and possibly inactivation of a DNA repair system, promotes hepatocellular carcinoma. These findings suggest that patients with GSNOR deficiency and concurrent iNOS overexpression in the liver may be at an increased risk of HCC, and inhibition of iNOS-derived *S*-nitrosylation in these patients may provide a therapeutic strategy to prevent liver cancer.

GENOME-WIDE ANALYSIS OF ETS TRANSCRIPTION FACTOR TARGET GENES AND REGULATORY POLYMORPHISMS

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The ETS transcription factors (TFs) represent one of the largest families of transcriptional regulators in mammalian genomes, playing diverse roles in various biological processes. Some ETS-factors such as ERG and ETS1 can act as oncogenes in malignant transformation and tumor development. All ETS-factors share a conserved ETS domain, which binds to a set of similar DNA sequence motifs *in vitro* but contributing to targeting specificities *in vivo*. To systematically decipher the regulatory networks downstream of ETS TFs, we set up a novel version of the enhancer-element locator program that allows identification of TF cistrome and regulatory SNPs. We globally predicted regulatory elements activated by the oncogenic ETS family members, and SNPs affecting TF binding sites within these elements based on TF DNA-binding specificity. We validated the ETS-regulated elements by ChIP-seq mapping of ETS-binding sites and epigenetic signatures in Ewing's sarcoma, leukemia and prostate cancer cells. RNAi followed by expression profiling were further applied to correlate ETS family DNA-binding and transcriptional regulation. To find causal rSNPs, we combined the rSNP predictions with genome-wide association studies, resulting in identification of a SNP in an ETS-regulated element upstream of the c-MYC gene that predisposes to prostate cancer (PCa). TMPRSS2-ERG fusion is present ~50% of all PCa cases. In addition to mapping TMPRSS2-ERG target genes as described above, we also systematically analyzed TMPRSS2-ERG-regulated miRNA networks and found that miR-25~106b cluster and its host gene MCM7 are potential ERG targets. Given evidence indicating that PTEN is a target of the miR-25~106b cluster, our results suggest a causal molecular link between the concurrent events of TMPRSS2-ERG translocation and decreased PTEN abundance observed in a subset of PCa cases.

Refs: Hallikas O, et al., Cell. 2006. 124:47; Han B, et al., Mod Pathol. 2009. 22:1083; Poliseno L, et al., Sci Signal. 2010. 3:ra29; Wei GH, et al., EMBO J. 2010 in press.

PARTIAL INACTIVATION OF THE DNA DAMAGE CHECKPOINT GENE HUS1 IMPAIRS TUMOR DEVELOPMENT IN A TWO-STEP SKIN CARCINOGENESIS MODEL

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Mammalian cells maintain genomic integrity despite frequent DNA damage due in part to the actions of checkpoint signaling pathways headed by the kinases Atm and Atr. While mutations in the well-characterized Atm pathway lead to increased cancer risk, the role of the Atr pathway in tumor suppression is less well understood, as deletion of any component of this pathway, including Hus1, results in embryonic lethality. As a member of the PCNA-like Rad9-Rad1-Hus1 (9-1-1) complex, Hus1 is recruited to sites of DNA damage and acts as a scaffold that promotes checkpoint signaling and DNA repair. In order to bypass the severe effects of complete Hus1 inactivation, we previously developed an allelic series in which mice express incrementally reduced levels of wild-type Hus1. Mice with reduced Hus1 expression show increased genomic instability and genotoxin hypersensitivity but nevertheless appear grossly normal and are not prone to spontaneous cancers. To investigate how partial Hus1 inactivation impacts tumor development, we assessed skin papilloma formation in Hus1 impaired and control mice following chemical carcinogenesis. A subset of mice with reduced Hus1 expression initially developed papillomas at a modestly accelerated rate relative to control animals, suggesting that genomic instability and checkpoint failure in initiated cells with reduced Hus1 expression can shorten the latency for papilloma development. However, at later timepoints mice expressing reduced levels of Hus1 had a decreased risk of papilloma development, and they ultimately showed significantly reduced papilloma size and number. These effects were not associated with acute cell death or decreased proliferation following carcinogen exposure, suggesting that the reduced papilloma formation may be due to an inability of cells with partial Hus1 impairment to survive the stresses of neoplastic proliferation because of insufficient genome maintenance. Consistent with this hypothesis, preliminary data suggest that cultured mouse embryonic fibroblasts with reduced Hus1 expression are partially defective for anchorage-independent growth and other characteristics of malignant transformation following expression of activated oncogenes. Overall, these studies highlight a fundamental requirement for intact Hus1 function during cell transformation and tumorigenesis, and suggest that targeting this checkpoint mechanism may be an effective strategy for cancer therapy.

THE TRUNCATED EPHA7 RECEPTOR ACTS AS A SECRETED TUMOR SUPPRESSOR IN FOLLICULAR LYMPHOMA

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Follicular lymphoma (FL) is the most common histological type of Non-Hodgkin's lymphoma. FLs are characterized by the t(14;18)(q32;q21) and increased expression of the anti-apoptotic Bcl2 protein. Lymphomagenesis and ultimate transformation towards aggressive lymphoma are driven by additional genetic events. In particular, deletions of Chr. 6q occur in ~ 30% and are linked to early progression and shortened survival of FL patients. To pinpoint genetic suppressors of FL and guided by an array-CGH analyses 70 FL specimens, we conducted a deletion-specific short-hairpin RNA screen. In addition to FoxO3 and TnfAip3/A20, which are also somatically mutated in FL, we identify a truncated variant of the Ephrin receptor (EphA7TR). EphA7TR acts as a secreted tumor suppressor in vitro and in a murine FL model. In addition to chromosomal deletions, we find that EphA7TR is epigenetically silenced in nearly all FL cases we examined. Strikingly, re-administration of the purified EphA7TR protein by injection suppresses Src and Mapk signaling and produces remissions in xenografted human lymphomas in vivo. Hence, the soluble EphA7TR tumor suppressor is widely inactivated in follicular lymphoma can be administered as a therapeutic agent.

FUNCTIONAL INTERACTIONS BETWEEN P19^{ARF} AND PDGFRB IN CULTURED CELLS AND *IN VIVO*

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p19^{Arf} is an critical tumor suppressor protein with roles regulating cell cycle arrest in development and suppressing oncogenesis. Previous studies established that p19^{Arf} regulates pericyte-like cells in the hyaloid vascular system (HVS) in the primary vitreous of the developing eye. Arf-deficient mice have hyperplasic expansion of pericytes in the vitreous, a developmental defect driven by excessive Pdgfrβ signaling. We have used complementary studies of cultured cells and mouse embryos to further explore the functional interactions between p19^{Arf} and Pdgfrβ. First, we determined if Pdgfrβ acts as a survival factor or a mitogen to foster the accumulation of pericytes in the Arf$^{-/-}$ vitreous using the Arf$^{Gfp/Gfp}$ knock-in reporter mouse. Quantitative studies of cell proliferation and apoptosis in E13.5 Arf$^{GFP/GFP}$ embryos that have or lack Pdgfrβ$^{-/-}$ indicated that cell proliferation was increased by threefold in the Arf expressing cells in the presence of Pdgfrβ. The presence or absence of Pdgfrβ did not change the small number of apoptotic cells in the vitreous of the eye. Second, we used a cell culture model in which retroviral expression of p19^{Arf} in 10T1/2 pericyte-like cells and Arf$^{-/-}$, Mdm2$^{-/-}$, and p53$^{-/-}$ "TKO" MEFS cells significantly repressed Pdgfrβ protein levels following transduction. Interestingly, Pdgfrβ was not repressed when p19^{Arf} was expressed in Arf$^{-/-}$, p53$^{-/-}$ MEFs, suggesting that endogenous Mdm2 may interfere with p19^{Arf} in the absence of p53. Conversely, knockdown of p19^{Arf} in wild type MEFs using pSIRPP retrovirus vector increased Pdgfrβ protein levels. Mature and primary transcript analysis indicates repression of the protein levels occurs earlier than repression of the transcript, suggesting p19^{Arf} primarily represses Pdgfrβ by a post-transcriptional mechanism. Consistent with this, transient co-transfection of p19^{Arf} and the 1.4kb minimal Pdgfrβ promoter-reporter plasmid failed to show consistent repression. Overall our data show that p19^{Arf} controls Pdgfrβ-driven proliferation in the developing eye by downregulation of Pdgfrβ protein levels via a post-transcriptional mechanism. On-going experiments are elucidating mechanisms underlying Pdgfrβ control by p19^{Arf}, and evaluating the importance of the Arf-Pdgfrβ pathway in cancer models.

DECIPHERING THE MOLECULAR EVENTS NECESSARY FOR SYNERGISTIC TUMOR CELL APOPTOSIS MEDIATED BY THE HISTONE DEACETYLASE INHIBITOR VORINOSTAT AND THE BH3 MIMETIC ABT-737.

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Therapeutic resistance afforded over expression of apoptotic pro-survival proteins is observed in over 50% of all cancers. This type of resistance is observed with the histone deacetylase inhibitor Vorinostat. However recently a small molecule inhibitor that neutralizes Bcl-2 pro-survival proteins has been developed called ABT-737. We have been able to show that pre-treatment with Vorinostat followed by further treatment with ABT-737 overcomes Bcl-2-mediated chemo-resistance in both haematological (Eμ-myc lymphomas) and solid tumours (4T1 breast carcinoma). However the molecular mechanisms underpinning the observed synergy were yet to be fully delineated. We observed that transcription of the BH3-only gene Bmf was induced in response to pre-treatment with Vorinostat and that Bmf expression was concomitant with increased acetylation at the Bmf promoter. Utilizing RNA-mediated knockdown of Bmf and Bmf knockout lymphoma cell lines we found that Bmf was essential for the synergistic apoptosis induced by Vorinostat and ABT- 737 in cells expressing high levels of Bcl-2. In defining the mechanism of HDACi-mediated priming we discovered a requirement for a functional p53 pathway for effective synergism. In line with previous studies we confirmed a requirement for the induction of p53 target gene (Noxa) expression for synergistic apoptosis involving ABT-737. Moreover the combined loss of Bmf and p53 expression completely ablated the synergistic apoptosis induced by Vorinostat and ABT-737. To determine the clinical potential of Vorinostat and ABT-737 in combination we undertook preliminary pre-clinical ex vivo therapy studies with human chronic lymphocytic leukemia (CLL) cells. Specific priming by BMF was not observed in the patient sample and could potentially account for the observed marginal resistance to Vorinostat and ABT-737 in this sample. Collectively the findings presented in this thesis provide new insight into the novel molecular mechanisms that underpin apoptosis induced by the combination therapy of Vorinostat and ABT-737. Moreover they demonstrate the potential therapeutic application of Vorinostat and ABT-737 against malignancies expressing high levels of Bcl-2.

URINARY BLADDER CANCER IN ATDC TRANSGENIC MICE

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The incidence of bladder cancer continues to rise and is the fourth most common cancer in men and ninth most common in women with over 70,000 cases diagnosed each year. The genetic profiles of different types of bladder cancer are being characterized with the aim of identifying new targets for therapy. Two genetic profiles have been characterized for different types of proliferative lesions in the bladder. Superficial hyperplastic and papillary lesions of low malignant potential (PUNLMP) are associated with activating mutations in HRAS and FGFR3 overexpression. Invasive carcinomas are associated with changes in p53, Rb, and PTEN pathways. Expression of downstream effectors of p53 such as p21 and upstream regulators such as MDM2 are also common in bladder cancer. The ataxia telangiectasia group D-complementing (ATDC) gene product, also known as TRIM29, is a member of the tripartite motif (TRIM) protein family. The ATDC gene product has been implicated in a variety of cellular processes including cell proliferation. We recently identified ATDC as a gene that is highly over-expresed in pancreatic adenocarcinomas. We also found that ATDC promoted cancer cell proliferation in vitro and enhanced tumor growth and metastasis in vivo via a β-catenin dependent mechanism. ATDC also appears to make pancreatic cancer cells resistant to current therapies. In order to study the oncogenic function of ATDC in vivo, we generated transgenic mice that expressed ATDC under the control of a ubiquitous promoter. Among the tissues that expressed high levels of ATDC was the urinary bladder, more specifically the urothelium. The ATDC transgenic mice developed a number of proliferative and neoplastic lesions but the most common was bladder lesions. Nearly 40% of CAG-ATDC tg mice developed severe bladder obstruction associated with urothelial hyperplasia or bladder cancer. The mice developed the full range of bladder lesions from simple to papillary hyperplasia, PUNLMP, carcinoma in situ, carcinomas, and muscle invasive carcinomas.

ANEUPLOIDY ALTERS THE IMMORTALIZATION PROCESS IN PRIMARY MAMMALIAN CELLS

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Aneuploidy, an incorrect chromosome number, is a hallmark of cancer. While almost all human cancers are aneuploid, the role of aneuploidy in the development of cancer is not well understood. To determine the effects of aneuploidy on cell physiology and growth, we generated a series of primary mouse cell lines that harbor an extra copy of one of four mouse chromosomes. In all trisomic lines analyzed, proliferation was impaired and metabolic properties were altered. To understand how aneuploidy might alter the process of tumorigenesis *in vitro*, the rate of spontaneous immortalization was determined in aneuploid cells and we observed that the rate of immortalization was altered; indicating the barrier of reduced proliferation caused by aneuploidy can be overcome. Presently, we have altered primary aneuploid cells to determine growth potential following genetic events inducing immortalization. We show that the growth defect of primary aneuploid cells is only partially rescued by reducing the expression of p53. Additionally, we observe that the expression of the adenoviral E1a protein alters the fitness of primary aneuploid cells compared to euploid controls. Our data indicate that aneuploidy can influence the efficiency and determine the genetic events required during immortalization primary cells.

GENOME-WIDE RNA-INTERFERENCE SCREEN IDENTIFIES MIR-19 TARGETS IN NOTCH-INDUCED T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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MicroRNAs (miRNAs) have emerged as novel cancer genes. In particular, the miR-17-92 cluster, containing six individual miRNAs, is highly expressed in hematopoietic cancers and promotes lymphomagenesis in vivo. Clinical use of these findings hinges on isolating the oncogenic activity within the 17-92 cluster and defining its relevant target genes. Here we show that miR-19 is sufficient to promote leukemogenesis in Notch1-induced T-cell acute lymphoblastic leukemia (T-ALL) in vivo. In concordance with the pathogenic importance of this interaction in T-ALL, we report a novel translocation that targets the 17-92 cluster and coincides with a second rearrangement that activates Notch1. To identify the miR-19 targets responsible for its oncogenic action, we conducted a large-scale short hairpin RNA screen for genes whose knockdown can phenocopy miR-19. Strikingly, the results of this screen were enriched for miR-19 target genes, and include Bim (Bcl2L11), AMP-activated kinase (Prkaa1) and the phosphatases Pten and PP2A (Ppp2r5e). Hence, an unbiased, functional genomics approach reveals a coordinate clampdown on several regulators of phosphatidylinositol-3-OH kinase-related survival signals by the leukemogenic miR-19.

THE IDENTIFICATION OF EPIGENETIC FEATURES FOR TUMOR SUPPRESSORS AND ONCOGENES IN OVARIAN CANCER TUMOR CELLS

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The identification of chromosomal alterations in primary tumor cells can provide insight into the regulation of the growth of the cancer cell. Epigenetic variations can also affect the cancer phenotype. We provide a coarse-grained bioinformatic analysis of copy number variation and DNA methylation covering the genetic landscape of ovarian cancer tumor cells. We individually identified the copy number variation and DNA methylation for each gene among 42 ovarian cancer tumor samples from our MOMA-ROMA technology and separately analyzed the epigenetic data for another 379 tumor samples presented in The Cancer Genome Atlas. Using the ROMA array method we determined copy number variability per chromosome for each of the 42 ovarian tumor samples. We also provide MOMA derived DNA methylation fold changes from normal to tumor sample for 11,975 genes. We have identified 346 genes with significant deletions or amplifications among the tumor samples. We also predict 156 genes with significantly altered copy number and correlated changes in expression. We show DNA methylation and gene expression dependence for each gene in the entire range of copy number deletion and amplification. And we have predicted 416 genes with either epigenetic tumor suppressor or oncogenic features as a relation to copy number variation. Finally, we apply functional analysis for significantly altered genes and examine possible biological mechanisms of tumorigenesis specific to the ovarian cancer cells.

E2F8 SYNERGIZES WITH RB TO MAINTAIN NORMAL HEMATOPOIESIS AND TO PREVENT HEMOLYSIS

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Rb tumor suppressor plays a critical role in regulating both embryonic hematopoiesis and bone marrow-derived hematopoiesis in mice. Since previous studies have shown that Rb function can be mediated by various E2F activities, we sought to determine whether genetic deletion of individual E2fs can modulate the hematopoietic defects induced by the loss of Rb. We found that among the activating E2F members, E2f2 plays the most important role in mediating Rb function in the control of hematopoiesis. Importantly, concomitant deletion of E2f8, one of the two newly identified repressor E2F family members, significantly exacerbates the hematopoietic defects resulted from the Rb loss. While mice deficient for E2f8 or Rb in hematopoietic stem cells (HSC) experience no or mild hematopoietic defects, mice with HSC deficient for Rb and E2f8 exhibit severe anemia and have substantially enlarged spleens that are almost completely filled with immature erythroids. The severe erythropoietic defects are accompanied by marked multi-lineage cytopenia in the bone marrow and peripheral blood. Furthermore, although both ineffective erythropoiesis and excessive red blood cell destruction resulted from hemolysis may contribute to the severe anemia observed in mice with HSC deficient for Rb and E2f8, hemolysis is only evident in the double knockout mice, suggesting a strong synergy between Rb and E2F8 in preventing hemolysis. Surprisingly, concomitant deletion of E2f7, which has very similar functional domains and biochemical features as E2f8, does not significantly impact on the hematopoietic defects resulted from Rb deficiency. Consistent with the notion that E2f7 makes little contribution to mediating Rb function in the control of hematopoiesis, concomitant deletion of E2f7 in mice with HSC deficient for Rb and E2f8 does not significantly enhance the hematopoietic defects observed in the double knockout mice. Taken together, our data suggest specificity of activating E2F members in mediating Rb function in the control of bone marrow-derived hematopoiesis, and reveal an intriguing synergistic role of Rb and E2F8 (but not E2F7) in maintaining normal hematopoiesis and preventing hemolysis.

IN VIVO RNAI SCREENING IDENTIFIES NEW REGULATORS OF LIVER REGENERATION

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During chronic liver damage repetitive waves of hepatocyte cell death and compensatory proliferation take place, eventually culminating in chronic liver failure and often in the development of hepatocellular carcinoma (HCC). A misregulated regenerative response to chronic liver injury may represent the base for development of HCC. Therefore, a more detailed understanding of signaling pathways involved in proliferation control of hepatocytes not only holds the great promise of informing new therapies to increase the hepatic regenerative potential but also to deduce new strategies for the treatment of HCC. We have established a unique system to perform in vivo RNAi screens to genetically dissect cellular signaling networks regulating hepatocyte proliferation during chronic liver damage. We show that FAH-/- mouse livers can be stably repopulated with complex microRNA based shRNA libraries. Quantification of shRNA representation in shRNA pools and chimeric mouse livers is accomplished by deep sequencing. Assuming that proliferation control during liver regeneration and hepatocyte transformation may engage overlapping signaling pathways, we performed a first screen using a thematically focused shRNA library consisting of 631 constructs targeting 362 genes which were found in focal genomic deletions of 98 human HCCs. Mouse livers were stably repopulated with the shRNA library and after repopulation, mice were subjected to chronic CCl4 treatment to induce liver damage. ShRNA representation in the starting library pool, in the liver after repopulation and after four weeks of CCl4 treatment was deconvoluted using deep sequencing. While the majority of shRNAs did not change representation during liver repopulation or CCl4 mediated liver damage, we identified some shRNAs which showed strong enrichment during regeneration and therefore pinpoint new regulators of liver regeneration. Interestingly, our top scoring candidate represents a kinase, which is accessible to pharmacological inhibition. Functional in vivo validation studies show that stable knockdown of the candidate gene by different shRNAs can significantly increase the repopulation efficiency of mouse hepatocytes and also increases the regenerative capacity of chronically damaged mouse livers. Despite the fact that some human HCCs show focal deletion of the candidate gene, a therapeutic window for regenerative therapy exists, as mice stably repopulated with shRNAs against the candidate did not develop liver tumors even after long term CCl4 challenge.

EVIDENCE THAT AN ALTERNATIVE LENGTHENING OF TELOMERES (ALT)-LIKE *STN1-M1* MUTANT OF *KLUYVEROMYCES LACTIS* MAINTAINS ITS TELOMERE LENGTH THROUGH A ROLL AND SPREAD MODEL.

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The unlimited proliferation of human cancer cells depends on the maintenance of telomere length. This is achieved either by upregulation of telomerase (in 85% of human cancers) or by a telomerase-independent mechanism referred to as Alternative Lengthening of Telomeres (ALT) (in 5-10% of human cancers). Although telomere elongation in ALT appears to be recombination dependent, details of the mechanism are not clear. Studies using the *stn1-M1* mutant of the yeast *K. lactis*, which has many parallels with ALT cells, may provide information for understanding the mechanism of telomere lengthening of ALT cancers. The similarities between *stn1-M1* cells and ALT cells include very long and heterogeneous telomeres, elevated level of telomere recombination and production of telomeric circles (t-circles).

Our work has now provided several new lines of evidence that support that recombinational telomere elongation in newly generated *stn1-M1* cells involves the Roll and Spread mechanism, where a t-circle is used as a template to make an initial long telomere with other telomeres becoming lengthened by copying its sequence. First, sequences from a transformed DNA circle containing telomeric repeats could be copied onto telomeres of *stn1-M1* cells in a tandem repeating manner. Second, formation of the long telomere phenotype in cells with telomeres tipped with composition of mutationally-tagged telomeric repeats commonly produced repeating patterns of the two repeat types. Third, studies using cells with a single telomere composed entirely of mutationally-tagged repeats has shown that the sequence from a single elongated telomere could be copied onto other telomeres via a Break Induced Replication (BIR) like event. Our findings support that the telomere lengthening in *stn1-M1* mutant may often occur through a Roll and Spread model and may suggest that formation of the ALT state could occur through a similar process.

INVESTIGATING THE NF-κB PATHWAY AS A NOVEL THERAPEUTIC TARGET IN PRE-CLINICAL LUNG CANCER MOUSE MODELS

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Lung cancer represents the most frequent cancer type and the leading cause of cancer death (1.3 million worldwide). Non-small cell lung cancer (NSCLC) accounts for 80% of all lung cancer cases. NSCLC development is associated with frequent mutations in well-defined oncogenes and tumor suppressor genes such as Kras (30%) and p53 (50-70%). We recently demonstrated that hyper-activated NF-κB pathway in NSCLC is required for the initiation and maintenance of lung cancer, indicating this pathway as a promising therapeutic target. In this study, we tested therapeutic delivery of proteasome inhibitor Bortezomib (Velcade) to inhibit NF-κB pathway in NSCLC mouse models. In mouse lung cancer cell lines harboring KrasG12D and p53 loss (KP cells), Bortezomib efficiently reduced nuclear p65, repressed NF-κB target genes such as Bcl2 and rapidly induced apoptosis. The cell lines with high NF-κB activity are especially sensitive to Bortezomib. Using autochothonous mouse models and microCT imaging, we showed that Bortezomib treatment at a well-tolerated dose leads to lung tumor regression in mice and prolonged survival. Our results demonstrated using preclinical mouse models to study cancer treatment response and supported NF-κB pathway as an effective therapeutic target in lung cancer.

LOSS OF HISTONE DEMETHYLASE RBP2 SUPPRESSES TUMORIGENESIS

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Aberrations in epigenetic processes, such as histone methylation, can lead to cancer. The JmjC domain protein Retinoblastoma Binding Protein 2 (RBP2) is capable of demethylating tri- and di-methylated lysine 4 in histone H3, which are epigenetic marks for transcriptionally active chromatin. Here we show that loss of RBP2 demethylase activity induces senescence and inhibits proliferation of mouse embryonic fibroblasts in a pRB-dependent manner. Furthermore, loss of RBP2 promotes the differentiation of murine myoblasts and embryonic stem cells in vitro. Genetic ablation of Rbp2 decreased tumor formation, and prolonged survival, in $Rb1^{+/-}$ mice. These studies provide insights into the roles of histone demethylase RBP2 in tumorigenesis and nominate RBP2 as a potential target for cancer therapy.

NETRIN-1 PROMOTES INVASIVENESS AND SURVIVAL OF HUMAN GLIOBLASTOMA CELLS

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Netrin-1 is a secreted extracellular matrix protein. It was discovered as an important regulator of embryogenic axon guidance. Recently it has been observed to act as a survival factor for different forms of cancer such as aggressive neuroblastoma, metastatic breast cancer and non small cell lung cancer. It also promotes tumorigenesis of colorectal cancer. According to microarray data on Oncomine database netrin-1 is upregulated in glioblastoma, most severe human brain cancer. So far, no efficient treatment has been discovered for glioblastoma. Glioblastoma is very invasive and aggressive cancer which invades diffusively among normal brain tissue and is therefore impossible to remove surgically. It is also resistant to chemotherapy. We analyzed the effects of netrin-1 on glioblastoma cells. According to Matrigel invasion assays overexpression of netrin-1 increased the invasiveness of human glioblastoma cells whereas partial knock-down of netrin-1 by shRNAs reduced the invasiveness. In wound healing assays netrin-1 overexpression increased and partial knockdown decreased migration of human glioblastoma cells. On the other hand, complete knock-down of netrin-1 led to apoptotic death of human glioblastoma cells. Our results suggest that netrin-1 is an important regulator of glioblastoma tumorigenesis and that the inhibition of netrin-1 signaling may prove out to be a treatment option for human glioblastoma.

INHIBITION OF HISTONE ACETYLATION BY JUN DIMERIZATION PROTEIN 2 (JDP2) INVOLVES IN SUPPRESSION OF CELL CYCLE PROGRESSION THROUGH DOWN-REGULATION OF CYCLIN A2

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Progression of the cell cycle in mammalian cells is regulated by the interplay of cyclin-dependent kinases (CDKs) and their respective cyclin partners, which act as positive coactivators or as negative regulators in the case of the so-called cdk inhibitors. Cyclin A is a rate-limiting component required for both the initiation of DNA synthesis and entry into mitosis. Jun dimerization protein 2 (JDP2), a member of the AP-1 family, is able to form homodimers, and, also, heterodimers with other members of the AP-1 family, such as c-Jun, JunB, JunD and ATF2, and with a member of the C/EBP family, C/EBPγ. JDP2 most likely participates in the repression of transcription via multiple mechanisms, which include DNA-binding competition and inactivation of formation of heterodimer with other members of the AP-1, recruitment of HDAC 3, inhibition of histone acetylation and the direct regulation of chromatin assembly (1). However, the details of the physiological role of JDP2 in cell fate remain unknown, and the mechanisms by which JDP2 acts as a regulator of the proliferation or transformation of cells remain to be clarified. We reported that "knock-out" of Jdp2 affects adipocyte differentiation (2) and resistance to replicative senescence (3).
We report here a novel role for JDP2 as a regulator of the progression of normal cells through the cell cycle. The healing of wounded skin of Jdp2 knockout mice (KO) mice proceeded more rapidly than that of control mice and more proliferating cells were found at wound margins. Fibroblasts derived from embryos of Jdp2KO mice proliferated more rapidly and formed more colonies than wild-type fibroblasts. JDP2 was recruited to the promoter of the gene for cyclin A2 (ccna2) at a previously unidentified AP-1 site. Cells lacking Jdp2 had elevated levels of cyclin A2 mRNA. Moreover, reintroduction of JDP2 resulted in repression of transcription of ccna2 and of cell cycle progression. Thus, transcription of the gene for cyclin A2 appears to be a direct target of JDP2 in the suppression of cell proliferation.

Refs; (1) C. Jin et al., Nat. Struc. Mol. Biol. 13, 331-338 (2006), (2) Nakade et al., Cell Death & Differ. 14, 1398-1405 (2007), (3) Nakade et al., J. Biol. Chem. 284, 10808-10817 (2009).

H3K9ME3-BASED CHIP-ON-CHIP ANALYSIS UNVEILS GLOBAL FUNCTIONAL RE-PROGRAMMING IN RAS-DRIVEN CELLULAR SENESCENCE

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Senescence is a permanent G1 cell-cycle arrest that can be triggered by acute stresses such as activated oncogenes and chemotherapeutic agents. In company with apoptosis, senescence serves as a barrier to cancer development. Senescent cells are metabolically active and exhibit distinct chromatin features compared with proliferating cells. Rb-associated nuclear complexes were reported to lead to chromatin remodeling in response to oncogenic Ras. Recruitment of a histone H3 lysine 9 methyltransferase into such complexes promotes the formation of senescence-associated heterochromatin foci, in which trimethylated H3K9(H3K9me3) imposes a repressive histone mark. Indeed, the enrichment of H3K9me3 on E2F-responsive growth promoting (mostly "S-phase") genes explains the firmly cell-cycle arrest status of senescent cells. However, senescent cells not only exhibit a cell-cycle block, but also display other functional capabilities, for example a senescence-associated secretory phenotype(SASP) and features of autophagy. Therefore, the senescent state – as controlled by a global chromatin remodeling process – reflects a functionally reprogrammed cell condition.

Here, we performed promoter tiling analysis on H3K9me3-immunoprecipitated lysates("ChIP-on-chip") from human diploid fibroblasts, upon transduction with oncogenic Ras vs. non-senescent empty vector-infected cells. As a result, we identified 193 genes whose H3K9me3 enrichment level was significantly changed(>2-fold). We also analyzed gene expression profiles on Affymetrix transcriptome chips. 50 genes exhibited significant changes(>2-fold), out of which 36 transcripts presented changes consistent with the ChIP-on-chip data. Gene ontology analysis clustered the 36 genes into several functional groups, including cell-cycle control, transcription regulation, protein metabolism, signal transduction and apoptosis. Notably, several candidates with reported effect on crucial signaling pathways such as NF-κB or Jak/Stat potentially serve as the pivotal regulators controlling SASP and other novel gain-of-function capabilities. We are currently conducting functional studies on these candidates and generating additional data by "ChIP-Seq" on drug-induced senescent lymphomas with and without H3K9me3 defects. In essence, our data indicate that genome-wide changes of senescence-associated H3K9me3 are informative regarding a functionally profoundly re-programmed phenotype of the senescent condition beyond a mere cell-cycle arrest.

SENESCENCE-ASSOCIATED DISASSOCIATION OF BRCA1 FROM CHROMATIN PROMOTES SENESCENCE ESCAPE.

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Cellular senescence suppresses tumorigenesis. Paradoxically, senescence inducers (such as activated oncogenes and tissue aging) are often known to promote cancer. Here we report a novel cell-intrinsic mechanism that promotes senescence while predisposes cells to senescence escape by allowing for secondary hits. We show that senescence inactivates the BRCA1 DNA repair complex by disassociating BRCA1 from chromatin. This event precedes senescence-associated cell cycle exit and coincides with DNA damage accumulation. Mechanistically, downregulation of BRIP1, a physiological partner of BRCA1, triggers BRCA1 chromatin disassociation. Conversely, ectopic BRIP1 rescues BRCA1 chromatin disassociation and suppresses senescence. Significantly, cells undergoing senescence do not exhibit a BRCA1-dependent DNA damage repair response when exposed to DNA damage, and DNA damage promotes senescence escape. These data place the altered BRCA1 DNA repair complex at the heart of senescence regulation, and provide a molecular basis whereby senescence inducers select for mutations that overcome senescence and ultimately lead to cancer.

THE PRB-SKP2-P27T187P TUMOR-SUPPRESSING PATHWAY DIVERGES AT P27T187P IN NEUROGENESIS .

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We recently showed that the pRb-Skp2-p27T187p pathway plays essential survival roles in tumorigenesis following loss of pRb in susceptible cells (Wang et al 2010, Nature Genetics). In addition to tumor suppression, pRb plays essential roles in normal development as Rb1-/- embryos die in midgestation. In this meeting, we will report our studies of the pRb-Skp2-p27T187p pathway in embryogenesis to determine whether the synthetic lethal relationships between Rb1 loss and Skp2 loss, and between Rb1 loss and p27T187A mutation during tumorigenesis are applicable to embryogenesis. We are studying this topic with germ line knockout, embryo proper knockout (Meox2-Cre), and tissue-specific knockout (Nestin-Cre) to determine the roles of the pRb-Skp2-p27T187p pathway at multiple levels. Our results so far demonstrate significant roles of Skp2 in Rb1-/- embryos consistent with its role in pituitary tumorigenesis following Rb1 loss. Rb1-/-Skp2-/- embryos die two days earlier than Rb1-/-Skp2+/+ embryos with massive apoptosis in neuroepithelium, providing the first case that inactivating a pRb target worsens Rb1-/- embryogenesis. The massive apoptosis in neuroepithelium appears cell autonomous as it is reproduced with targeted Rb1 deletion by Nestin-Cre. Downstream of Skp2, the pathway diverges from what was seen in pituitary tumorigenesis. p27T187A knockin does not shorten Rb1-/- embryo viability, while still lead to massive apoptosis in neuroepithelium as well as in other organs such as the lung. The massive apoptosis in neuroepithelium however is not reproduced with targeted Rb1 deletion by Nestin-Cre. These results establish that Skp2 also plays essential survival roles following Rb1 loss in susceptible cells during normal development, but how Skp2 functions in this capacity diverges. In some cells, Skp2 still functions through the SCFSkp2-p27T187p mechanism, in others, Skp2 appears to function via other mechanisms. Further genetic and biochemical studies are ongoing to delineate and compare the functional roles and mechanisms of the pRb-Skp2-p27T187p pathway in tumorigenesis and embryogenesis.

P53 LOSS PROMOTES ACUTE MYELOID LEUKEMIA BY ENABLING ABERRANT SELF-RENEWAL

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The p53 tumor suppressor limits proliferation in response to cellular stress through several mechanisms. Here, we test whether the recently described ability of p53 to limit stem cell self-renewal suppresses tumorigenesis in acute myeloid leukemia (AML), an aggressive cancer where p53 mutations are associated with drug resistance and adverse outcome. Our approach combined mosaic mouse models, Cre-lox technology and in vivo RNAi to disable p53 in concert with endogenous KrasG12D – a common AML lesion that promotes proliferation but not self-renewal. We show that p53 inactivation strongly cooperates with oncogenic KrasG12D to induce aggressive AML, while both lesions on their own induce T-cell malignancies with long latency. This synergy is based on a pivotal role of p53 in limiting aberrant self-renewal of myeloid progenitor cells, such that loss of p53 counters the deleterious effects of oncogenic Kras on these cells and enables them to indefinitely self-renew. Consequently, myeloid progenitor cells expressing oncogenic Kras and lacking p53 become leukemia-initiating cells, resembling cancer stem cells capable of maintaining AML in vivo. Our results establish an efficient new strategy for interrogating oncogene cooperation, and provide strong evidence that the ability of p53 to limit aberrant self-renewal contributes to its tumor suppressor activity.

PROTEIN TYROSINE KINASE 6 REGULATES AKT ACTIVATION BY PHOSPHORYLATING AKT ON TYROSINE RESIDUES 315 AND 326

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Protein tyrosine kinase 6 (PTK6) is a nonmyristoylated Src-related intracellular tyrosine kinase, which is localized to different cellular compartments including the nucleus. Although not expressed in the normal mammary gland, PTK6 is expressed in a majority of human breast tumors examined, and its expression has been correlated with ErbB2 expression in human breast cancer. Expression of PTK6 is also upregulated in other types of cancer including colon cancer, ovarian cancer, head and neck cancer and metastatic melanoma. PTK6 has been linked to ErbB receptor signaling and AKT activation in breast cancer cell models, but the exact role of PTK6 in regulating AKT activation is not clear. We demonstrate that AKT is a direct substrate of PTK6, and that AKT tyrosine residues 315 and 326 are phosphorylated by PTK6. Association of PTK6 with AKT occurs through the SH3 domain of PTK6, and is enhanced through SH2 domain mediated interactions following tyrosine phosphorylation of AKT. Using Src, Yes, and Fyn null mouse embryonic fibroblasts (SYF cells), we show that PTK6 phosphorylates AKT in a Src-family kinase independent manner. Introduction of PTK6 into SYF cells sensitized these cells to physiological levels of EGF and increased AKT activation. Stable introduction of active PTK6 into SYF cells also resulted in increased proliferation. Knockdown of PTK6 in BPH-1 human prostate epithelial cell line led to decreased AKT activation in response to EGF. Our data indicate that in addition to promoting growth factor receptor mediated activation of AKT, PTK6 can directly activate AKT to promote oncogenic signaling. These studies provide insight about the potential benefits of targeting PTK6 as part of a therapeutic regime to treat different types of cancer that have up-regulated PTK6 expression and activity.

NEURAL STEM CELLS AND CANCER STEM CELLS.

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It is only in the last decade that the existence of self-renewing cells in the brain has become fully appreciated. As a consequence, classic models of tumor development in the nervous system have invoked dedifferentiation as a requisite for tumor initiation. New concepts have arisen, however, with the continuing study of “adult” neural stem cells in vivo and in vitro. For example, preparation of neurosphere cultures from primary glioma tissue from human tumors and mouse genetic models permits detailed analysis and the hope for a full molecular understanding of these cells and how they compare to normal stem cells. A central question remains the identification of the cell of tumor origin. Our own work using tumor suppressor models of gliomagenesis or neurofibromas leads us to propose that the cell of origin in these tumors is an early progenitor cell or possibly the primary stem cell. I will discuss the nature of these mouse models, recent advances, how we hope to resolve our current working hypothesis, and implications for sporadic human cancer. If indeed stem or progenitor cells give rise to glioma, it becomes critical to fully understand their properties and physiological roles. Importantly, the ability to isolate these stem/progenitor cells and expand them in culture gives us a powerful resource for high-throughput screens that could yield potentially novel therapies for cancer treatment.

SUPPRESSION OF LUNG ADENOCARCINOMA PROGRESSION BY NKX2-1

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Despite the high prevalence and poor outcome of patients with metastatic lung cancer, the mechanisms of tumor progression and metastasis remain largely uncharacterized. We modeled human lung adenocarcinoma, which frequently harbors activating point mutations in KRAS and inactivation of the p53-pathway, using conditional alleles in mice. Lentiviral-mediated somatic activation of oncogenic Kras and deletion of p53 in the lung epithelial cells of $Kras^{LSLG12D/+};p53^{flox/flox}$ mice initiates lung adenocarcinoma development. Although tumors are instigated synchronously by defined genetic alterations only a subset become malignant, suggesting that disease progression requires additional alterations. We developed a method, based on identification of the lentiviral integration sites, to distinguish metastatic from non-metastatic tumors and determined the gene expression alterations that distinguish these tumor types. Cross-species analysis identified the NK-2 related homeobox transcription factor, Nkx2-1 (Ttf-1/Titf1) as a candidate suppressor of malignant progression. In our model, downregulation of Nkx2-1 is pathognomonic for high-grade poorly differentiated lung adenocarcinoma. Gain- and loss-of-function experiments in cells derived from metastatic and non-metastatic tumors demonstrate that Nkx2-1 controls tumor differentiation and limits metastatic potential *in vivo*. Interrogation of Nkx2-1 regulated genes and analysis of tumors at defined developmental stages indicates that Nkx2-1 constrains progression in part by repressing the embryonically-restricted transcriptional regulator Hmga2. While focal amplification of NKX2-1 in a fraction of human lung adenocarcinomas has focused attention on its oncogenic function, our data specifically link Nkx2-1 downregulation to loss of differentiation, gain of stem cell characteristic and enhanced metastatic proclivity. Thus, the oncogenic and suppressive functions of Nkx2-1 in the same tumor type substantiate its role as a dual function lineage factor.

MODELING OF ONCOGENE ADDICTION IN TRANSGENIC MOUSE MODELS

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The targeted inactivation of oncogenes can elicit sustained tumor regression, associated with the phenomenon that has been described as oncogene addiction. Utilizing conditional transgenic mouse models, we have gleaned some insight into the mechanisms of oncogene addiction illustrating that upon oncogene inactivation tumors undergo proliferative arrest, apoptosis, differentiation and/or senescence. The specific consequences of oncogene addiction appear to depend upon both cellular and genetic context and both tumor cell intrinsic and host-dependent mechanisms appear to be critical. Recently, we have utilized a conditional mouse model of MYC induced T-acute lymphoblastic lymphoma to dissect the role of host immune effectors in tumor regression associated with MYC inactivation. We have found that defects in host immune effectors cause a dramatic reduction in the kinetics, degree and duration of tumor elimination. Moreover, we have found that upon MYC inactivation immune cells are recruited to the tumor site. Finally, we have identified that discrete immune effectors are required for MYC inactivation to induce cellular senescence in tumor cells and the shut down of angiogenesis in the host. We have found that the adoptive reconstitution of cellular effectors can restore the ability of MYC inactivation to induce sustained tumor regression. Our results illustrate that oncogene addiction is not cell autonomous and that the interaction between the host and tumor microenvironment may play a critical role in the mechanism by which oncogene inactivation elicits tumor regression. We will describe how specific immune effectors and specific secreted cytokines appear to be essential to regulate the tumor microenvironment. We speculate that targeted oncogene inactivation leads to tumor regression through mechanisms that require a restoration of the normal tissue micronenvironment.

KRAS-MEDIATED MODULATION OF THE IMMUNE RESPONSE IN PANCREATIC NEOPLASIA.

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It is becoming increasingly clear that stromal responses elicited by early-stage neoplastic lesions can promote tumor progression towards a more invasive and potentially metastatic state. However, the molecular mechanisms that underlie the early recruitment of stroma, and in particular immune cells, to sites of neoplasia remain poorly understood. To address this question in the context of pancreatic adenocarcinoma, we make use of orthotopic pancreatic grafts of primary murine pancreatic ductal epithelial cells (PDEC) harboring oncogenic KRas (KRasG12D). We find that engrafted KRasG12D-expressing PDEC can elicit a significant inflammatory response. The nature and composition of inflammatory infiltrates was assessed using FACS sorting as well as immunohistochemical techniques and was directly compared to those derived from p48-Cre;LSL-KRasG12D mice. Our observations to date indicate that the expression of KRasG12D promotes the recruitment of immunosuppressive leukocytes in the absence of a notable increase in the cytotoxic T cell population. These results support the hypothesis that effective tumor immunity mediated by an adaptive T cell response is actively suppressed at the very early stage of pancreatic neoplasia. We are currently investigating the molecular mechanisms by which oncogenic KRas modulates the intratumoral immune response.

DNA DAMAGE-MEDIATED INDUCTION OF A CHEMORESISTANT NICHE

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While numerous cell-intrinsic processes are known to play decisive roles in chemotherapeutic response, relatively little is known about the impact of the tumor microenvironment on therapeutic outcome. Here, we use a well-established mouse model of Burkitt's lymphoma to show that paracrine factors in the tumor microenvironment modulate lymphoma cell survival following the administration of the genotoxic chemotherapeutic doxorubicin. Specifically, IL-6 and Timp-1 are released in the thymus in response to DNA damage, creating a "chemo-resistant niche" that promotes the survival of a minimal residual tumor burden and serves as a reservoir for eventual tumor relapse. Disruption of this chemo-protective cytokine signaling or ablation of the protective microenvironment potentiates the action of doxorubicin, as assessed by both lymphoma cell survival and disease progression. Notably, IL-6 is released acutely from thymic endothelial cells in a p38-dependent manner following genotoxic stress, and this acute secretory response precedes the gradual induction of senescence in tumor-associated stromal cells. Thus, conventional chemotherapies can induce tumor regression while simultaneously eliciting stress responses that protect subsets of tumor cells in distinct anatomical locations from drug action.

TUMOR SUPPRESSOR FUNCTION OF LKB1 IS LINKED TO THE CONTROL OF POLARIZED EPITHELIAL ARCHITECTURE

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Cellular organization into epithelial architecture maintains structural integrity and homeostasis by suppressing cell proliferation and apoptosis. We have previously shown that differentiated epithelial organization of MCF10A 3D acini blocks the ability of c-Myc to re-initiate cell cycle progression and induce transformation. This epithelial architecture imposed restraint to the oncogenic c-Myc is dismantled upon silencing of LKB1, the mammalian homologue of polarity protein PAR4 (Partanen et al., PNAS 2007). We have further explored the role of LKB1 in formation and maintenance of epithelial architecture in vivo using mice, in which Lkb1 alleles can be conditionally deleted in the luminal epithelial cells of mammary gland. We show that loss of Lkb1 induces gross alterations in the epithelial architecture of 3D structures and in the mammary gland as manifested by loss of cell polarity, defects in the basement membrane structure and spontaneous hyperbranching. Loss of Lkb1, when combined with mammary specific overexpression of c-Myc, led to dramatic acceleration of c-Myc-induced mammary tumorigenesis. These mice exhibit increased tumor multiplicity and volume, and in addition the tumors show histopathological features distinct from Myc-induced mammary tumors. Furthermore, the cooperation of Lkb1 loss and c-Myc activation resulted in strikingly transformed 3D acini, which allows studies on molecular mechanisms underlying the synergy. In conclusion, our data show evidence that LKB1 has a crucial role in formation of epithelial architecture and apicobasal polarity and that loss of LKB1 significantly cooperates with activated c-Myc oncogene in tumorigenesis.

THE OSTEOCLAST DIFFERENTIATION FACTORS RANKL/RANK CONTROL DEVELOPMENT OF PROGESTIN-DRIVEN MAMMARY CANCER

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Breast cancer is one of the most common cancers in humans and will on average affect up to one in eight women in their life time in the US and Europe. The Women's Health Initiative (WHI) and the Million Women Study have shown that hormone replacement therapy (HRT) is associated with an increased risk of incident and fatal breast cancer. In particular synthetic progesterone derivatives (progestins) such as medroxyprogesterone acetate (MPA), used in millions of women for HRT and contraceptives, markedly increase the risk of developing breast cancer. Here we report that in vivo administration of MPA triggers massive induction of the key osteoclast differentiation factor Receptor Activator of NF-κB Ligand (RANKL) in mammary gland epithelial cells. Genetic inactivation of the RANKL receptor RANK in mammary gland epithelial cells prevents MPA-induced epithelial proliferation, impairs expansion of the $CD49f^{hi}$ stem cell-enriched population, and protects these cells from DNA damage induced cell death. Importantly, RANK deletion in the mammary epithelium results in a markedly reduced incidence and delayed onset of MPA-driven mammary cancer. Mechanistically, RANK/RANKL increases the self-renewal capacity of mammary cancer stem cells and induces anchorage-independent growth of breast cancer cells. Interestingly, whereas RANKL-RANK triggers osteoclastogenesis via both NF-κB and NFATc1, MPA-driven mammary cancer was only delayed in mice that carry IKKα, but not NFATc1, deletions in the mammary epithelium. These data identify the essential osteoclast differentiation factors RANKL/RANK as a key pathway that controls the incidence and onset of progestin-driven breast cancer.

IDENTIFICATION OF PHLPP AS A TUMOUR SUPPRESSOR REVEALS THE ROLE OF P53 AS A GATEKEEPER OF LETHAL PROSTATE CANCER PROGRESSION

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Hyper-activation of the PI 3-Kinase/ AKT pathway is common in many cancer types. Tumourigenesis through this pathway is prevented by concerted action of multiple tumour suppressor genes. Most notably, PTEN reverts PI 3-Kinase activity while excessive pathway activation triggers the p53-mediated senescence response, which constitutes a fail-safe mechanism against cancer. However, it remains ill defined if & at what stage this response acts in human prostate cancer. Here we identify the AKT-inactivating phosphatase PHLPP as a tumour suppressor & demonstrate that the p53-response prevents co-deletion of PTEN & PHLPP to form a barrier against progression to aggressive prostate cancer. We show that Phlpp-loss causes neoplasia & upon partial Pten-loss, carcinoma in mouse prostate. In this setting, Phlpp-deficiency shifts active Akt localisation from cytoplasm to the membrane & triggers growth arrest via mTor-dependent translation of p53. As a consequence, we find that co-deletion of Pten & Phlpp requires inactivation of p53 in prostate, as shown by its spontaneous mutation & loss of p21 transcription. Finally, we validate this conditional gene inactivation scheme for prostate in a gene-copy number & expression dataset on 218 patient samples. Surprisingly, we find that co-deletion of the PTEN & PHLPP loci is almost exclusively observed in metastatic prostate cancer & in complete agreement with our results from mouse, is tightly correlated to deletion of TP53. Our findings demonstrate how combining large-scale human prostate cancer genomics with mouse genetics allows for discovery of progression principles that will help identify patients at risk for developing lethal disease through molecular signatures. Identification of PHLPP-loss as a critical determinant of metastatic disease progression highlights the potential of drug-based inhibition of the mTORC2 kinase complex, which directly reverts PHLPP phosphatase activity on AKT.

MATRIX METALLOPROTEINASE-DEPENDENT REMODELING OF THE COLLAGEN SCAFFOLD REGULATES MAMMARY EPITHELIAL INVASION

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Type I collagen is the most abundant extracellular matrix protein, providing a scaffold for organs. Its expression is dramatically increased in primary tumors of metastatic cancer, yet, degradation of the collagen scaffold is necessary for epithelial invasion. By targeting both type I collagen and the matrix metalloproteinases (MMPs) that cleaves it, we show here that stromal cleavage of collagen by MMPs controls ductal epithelial invasion in mammary gland development. Epithelial invasion is reduced in the absence of stromally expressed MMP14 (MT1-MMP), when collagen is MMP-resistant, or when collagen is hypersensitive to non-MMP mediated proteolysis. Therefore, epithelial ductal invasion is reduced when MMP-dependent collagen remodeling is reduced. To address the role of collagen remodeling for epithelial invasion in cancer, we cross-bred MMTV-PyMT mice, which develop metastatic mammary carcinoma, with mice with MMP-resistant collagen. Primary tumor size was not affected, but metastasis to the lungs was significantly reduced. Thus, MMP-specific remodeling of the collagen scaffold regulates epithelial invasion in development and cancer.

THE ROLE OF THE RAC-SPECIFIC GEF, P-REX1, DURING DEVELOPMENT AND MELANOMA PROGRESSION

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The incidence of malignant melanoma has continued to rise over the past several decades. It is the most deadly form of skin cancer primarily due to treatment resistant metastases. The progression to melanoma is driven by activating mutations in either BRAF (60%) or NRAS (30%) that initiate the formation of nevi, some of which convert to full blown melanoma accompanied by further genetic and epigenetic changes in gene expression; most commonly the loss of tumour suppressors p16INK4A or PTEN, which facilitate an escape from senescence and an increase in survival and invasion, respectively. There may be a link between the highly migratory behavior of neural crest derived melanocyte precursors during development and the propensity of melanomas to metastasize early. Rho-family GTPases determine the mode of motility that human melanoma-derived cells use to migrate through 3D ECM. Rho regulates amoeboid migration, whilst Rac regulates mesenchymal migration. The Rac-specific GEF, P-Rex1, though not expressed in human melanocytes in tissue culture, is expressed in most human melanoma derived cell lines, where it is necessary for the mesenchymal mode of 3D migration. P-Rex1 is not detectable by immunohistochemical staining of normal skin, but is detected in most nevi, primary melanomas and metastases. Mouse models are being used to investigate the role of P-Rex1 during melanocyte development and melanoma progression. C57/Black P-Rex1-/- mice have a melanocyte migration defect evidenced by a white belly stripe resulting from retarded melanoblast migration observed by the location of DCT-LacZ melanocyte precursor cells in 15.5 day embryos. Mouse melanoma models driven by tyrosinase (Tyr)-NRASQ61K, or Tyr-CRE recombinase activated BRAFV600E expression have been used in conjunction with P-Rex1-/- mice. Cutaneous melanomas develop with equal frequency and similar latency in Tyr-NRAS, INK4A-/- mice regardless of the presence or absence of P-Rex1, however progression to metastasis is dramatically suppressed in P-Rex1-/- mice (P=0.001).
P-Rex1-/- mice with melanomas had a significant increase in survival (P=0.037). We conclude that P-Rex1 plays a significant role in melanoblast migration during development and progression from primary melanoma to metastasis.

CD95/FAS AND NFκB SIGNALING MODULATE DEPENDENCE OF LUNG CANCERS ON MUTANT EGFR

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Human lung adenocarcinomas harboring activating mutations in the epidermal growth factor receptor (EGFR) often regress when treated with EGFR tyrosine kinase inhibitors (TKIs) but the clinical benefit of EGFR TKIs is variable and transient. We hypothesized that the heterogeneity of treatment response may be explained by genetic modifiers of dependence on mutant EGFR. Through a pooled RNA interference screen, we identified 36 genes that, when knocked down, specifically enhance cell death induced by the EGFR TKI erlotinib. Curiously, silencing of many of these genes, such as the TNF receptor family member CD95/Fas, promoted growth in conjunction with mutant EGFR in the absence of erlotinib. FAS-mediated activation of NFκB, through the intermediate signaling molecules FLIP, IKK and IκB, was necessary and sufficient to confer erlotinib resistance in EGFR mutant tumor cells. Furthermore, combined inhibition of NFκB and EGFR resulted in enhanced apoptosis in erlotinib-sensitive and erlotinib-resistant EGFR-mutant lung cancer models. These findings provide novel insights into the mechanisms by which tumor cells acquire oncogene dependence and subsequently escape from oncogene inhibition.

ASSESSING THERAPEUTIC RESPONSES IN PANCREATIC CANCER USING GENETICALLY ENGINEERED MOUSE MODELS

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Pancreatic adenocarcinoma (PDAC) represents a significant unmet medical need with a high failure rate experienced in clinical trials, underscoring a need for predictive preclinical models of therapeutic response in this disease. Genetically engineered mouse models (GEMMs) of cancer are extensively used to mimic human tumorigenesis but despite their potential, there is limited evidence of their utility in preclinical drug development. To address this deficiency, we recently examined responses to existing standard-of-care chemotherapy with gemcitabine and the targeted EGFR inhibitor erlotinib (alone and in combination) in a mutant Kras-driven GEMM of pancreatic cancer: *Kras*$^{LSL\text{-}G12D}$*; p16/p19*$^{fl/fl}$*; Pdx1-Cre*. Comparisons of progression-free and overall survival patterns observed in corresponding clinical trials indicate that the GEMM appropriately models human responses. To evaluate new treatment strategies for pancreatic cancer, we then assessed responses in this model to anti-angiogenic therapy starting with anti-VEGF alone and in combination with gemcitabine and erlotinib. Bevacizumab (Avastin®) in combination with gemcitabine was not efficacious in human pancreatic cancer, unlike the positive results observed with this combination in human pancreatic xenografts. In the PDAC GEMM, the combination of gemcitabine and anti-VEGF resulted in partial efficacy (as compared to gemcitabine alone), and therapy with the triple combination of erlotinib, gemcitabine, and anti-VEGF resulted in an incremental benefit recapitulating recent results seen with the latter regimen in the clinic. We then tested the hypothesis that pancreatic tumor vasculature may be sustained by additional angiogenic factors along with VEGF by treating tumor-bearing GEMMs with a novel inhibitor of tumor angiogenesis. When this new agent was combined with anti-VEGF, we observed a significant impact on tumor growth and on overall survival. The triple combination of gemcitabine, anti-VEGF, and this novel therapy was significantly efficacious in comparison to gemcitabine treatment alone, implicating a new therapeutic target that is required for pancreatic tumor progression and angiogenesis. These results will be presented along with analyses of treatment effects on tumor vasculature and metastasis. These studies lay the foundation for the use of this model in predicting clinical outcomes as well as interrogating mechanisms of therapeutic response and resistance.

THE IMPACT OF DEFINED *BRCA1* MUTATIONS ON TUMOR DEVELOPMENT, DRUG RESPONSE AND ACQUIRED RESISTANCE.

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Women with heterozygous germline mutations in *BRCA1* have a strongly increased lifetime risk of developing breast and/or ovarian cancer. BRCA-related cancers show a good response to DNA-damaging agents, like platinum compounds. But resistance to platinum agents is still a serious problem in the treatment of BRCA-related cancers. In the human situation, genetic reversion of the *Brca1* mutation may serve as an important mechanism underlying the induction of platinum resistance. Strikingly, mammary tumors arising in the *K14Cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ mouse model (which lack *Brca1* exons 5-13) never become resistant against platinum drugs, suggesting that (partial) BRCA1 function is required for platinum resistance. To investigate whether BRCA1 function is causally related to acquired platinum resistance, mice carrying various *Brca1* mutations were bred with *K14Cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ mice. One of these mutations is *Brca1*^{C61G}, which is a pathogenic missense mutation disrupting the interaction between BRCA1 and BARD1 and thereby the E3 ubiquitin ligase activity of BRCA1. Mammary tumor development was observed in the *K14Cre;Brca1*$^{C61G/F}$*;p53*$^{F/F}$ model. Sequencing revealed that these mammary tumors still carried the *Brca1*^{C61G} mutation. *Brca1*$^{C61G/del}$*;p53*^{del} mammary tumors displayed similar characteristics as *Brca1*^{del}*;p53*^{del} tumors, like "triple-negativity" and genomic instability. Spontaneous *Brca1*$^{C61G/del}$*;p53*^{del} mammary tumors were transplanted orthotopically into immunocompetent mice and intervention studies with platinum compounds were performed. In contrast to *Brca1*^{del}*;p53*^{del} tumors, resistance to platinum agents was observed for various independent *Brca1*$^{C61G/del}$*;p53*^{del} tumors. Thus far, we have not observed genetic reversion of the *Brca1*^{C61G} mutation as a mechanism of platinum resistance. We are currently investigating which other mechanisms can cause resistance to platinum agents in these tumors. Identical experiments are performed for tumors carrying to other *BRCA1* founder mutations, respectively the *Brca1*^{185delAG} and *Brca1*^{5382insC} mutation. This research could reveal the most important mechanisms of platinum resistance in BRCA-related cancers and show possible variation in sensitivity to platinum drugs of different *BRCA1* mutations. These novel mouse models could offer an ideal platform to test new treatment strategies for *BRCA1* mutation carriers. This insight could lead to various treatment strategies for carriers of different *BRCA* mutations and thereby hopefully to a better survival.

DEVELOPING A NOVEL MTOR-INHIBITOR BASED COMBINATION CANCER THERAPY FOR RAS-DRIVEN CANCERS

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The mTOR kinase is frequently deregulated in human cancer due to genetic alterations in various tumor suppressors and oncogenes including PTEN, TSC1/2, LKB, NF1, PI3K and RAS. Consequently, mTOR inhibitors have been extensively evaluated as anti-cancer agents in the clinic. While these agents exhibit efficacy in a subset of tumor-types, responses are typically cytostatic and temporary, suggesting that mTOR inhibitors might be more effective when combined with other therapies. We now demonstrate the potent therapeutic effects of combining an mTOR inhibitor with agents that induce ER stress. Using a genetically engineered mouse cancer model we show that ER stress-inducing agents, including the Hsp90 inhibitor IPI-504, trigger the rapid regression of highly aggressive NF1-deficient malignancies in vivo, but only when combined with rapamycin. Together, these agents cause pronounced effects on the ER and the mitochondria in tumor cells in vivo, induce excessive levels of autophagy and autophagic-associated cell death, and promote tumor regression via a caspase-3 independent mechanism. Rapamycin/IPI-504 treatment similarly causes tumor regression in a KrasG12D/p53 genetically engineered model of non-small cell lung cancer (NSCLC), suggesting that the therapeutic potential of this combination therapy may extend to other cancers. Importantly, the combined efficacy of these compounds would not have been predicted by in vitro studies, highlighting the utility of mouse models in preclinical discovery. Collectively, these findings reveal a promising strategy for developing mTOR inhibitor-based combination therapies for two Ras-driven malignancies for which there are currently no effective treatments.

INNATE CHEMOTHERAPY RESISTANCE AND "CANCER STEM CELLS" ARE ENRICHED IN OVERLAPPING BUT DISTINCT POPULATIONS IN MURINE LUNG CANCER

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In many cases, treatment of solid tumors with standard chemotherapy leads only to partial response. These recurrent tumors usually prove to be resistant to the chemotherapeutic agents initially used to treat the cancer. Thus, tumor recurrence driven by tumor-reinitiating cells (TRICs) is a central problem in cancer therapy. Despite the importance of this problem, the mechanisms accounting for variable chemotherapy response and tumor re-initiation in vivo are poorly understood. For example, it is unclear whether tumor-reinitiating cells are intrinsically therapy resistant cells with distinct genetic or epigenetic characteristics or whether they acquire genetic changes that allow them to become therapy resistant. In addition, controversy exists regarding whether tumor re-initiating cells arise stochastically from a genetically diverse tumor population or whether "cancer stem cells" are present in tumors and represent the primary reservoir for intrinsic chemoresistance. Surprisingly, relatively little attention has been focused on studying the process of tumor re-initiation in vivo. Mouse models of cancer that closely mimic the human disease are valuable tools to study the process of tumor re-initiation after chemotherapy.

Using one such mouse model of human lung cancer, we have identified a subset of tumor cells that can be isolated by their cell surface characteristics and have an increased intrinsic resistance to chemotherapy. Our studies were carried out in Kras-driven lung tumor model crossed to a conditional fluorescent transgenic reporter (tdRFPLSL) to facilitate isolation of tumor cells by FACs. We first noted that cisplatin-treatment of these mice led to a dramatic decrease in the number of CD44+;RFP+ cells, suggesting that CD44- cells are chemotherapy resistant. Interestingly, chemoresistance in this model is associated with a significant increase in tumor re-initiation capacity in transplantation experiments in vivo and "pulmosphere" formation in vitro. Gene expression analysis comparing FACS-sorted "chemoresistor" cells them to the bulk control population has identified a large number of differentially expressed genes that are currently being validated. Gene expression analysis identified the cell surface marker CD24 as upregulated in chemoresistant cells. Subsequent analysis demonstrated that in vitro sphere-forming ability is greatly enriched in a CD44-/CD24+ subpopulation. Thus, we have uncovered a relationship between chemoresistance and characteristics of cancer stem cells (transplantation, sphere formation) in this model. Further supporting a link between cancer "stemness" and chemoresistance, gene expression and functional studies suggest a role for the iPS transcription factor Sox2 in chemotherapy resistance in vivo.

COPPER PROMOTES TUMOR GROWTH BY INCREASING OXIDATIVE PHOSPHORYLATION

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Copper exposure and intake levels have been associated with cancer incidence and mortality. Cancer patients exhibit elevated levels of serum and tumor copper; agents that reduce systemic copper levels have been demonstrated to retard angiogenesis and tumor growth in mice, underscoring the importance of copper in tumor development.

We have previously demonstrated in a mouse model of human cervical cancer that tumors overexpress the copper transporter Ctr1 and upregulate its activity in response to a decrease in plasma copper levels by a copper chelator, but not normal tissues. These observations led us to propose that tumors are particularly sensitive to changes in copper levels and may have a higher demand for copper.

We dissected the role of copper at different stages of tumorigenesis using the mouse model of pancreatic islet carcinoma, RIP-Tag2. A copper chelator inhibited angiogenesis at the early stage of tumor development, and retarded tumor growth at the late stage. Remarkably, when mice were chronically exposed to 20 μM copper in drinking water, which is the maximal level of copper allowed in our drinking water by the Environmental Protection Agency, there was a two-fold increase in tumor volume. Analysis of tumors revealed that copper increased the rate of tumor cell proliferation, but did not affect apoptosis or vessel density. In vitro, the copper chelator arrested tumor cells in G2, reminiscent of ATP deficiency. Copper is a co-factor for cytochrome c oxidase, the last enzyme in the respiratory chain of oxidative phosphorylation. We found that the copper chelator decreased cytochrome c oxidase activity and increased lactate production in tumor cells. We propose that tumor cells use oxidative phosphorylation for ATP source in the presence of oxygen and copper, but depend on glycolysis when copper becomes scarce. This may explain aerobic glycolysis of tumors described by Warburg, and suggests a copper chelation therapy as a promising cure for cancer.

UBIQUITINATION AS A NOVEL MECHANISM OF ONCOGENIC TRANSFORMATION IN LEUKEMIA

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T cell acute lymphoblastic leukemia (T-ALL) is a devastating blood tumor that afflicts both children and adults. Several laboratories including ours have shown that the main oncogenic trigger in T-ALL is the activation of the Notch signaling pathway. In a recent screen for silencers of the Notch oncogenic activity we have identified the ubiquitin ligase Fbw7 and a potent tumor suppressor that ubiquitinates and degrades oncogenic Notch1. We have also shown that Fbw7 is inactivated by mutations that target its substrate binding pockets leading to the stabilization and over-expression of oncogenic substrates, including Notch1 and c-Myc. Generation of Fbw7 knock-out and knock-in (carrying the human mutations) mouse mutants mimics human disease and provides clues on the molecular mechanism of cell transformation. Using these mice as well as knock-in models of Notch1 activation (carrying the human mutants found in T-ALL) we were able to show that Notch1 activation leads to the hyper-activation of the IKK kinase complex and the nuclear translocation of NF-kB transcription factors leading to aberrant gene expression. Genetic targeting of the IKK complex in already established disease showed that T-ALL maintenance depends on IKK signaling suggesting that IKK targeting could lead to future therapies. Finally, further biochemical screens have identified another member of the ubiquitin complex, the deubiquitinase CYLD as the link between Notch signaling and IKK hyper-activation. These studies are important for the therapeutic targeting of T-ALL and suggest that the ubiquitin complex adds a novel level of complexity in the regulation of blood stem and progenitor transformation.

THERAPEUTIC EFFECT OF γ-SECRETASE INHIBITION IN A MOUSE MODEL OF LUNG ADENOCARCINOMA

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The γ-secretase protease complex regulates a variety of cell processes [1], notably including the Notch pathway, which is critical for the function of stem cells and can be oncogenic when constitutively active [2-5]. A large proportion of human and murine lung adenocarcinomas have the Notch pathway hyperactive, and here we examine its role in lung stem cells, tumour initiation and cancer maintenance. The bronchioalveolar stem cells (BASCs) have been implicated in the initiation of lung tumorigenesis driven by oncogenic Kras [6]. Interestingly, genetic disruption of the γ-secretase complex by combined deletion of its key components presenilins 1 and 2, results in impaired BASC expansion upon lung injury and in complete protection from KrasG12V-driven lung adenocarcinomas. We show that the activity of the Notch pathway increases during tumour progression and, based on this, we have tested the role of γ-secretase in cancer maintenance. Importantly, mice with endogenous lung adenocarcinomas were treated with a small molecule γ-secretase inhibitor [7] and this resulted in complete arrest of cancer growth, often accompanied by cancer regression, as measured by positron electron tomography (PET). Immunohistochemical analyses of treated cancers indicated loss of Hes1 and active Erk, accompanied by decreased proliferation and higher apoptosis. Together, these results support a critical role for γ-secretase activity in lung cancer initiation and maintenance, and provide proof for the therapeutic potential of γ -secretase inhibitors against lung adenocarcinoma.

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SUPPRESSION OF GATA2 INHIBITS ONCOGENIC *KRAS*- AND *EGFR*-DRIVEN NON-SMALL CELL LUNG CANCER

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We previously performed a RNA interference screen to discover factors that selectively kill cells with activating mutations of *KRAS*, in which we observed synthetic lethality of *KRAS* mutant cancer cells with knockdown of the transcription factor GATA2. We explored this synthetic lethality in non-small cell lung cancer (NSCLC) cells with mutations in either *KRAS* or *EGFR*, two of the most common oncogenic mutations in NSCLC. Surprisingly, we observed that knockdown of GATA2 reduces the viability of NSCLC cell lines with activating mutations in either *KRAS* or *EGFR*. Notably, GATA2 knockdown inhibited the growth of *EGFR* mutant cells resistant to erlotinib. In contrast, suppression of GATA2 caused little change in the viability of NSCLC cells wild type for *KRAS* and *EGFR*, suggesting that GATA2 silencing is specifically required in these oncogene-driven NSCLC cells.

To understand the consequences of GATA2 suppression on gene expression, we examined the transcript profiles of *KRAS* and *EGFR* mutant cells in the presence or absence of GATA2 knockdown. Interestingly, we observed a significant down-regulation of multiple factors which exhibit synthetic lethality with oncogenic *KRAS*. Using gene set enrichment analysis, we found observed down-regulation of several signalling pathways and molecular complexes known to be requisite for *KRAS*-driven cancers. Furthermore, we generated a gene expression signature for GATA2 knockdown for assessment in NSCLC cells and primary tumor data sets. Coordinate with gene expression analysis, we mapped GATA-2 genome occupancy via chromatin immunoprecipitation coupled to 'deep' sequencing (ChIP-seq). In particular, we performed ChIP-seq of GATA-2 in NSCLC cells containing either oncogenic mutations in *KRAS* or *EGFR* or wild type for both genes. We found substantial GATA-2 binding across the genome, with enrichment of specific gene networks. To characterize the role of GATA-2 in transcriptional control, we also performed ChIP-seq for RNA polymerase II and histone modifications involved in transcription initiation, Polycomb-mediated repression, and regulation of enhancer activity. Analysis of these occupancies should clarify the role of GATA-2 in the broader chromatin state of NSCLC.

Taken together, these findings suggest that GATA2 is required for the survival of NSCLC cells with oncogenic mutations in either *KRAS* or *EGFR*. Beyond these initial results, we will test candidate transcripts and signalling pathways for their functional relevance to GATA2-mediated cell viability. In addition, we will determine the role of GATA2 suppression on *KRAS* and *EGFR*-driven tumorigenesis *in vivo*.

BIOLOGICAL INSIGHTS FROM QUANTITATIVE ANALYSIS OF RECEPTOR TYROSINE KINASE SIGNALING NETWORKS

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To effectively monitor protein phosphorylation events governing signaling cascades, we have developed a mass spectrometry-based methodology enabling the simultaneous quantification of tyrosine phosphorylation of specific residues on dozens of key proteins at multiple time points under a variety of perturbations. We have recently applied this technique to identify key signaling nodes regulating EGFR, Insulin Receptor, and T Cell Receptor signaling network response to stimulation.
Here we have performed an in-depth characterization of the network-wide effects of single-point mutations on the oncogenic, constitutively active, EGFRvIII receptor tyrosine kinase. This study quantifies the adaptive capacity of cellular signaling networks and the pleiotropic effects of knocking out individual phosphorylation sites with the network. Computational analysis of the phosphorylation data relative to phenotypic data highlights the role of selected protein phosphorylation sites in regulating biological outcome. We demonstrate the utility of a data-driven computational model that is capable of fully describing glioblastoma cell growth based on just 13 phosphoproteins. Overall, we have now demonstrated that the combination of mass spectrometry-based analysis of protein phosphorylation with phenotypic measurements and computational modeling yields novel insights into the regulation of cellular signaling on a network scale

IDENTIFICATION OF A NOVEL TUMOR SUPPRESSOR NETWORK REVEALS A ROLE FOR PROTO-ONCOGENIC RECEPTOR TYROSINE KINASES IN TRIPLE-NEGATIVE BREAST CANCER

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Breast cancer is a collection of diseases with distinct clinical behaviors and underlying genetic causes. Triple-negative breast cancer (TNBC) is a common subtype of breast cancer that confers a particularly poor prognosis and is refractory to current targeted therapies. Unfortunately, the molecular determinants driving this aggressive malignancy are poorly understood. Using an unbiased genetic screen, we have identified a novel tumor suppressor network that governs proliferation and transformation of TNBCs in vitro and in vivo. We define SECT21 as a core component in this network and a commonly inactivated tumor suppressor in TNBC. SECT21 is a potent suppressor of human mammary epithelial cell proliferation and transformation. SECT21 function is frequently compromised in human TNBCs by inactivating mutations or loss of protein expression. Mechanistically, SECT21 is a tyrosine phosphatase that suppresses cellular transformation by interacting with and inhibiting several oncogenic receptor tyrosine kinases including HER2 and EGFR. Notably, the tumorigenic and metastatic potential of SECT21-deficient TNBCs is severely impaired by restoring SECT21 function or by inhibiting kinase targets of SECT21, suggesting that TNBCs are dependent on the proto-oncogenic tyrosine kinases constrained by SECT21. Collectively, these data identify SECT21 as a commonly inactivated tumor suppressor and provide a rationale for combinatorially targeting tyrosine kinases in TNBC and other cancers based on their profile of tyrosine phosphatase activity.

THE SUPER ELONGATION COMPLEX (SEC) AND ITS ROLE IN MLL TRANSLOCATION-BASED LEUKEMIA

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Chromosomal translocations involving the MLL gene are associated with infant acute lymphoblastic and mixed lineage leukemia. There are a large number of translocation partners of MLL that share very little sequence or seemingly functional similarities; however, their translocations into MLL result in the pathogenesis of leukemia. To define the molecular reason why these translocations result in leukemogenesis, we have purified several of the commonly occurring MLL chimeras. We have identified many of the MLL partners in a super complex with several of the known RNA polymerase II elongation factors. We have named this complex, the Super Elongation Complex (SEC). SEC includes ELLs, P-TEFb, AFF4, and other MLL partners. We have shown that AFF4 is required for SEC stability and proper transcription by poised RNA polymerase II in metazoans. Knockdown of AFF4 in leukemic cells shows reduction in MLL chimera target gene expression, suggesting that AFF4/SEC could be a key regulator in the pathogenesis of leukemia through many of the MLL partners. Our studies have provided a molecular insight to why translocations of MLL into a large number of genes with seemingly little sequence similarities, result in leukemogenesis. Our model in this regard will be discussed.

MYB INHIBITION CURES CHEMOTHERAPY-RESISTANT MLL/AF9-INDUCED AML

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Acute myeloid leukemia (AML) exemplifies the genetic heterogeneity and complexity of human cancer; however, our growing genetic knowledge has not yet translated into effective targeted therapies. Translocations involving the MLL gene on 11q23 induce chemotherapy resistance and define one of the most adverse prognostic markers. Here we use an approach combining mosaic mouse models, conventional Tet-Off oncogene addiction studies and novel Tet-On in-vivo RNAi to systematically survey genes that are required for maintenance of MLL/AF9-induced AML. Withdrawal of MLL/AF9 itself triggers a terminal differentiation program resulting in cure of leukemic mice. Using transcriptome profiling, we identify a group of transcription factors that act as direct MLL/AF9 targets. Besides known targets such HoxA9 and Meis1, these include two human oncogenes – Myc and Myb. To test a possible role of Myc and Myb in maintenance of MLL/AF9-driven AML in vivo, we developed a TetOn-shRNA expression system (TRMPV) and show that shRNA-mediated repression of Myc or Myb effectively eliminates MLL/AF9 blasts in vivo. To determine if this sensitivity was leukemia specific, we tested the impact of Myc and Myb shRNAs in normal hematopoiesis: While Myc suppression completely abrogates reconstitution of the hematopoietic compartment, suppression of Myb does not interfere with myelo- and eythropoiesis, while partially suppressing lymphopoiesis. To further characterize Myb as potential drug target, we transplanted leukemia cells that were selected for inducible shRNAs. Strikingly, induction of Myb shRNAs in leukemic mice triggers a terminal differentiation program that precisely phenocopies MLL/AF9 withdrawal and results in complete remission, which is permanent even after discontinuing doxycycline treatment. In summary, our study identifies Myb as a direct and essential transcriptional target in MLL/AF9 leukemogenesis and shows that transient and subtotal repression of Myb – e.g. using emerging RNAi therapeutics - is a promising therapeutic strategy in high-risk MLL-rearranged AML. More broadly, we provide evidence that partial and transient suppression of a single non-essential gene can cure aggressive cancers that are resistant to current therapies.

LOSS OF THE NOVEL TUMOR SUPPRESSOR PRDM11 COLLABORATES WITH MYC IN LYMPHOMAGENESIS

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Deregulation of epigenetic factors collaborates with genetic alterations in carcinogenesis. The PR domain containing proteins (PRDMs) constitute a sub-family of the SET domain family of histone methyl transferases, which are involved in epigenetic gene regulation by methylation of histones. As several SET/PR domain proteins have been associated with tumorigenesis either as oncogenes or tumor suppressors, we undertook a functional screening approach to identify novel tumor suppressors amongst the SET/PRDMs. The screen was performed as an oncogenic collaboration screen, in which MYC was co-transduced into primary mouse embryo fibroblasts (MEFs) together with a retroviral vector-based shRNA library targeting a set of 61 SET and PR domain containing genes. One of the putative tumor suppressors identified was Prdm11, a previously uncharacterized member of the PRDMs.
A *Prdm11* knockout (KO) mouse model was generated in order to study the function of Prdm11 in vivo. These mice are viable, born in Mendelian ratios and morphologically normal. In accordance with a tumor-suppressive function of PRDM11, we find that over-expression of PRDM11 in MEFs reduces cellular growth and induces apoptosis. In agreement with this, *Prdm11* KO MEFs grow faster than wt littermate controls and exhibit significantly higher susceptibility to transformation by MYC.
To evaluate the tumor suppressor potential of Prdm11 *in vivo*, we crossed the *Prdm11* KO strain with the tumor-prone Eμ-Myc mouse strain. The Eμ-Myc mouse model mimics Burkitt's lymphoma by selective expression of a *Myc* transgene in the B-cell lineage. These mice develop malignant lymphomas with a mean latency of 100-120 days. Importantly, we found that loss of *Prdm11* potently accelerates lymphomagenesis in the Eμ-Myc mouse to a mean latency of 75 days (P=0.00006). To evaluate a role in human cancer, *PRDM11* expression levels were evaluated in a panel of human lymphoma cell lines. Compared to normal B-cells and reactive lymph nodes, *PRDM11* levels are low in most cell lines suggesting a tumor suppressor function also in human B cells malignancies. Tissue microarrays are being setup to evaluate the expression of PRDM11 across a panel of human tumor types. Current mechanistic insight places PRDM11 in the TNFα/NF-κB pathway with direct binding to several central components. In conclusion, this study identifies the PR-domain containing gene, PRDM11, as a novel tumorsuppressor.

THE PIAS1 SUMO-E3 LIGASE REGULATES TUMORIGENESIS THROUGH SUMOYLATION OF THE PML TUMOR SUPPRESSOR

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The Promyelocytic Leukemia gene (PML) is a tumor suppressor originally identified as a component of the PML-RARα oncoprotein of acute promyelocytic leukemia (APL). PML critically regulates cellular proliferation and apoptosis. In human tumors, PML deficiency is achieved through aberrant ubiquitin/proteasomal degradation or by physical interaction with PML-RARα.
It is well known that PML and PML-RARα undergo SUMOylation, however the functional relevance of this event has been contentious. It has been reported that SUMOylation promotes PML tumor suppressive function, while others have indicated that SUMOylation promotes PML degradation. SUMOylation of PML-RARα has been linked to the therapeutic effect of arsenic trioxide.
To gain insight into the biological significance of PML SUMOylation, we aimed at the identification of the PML SUMO-E3 ligase.
We discovered that PIAS1, a member of the Protein Inhibitor of Activated STAT family, physically interacts and co-localizes with PML in PML nuclear bodies. PIAS1 promotes PML SUMOylation in cell free systems. Furthermore, PIAS1 dependent PML SUMOylation leads to ubiquitin mediated PML degradation in non-small cell lung cancer (NSCLC) cells. Moreover, an inverse correlation exists between the levels of PIAS1 and PML proteins in NSCLC cells. Finally, RNAi mediated silencing of PIAS1 leads to PML protein upregulation and PML dependent inhibition of cell proliferation.
We also determined that PIAS1 efficiently mediates SUMOylation of the PML moiety of the PML-RARα oncoprotein leading to its degradation in APL cells after arsenic trioxide treatment. Thus, PIAS1 is required for the therapeutic effect of arsenic trioxide in APL cells.
We conclude that PIAS1 is a PML SUMO-E3 ligase whose biological output depends on cellular context. We propose that PIAS1 has oncogenic properties when targeting wild type PML, while it may oppose leukemogenesis when expressed in APL cells where PML is a component of the PML-RARα oncoprotein. These findings reveal a novel function for the SUMOylation machinery in tumorigenesis providing the rationale for pharmacologic targeting of PIAS1.

IDENTIFICATION OF DISTAL SILENCING ELEMENTS AND *TRANS*-ACTING REPRESSORS FOR THE EPIGENETIC REGULATION OF THE *HTERT* GENE

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The *hTERT* gene, which encodes the limiting subunit of human telomerase, is highly expressed during embryogenesis, but strictly repressed in most adult somatic cells. While hTERT reactivation in cancer cells is critical for tumorigenesis, telomere attrition in somatic tissues due to hTERT silencing contributes to human aging. In contrast, the *mTERT* gene is widely expressed in adult mouse tissues. This species-specific regulation likely contributes to the much longer telomeres in mouse cells and some of the distinct characteristics of tumor development in mice vs. humans.
We previously reported that the endogenous *hTERT* locus was embedded in a condensed chromatin domain in many somatic cells, while an equivalent chromatin domain did not exist for the less repressed *mTERT* gene. To determine the *cis* elements required for hTERT repression, we developed a novel technical platform, recombinase-mediated BAC targeting (RMBT), for integrating single-copy BAC reporters into specified chromosomal sites. Using RMBT, we found that chromatinized BAC reporters containing corresponding *hTERT* and *mTERT* loci recapitulated their respective native genes, indicating that cis regulatory elements were responsible for the species-dependent hTERT repression. By analyzing human and mouse chimera BAC reporters, we discovered that multiple distal elements, but not the proximal promoter, were involved in the stringent hTERT regulation in somatic cells. In addition, using the chromatinized BAC reporters and RNA interference-mediated genetic screenings, we identified several novel repressors of the *hTERT* gene that acted through the distal silencers. These repressors were previously reported to participate in multiple epigenetic silencing pathways and their knockdown by siRNAs activated both the transgenic hTERT reporters and endogenous hTERT mRNA expression. Thus, with these new findings, we are beginning to dissect the pathways involved in the human-specific epigenetic hTERT silencing in somatic cells as well as its activation during tumorigenesis.

BRCA1-ASSOCIATED EPIGENETIC REGULATION OF P73 MEDIATES AN EFFECTOR PATHWAY FOR CHEMOSENSITIVITY IN OVARIAN CARCINOMA.

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The majority of tumors arising in BRCA1 mutation carriers exhibit inactivation of p53, a key effector of cell death following DNA damage. Despite the loss of p53, BRCA1-deficient tumor cells exhibit increased sensitivity to cisplatin, and patients with BRCA1-associated ovarian carcinomas experience improved outcomes with platinum-based chemotherapy compared to sporadic cases. While it is known that chemosensitivity in BRCA1-associated cancers is associated with unrepaired DNA damage, the specific effector pathway mediating the cellular response to platinum-induced damage in these tumors is poorly understood. Here we demonstrate that the p53-related gene p73, encoding a pro-apoptotic protein which is linked to chemosensitivity in many settings, is upregulated through a novel epigenetic mechanism in both human and murine models of BRCA1-associated ovarian carcinoma. BRCA1-deficient ovarian carcinoma cells exhibit hypermethylation within a p73 regulatory region which includes the binding site for the p73 transcriptional repressor ZEB1, leading to abrogation of ZEB1 binding and increased expression of transactivating p73 isoforms (TAp73). Cisplatin chemotherapy induces TAp73 target genes specifically in BRCA1-deficient cells, and knockdown of TAp73 in these cells causes chemoresistance while having little or no effect on BRCA1-expressing tumor cells. In primary ovarian carcinomas, ZEB1 binding site methylation and TAp73 expression correlate with BRCA1 status and with clinical response. Together, these findings uncover a novel regulatory mechanism that supports the contribution of TAp73 as an important mediator of the response to platinum chemotherapy in a subset of ovarian carcinomas. TAp73 may represent a response predictor and potential therapeutic target for enhancing chemosensitivity in this disease.

SYNTHETIC LETHAL INTERACTION OF ARK5 DEPLETION WITH DEREGULATED MYC SUGGESTS THAT THE HIPPO PATHWAY MEDIATES MYC-INDUCED APOPTOSIS

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Identification of synthetic lethal interactions with key oncogenic mutations represents a strategy to identify novel targets for tumor therapy. We present here the results of an siRNA screen of the human kinome aimed at identifying synthetic lethal interactions with deregulated expression of Myc. U2OS cells that carry a hormone-inducible allele of Myc (MycER) undergo apoptosis in a p53-independent manner upon depletion of growth factors. We have now screened an siRNA library of the human kinome for kinases that are specifically required for cell survival in the presence of active Myc, but not in its absence. The screen identified a small number of kinases: prominent among them are Ark5, a kinase related to AMPK that has previously been implicated in invasion and metastasis, and AMPK itself. Multiple siRNAs and shRNAs targeting Ark5 strongly suppress growth in a Myc-dependent manner. The interaction is observed not only in U20S cells, but also in immortalized lung fibroblasts and in cells expressing a constitutive allele of Myc. FACS analyses demonstrate that suppression of Ark5 induces Myc-dependent apoptosis.
Rescue experiments show that both the kinase activity of Ark5 and its activation by LKB1 are required to suppress Myc-induced apoptosis. Recent work by others has shown that Ark5 is a negative regulator of LATS, a kinase that is a central player in the Hippo signaling pathway. Consistent with these observations, depletion of Ark5 leads to a dramatic increase in LATS1 levels. Activation of Myc and depletion of Ark5 synergize to activate expression of the downstream target of the pro-apoptotic branch of the Hippo pathway, Puma.
Experiments are currently underway to validate the relevance of this pathway in mouse models of Myc-dependent tumorigenesis.

IDENTIFICATION OF MYC-REGULATED GENES DURING MITOGENIC STIMULATION AND LYMPHOMA ONSET

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The Myc oncoprotein is a transcription factor that targets thousands of genomic loci. While its expression is frequently deregulated in cancer cells, Myc is tightly controlled by growth-regulatory signals in normal cells, and is required for a normal cellular response to mitogenic stimuli. In spite of a wealth of information on numerous Myc-target genes, the Myc-dependent transcriptional programs involved in mitogenesis and cancer progression, as well as their overlap, largely remain to be identified. We have undertaken a concerted genome-wide analysis in two different model systems: (i.) serum-stimulated fibroblasts and (ii.) Eμ-myc transgenic mice. In each system, we combined genome-wide Myc-DNA interaction maps generated by ChIP-seq (in collaboration with Dr. Chia-Lin Wei, GIS, Singapore) with gene expression profiles generated on Affymetrix GeneChip Arrays. (i.) We used 3T9 fibroblasts homozygous for a conditional KO allele of c-myc (c-mycf/f): Cre-mediated deletion was induced in quiescent cells, prior to serum stimulation. mRNA profiling identified a group of several hundred Myc-dependent serum response (MDSR) genes, the majority of which where direct Myc targets. (ii.) B-cells were isolated from control non-transgenic mice, young Eμ-myc mice at the pre-tumoral stage, or lymphomas, and directly processed (without any in vitro culture) for either ChIP-seq or expression profiling, allowing identification of the genes directly induced by Myc at the two different disease stages. Either MDSR genes in fibroblasts or Myc-induced genes in B-cells constitute a small fraction of all Myc-bound loci. These represent the “core” Myc targets likely to be involved in the mitogenic response, on one hand, and tumor promotion and/or suppression, on the other.

MYC INHIBITION HAS DRAMATIC THERAPEUTIC IMPACT IN DIVERSE MOUSE MODELS OF CANCER

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Myc is a pleiotropic transcription factor whose levels are elevated and deregulated in most cancers, suggesting a pivotal role for Myc in oncogenic signaling. De-activation of Myc function in tumors driven by oncogenically activated Myc triggers their rapid regression through a variety of intracellular and extracellular mechanisms, including differentiation, apoptosis and vascular collapse. However, Myc mutations are relatively rare and deregulated Myc in most tumours appears to be a consequence of aberrant upstream signals. The extent to which such endogenous Myc, acting as a client of upstream oncogenes, is a therapeutic target will depend on the degree to which it coordinates functions essential for tumor maintenance and what those functions are, neither of which is known. To investigate the therapeutic potential of Myc inhibition we have constructed switchable genetic mouse models in which endogenous Myc can be systemically and reversibly inhibited in normal and tumor tissues in vivo. Our data indicate that inhibiting Myc has a remarkably efficacious therapeutic impact on multiple cancer types, independent of the tissue or driving oncogenic lesion (various examples will be shown). Importantly, Myc inhibition triggers widespread tumour cell apoptosis while elicits surprisingly mild, reversible and non-cytotoxic side effects in normal tissues. The nature of this differential response between tumor and normal tissues will be explored.
The p53 tumor suppressor, or its attendant pathway, is functionally inactivated in almost all human cancers. p53 mediates the cellular apoptotic and senescence response to DNA damage, and this is thought to be critical for tumor suppression. Using a unique mouse model in which the endogenous p53 gene is replaced by one encoding a ligand-dependent, reversibly switchable variant of p53, we assessed the contribution of this tumor suppressor to the therapeutic impact of Myc inhibition.
Our data show that targeting essential and non redundant pivotal nodes, such as Myc, in tumor cells could have enormous therapeutic impact in multiple, if not all, types of cancer.

TARGETING THE MYCN ONCOGENE TO COMBAT CHILDHOOD NEUROBLASTOMA

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MYCN, a proto-oncogene normally expressed in the migrating neural crest, is in its amplified state a key factor in the genesis of human neuroblastoma (NB). However, the mechanisms underlying MYCN-mediated NB progression are poorly understood. We have identified a MYCN-induced miRNA signature in human NB involving the activation and transrepression of several miRNA genes from paralogous clusters. Several family members derived from the miR-17-92 cluster, including miR-18a and miR-19a, were among the up-regulated miRNAs. Expression analysis in NB tumors confirmed increased levels of these miRNAs in MYCN-amplified samples. Specifically, we show that miR-18a and miR-19a target and repress the expression of estrogen receptor-α(ESR1). Furthermore, we demonstrated ESR1 expression in human fetal sympathetic ganglia, suggesting a role for ESR1 during sympathetic nervous system development. Reconstitution of ESR1 expression resulted in marked growth arrest and neuronal differentiation. Similarly, inhibition of miR-18a in NB cells led to severe growth retardation, outgrowth of varicosity-containing neurites, and induction of neuronal sympathetic differentiation markers. We propose that MYCN amplification may disrupt estrogen-signaling sensitivity in primitive sympathetic cells through deregulation of ESR1, thereby preventing the normal induction of neuroblast differentiation. Collectively, our findings demonstrate the molecular consequences of abnormal miRNA transcription in a MYCN-driven tumor and offer unique insights into the pathology underlying MYCN-amplified NB. We are presently investigating interacting growth factors and downstream targets involved in the regulation of differentiation by ESR1 in NB cells.
In parallel, we have characterized one low molecular weight compound that resulted in downregulation of MYCN followed by apoptosis of MYCN amplified NB cells. In vivo treatment of MYCN driven tumors in different mouse models delayed their growth. Taken together, our data suggest that targeting MYCN is an attractive approach for treatment of childhood NB.

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MYC-NICK: A CYTOPLASMIC FORM OF MYC THAT PROMOTES TUBULIN ACETYLATION AND MUSCLE DIFFERENTIATION

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Myc is a BHLH transcription factor that plays a critical role in major biological processes, such as proliferation, growth, apoptosis, and differentiation. Recently, we discovered Myc-nick, a transcriptionally inactive form of Myc that is localized in the cytoplasm. Myc-nick is generated by a proteolytic cleavage of the full-length Myc that is carried out by members of the calpain family of calcium dependant proteases. Myc-nick lacks the C-terminal region (nuclear localization signal, Max dimerization domain, and DNA binding domain) of Myc but contains an intact N-terminus. Ectopic expression of Myc-nick in fibroblasts and epithelial cells promotes changes in cell morphology marked by the induction of cellular protrusions. We found that GCN5 functions as an alpha-tubulin acetyl transferase and it can cooperate with Myc-nick to promote alpha-tubulin acetylation. Since induction of alpha-tubulin acetylation, calpain activation, and decrease in full length Myc all occur during terminal differentiation we investigated the role of Myc-nick in cell differentiation. We found that Myc-nick is augmented during the differentiation of human and mouse primary myoblasts. In addition, we found that while full-length Myc levels diminish, Myc-nick levels remain elevated in adult mouse differentiated muscles, brain and cerebellum. Ectopic expression of Myc-nick accelerates myoblast fusion and the expression of myogenic markers in mouse and human myoblasts and in Rhaddomyosarcomas. Importantly we found that the expression of Myc-nick renders myc-null fibroblasts competent to MyoD induced transdifferentiation. Based on our results we propose that while full-length Myc blocks differentiation at the transcriptional level, Myc-nick is involved in promoting differentiation through transcription independent mechanisms.

GENETIC DISSECTION OF THE MIR-17~92 CLUSTER IN MICE

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MiR-17~92 has emerged as the prototypical oncogenic microRNA cluster in humans and mice. It encodes six distinct miRNAs that can be grouped into four "seed families" (miR17/20, miR-18, miR-19a/b and miR-92). We have previously reported the generation and characterization of mice carrying a targeted deletion of the entire cluster. Homozygous mutant mice are significantly smaller than their wild type counterpart, die soon after birth and display a complex array of defects involving heart lungs and lymphocyte development. One limitation of such an analysis is that these results were obtained by deleting the entire miR-17~92 locus. As such, little is known with respect to the relative role of each of the six microRNAs encoded by miR-17~92. We will discuss the results of two parallel lines of investigation that our laboratory is undertaking to address this important issue:

a) We are carrying out a systematic genetic analysis by creating an allelic series of knock-in mice, each lacking only one of the four "seed families" encoded by miR-17~92. The analysis of these mice is providing key information regarding the specific functions of individual components of miR-17~92 .

b) In a parallel line of investigation, we have used a conditional miR-17~92 knockout allele to determine the role of this cluster in Myc-induced B cell lymphomas. By using this approach we show that endogenous miR-17~92 is required in lymphoma to suppress apoptosis via the concerted action on a number of genes and that this effect is largely, if not exclusively attributable to the miR-19 seed family.

THERAPEUTIC SENESCENCE – INDUCTION OF TUMOR CELL SENESCENCE BY TARGETING MTOR TO PREVENT AND TREAT B CELL LYMPHOMAS

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The PI3K/mTOR pathway couples signals from oncoproteins such as c-myc, growth factor receptors and nutrient sensors to cell machinery involved in growth, survival and proliferation. PI3K/mTOR activation is a feature of many haematological malignancies, and agents targeting this pathway have an emerging therapeutic role. Here we used the allosteric mTORC1 inhibitor everolimus (RAD001) and BEZ235 a small molecule non-allosteric kinase inhibitor that inhibits PI3K, mTORC1, mTORC2 to target the PI3K/mTOR pathway in the Eμ-myc mouse model of B cell lymphoma. "Compound mutant" Eμ-myc lymphomas with deletions or overexpression of important tumor suppressor genes and oncogenes (i.e. p53, Bcl-2) were employed to identify the molecular pathways necessary for the biological and therapeutic effects of everolimus and BEZ235. Chronic dosing of Eμ-myc transgenic mice with everolimus reduced B cell expansion in the bone marrow and spleen and restored B cell differentiation. Everolimus significantly improved lymphoma-free survival relative to placebo (median survival not reached after 200 days for mice treated with everolimus versus 73 days for placebo-treated mice, p=0.004). The clearance of premalignant cells was associated with induction of cellular senescence as assessed by β-gal staining. Treatment of mice bearing established Eμ-Myc lymphomas with everolimus or BEZ235 resulted in a significant increase in survival of mice compared to placebo treatment. Importantly, everolimus treatment caused tumor cell cycle arrest in G1 and induction of senescence with subsequent tumor regression concomitant with infiltration of host immune cells. In contrast, BEZ235 caused tumor regression associated with robust apoptosis and no sign of cellular senescence. Both everolimus and BEZ235 required a functional p53 pathway to mediate their biological and therapeutic effects. Interestingly, overexpression of Bcl-2 abrogated the apoptotic and therapeutic effects of BEZ235 but had no effect on the ability of everolimus to induce senescence or provide a significant survival benefit to treated mice.
Our data demonstrate that targeting the PI3K/mTOR pathway at different points using everolimus and BEZ235 results in therapeutic efficacy however this is achieved by inducing differential biological responses. The therapeutic benefit of everolimus-induced senescence was as robust as that observed following induction of tumor cell apoptosis by BEZ235. Both agents required a functional p53 pathway to mediate their biological and therapeutic effects however everolimus but not BEZ235 retained its anti-tumor effects in cells overexpressing Bcl-2. These data demonstrate the potential of 'pro-senescence' therapy for cancer prevention and treatment.

MECHANISM OF CYCLIN D1-DEPENDENT GENOMIC INSTABILITY AND NEOPLASTIC TRANSFORMATION

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Cyclin D1 deregulation is implicated in the genesis of multiple human cancers. Importantly, cyclin D1 nuclear accumulation promotes DNA re-replication and subsequent genomic instability mediated by *cul4* repression and Cdt1 stabilization. Recent molecular dissection of these processes identified the protein arginine methyltransferase 5 (PRMT5)/MEP50 complex as a substrate and effector of cyclin D1-dependent neoplasia. Specifically, nuclear cyclin D1 retention during S-phase increases PRMT5-dependent histone methylation at key target promoters, providing a direct correlation between aberrant cyclin D1/CDK4 activity, transcriptional regulation, and double strand DNA break (DSB) induction; together, these molecular events catalyze the genomic instability necessary for cellular transformation. Given that replication-associated DNA damage is central to cyclin D1-coupled neoplasia, inactivation of critical checkpoint mediators should accelerate cyclin D1-dependent tumorigenesis. To interrogate the potential synergy between nuclear cyclin D1 and impaired checkpoint integrity, ATM deficient mice harboring the Eμ-D1/T286A transgene were generated and evaluated for tumor onset. Eμ-D1/T286A/ATM-/- mice exhibit accelerated development of thymic and B-cell lymphomas relative to Eμ-D1/T286A or ATM-/- cohorts. Lymphomas harbor clonal chromosomal alterations distinct from ATM-null mice that typically acquire translocations involving the Tcrα/δ locus during V(D)J recombination. Collectively, these findings reveal an intricate relationship wherein nuclear cyclin D1/CDK4 activity modulates genetic alterations necessary for perturbed DNA replication, genomic instability, and ultimately neoplastic transformation.

DORMANT REPLICATION ORIGINS ARE REQUIRED FOR CHROMOSOME STABILITY AND TUMOR SUPPRESSION

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Origin licensing is an essential process to prime replication origins for genome duplication through loading of the MCM2-7 complex, the replicative helicase. Licensed origins exist in a large excess over the number that will actually fire during S phase. While the majority of these excess origins remain dormant in unperturbed S phase, we show here that a fraction of them are required for the recovery of stalled replication forks, contributing to tumor suppression.

We previously reported that homozygosity for *Mcm4*^{Chaos3}, the first viable allele of any *Mcm* gene in vertebrates, causes spontaneous tumors in mice with nearly complete penetrance. In this study, we found that primary *Mcm4*$^{Chaos3/Chaos3}$ embryonic fibroblasts suffer from a ~50% loss of dormant origins, accumulating stalled replication forks in S phase. A substantial fraction of these stalled forks apparently persist into early M phase, as the number of FANCD2 sister foci increases dramatically. The incidence of lagging chromosomes is also significantly elevated in anaphase, leading to a two-fold increase in micronucleus formation in *Mcm4*$^{Chaos3/Chaos3}$ cells.

These data suggest that insufficient origin licensing drives tumorigenesis by increasing the occurrence of chromosome missegregation. Furthermore, our findings strengthen the current view that deregulated origin licensing is a cause of genome instability in cancer.

SPINDLE ASSEMBLY CHECKPOINT AND TUMORIGENESIS: MICE HETEROZYGOUS FOR ACETYLATION-DEFECTIVE BUBR1 ALLELE (K243R) DEVELOP SPONTANEOUS TUMORS

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The regulation of BubR1 is central to the control of APC/C activity. We found that BubR1 forms a complex with PCAF and is acetylated at lysine 250. With extensive analyses, we showed that BubR1 acetylation is crucial in the control of anaphase onset. Based on our collective results, we proposed that the acetylation status of BubR1 is a molecular switch that changes BubR1 from an inhibitor to a substrate of APC/C, thus providing an efficient way to modulate APC/C activity in a signal-dependent and timely-controlled manner (EMBO J., 2009. 28: 2077-2090). In order to investigate the physiological role of BubR1 acetylation, we generated mutant mice where the acetylation site (K243) was substituted to arginine to interfere with the acetylation (K243R). Embryos homozygous for K243R allele (BubR1K243R/K243R) exhibited early embryonic death. Heterozygous mice (BubR1K243R/+) survived to birth; however they displayed poor embryonic growth and generated small-sized littermates. Paradoxically, various types of tumors developed in BubR1K243R/+ mice around 60 weeks after birth. MEFs from BubR1K243R/+ mice displayed aneuploidy, premature sister chromatid breakages (PMSCs), and abnormal spindle formation, the hallmarks of mitotic infidelity. Indeed, the spindle assembly checkpoint (SAC) was compromised and the mitotic timing was shortened in MEFs isolated from BubR1K243R/+ mice. Analyses of the large B cell lymphomas and liver cancers from BubR1K243R/+ mice indicated massive genomic alterations and chromosome instability. Given that the acetylation-defective BubR1 results in decreased BubR1 level in mitosis, K243R allele was initially thought to be loss-of-function allele. However, the high incidence of cancer development in BubR1K243R/+ mice suggests otherwise: the results indicate that BubR1K243R/+ allele may be a gain-of-function or a dominant negative allele. The as-yet-unidentified potential roles of BubR1, and its acetylation/deacetylation, in the maintenance of genome integrity will be discussed.

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Ambulance	**(9) 742-3300**	**(9) 692-4747**
Poison	**(9) 542-2323**	**(9) 542-2323**
Police	**(9) 911**	**(9) 549-8800**
Safety-Security	**Extension 8870**	

Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2300** **(1037)**
Dentists Dr. William Berg Dr. Robert Zeman	 **631-271-2310** **631-271-8090**
Doctor MediCenter 234 W. Jericho Tpke., Huntington Station	**631-423-5400** (**1034**)
Drugs - 24 hours, 7 days Rite-Aid 391 W. Main Street, Huntington	**631-549-9400** (**1039**)

Free Speed Dial
Dial the four numbers (********) from any **tan house phone** to place a free call.

GENERAL INFORMATION

Books, Gifts, Snacks, Clothing, Newspapers
BOOKSTORE 367-8837 (hours posted on door)
Located in Grace Auditorium, lower level.

Photocopiers, Journals, Periodicals, Books, Newspapers
Photocopying – Main Library
Hours: 8:00 a.m. – 9:00 p.m. Mon-Fri
10:00 a.m. – 6:00 p.m. Saturday
Helpful tips - Obtain PIN from Meetings & Courses Office to enter Library after hours. See Library staff for photocopier code.

Computers, E-mail, Internet access
Grace Auditorium
Upper level: E-mail only
Lower level: Word processing and printing.
STMP server address: mail.optonline.net
To access your E-mail, you must know the name of your home server.

Dining, Bar
Blackford Hall
Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
Bar 5:00 p.m. until late
Helpful tip - If there is a line at the upper dining area, try the lower dining room

Messages, Mail, Faxes

Message Board, Grace, lower level

Swimming, Tennis, Jogging, Hiking

June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m. Two tennis courts open daily.

Russell Fitness Center

Dolan Hall, west wing, lower level
PIN#: **Press 64475 (then enter #)**

Concierge

On duty daily at Meetings & Courses Office.
After hours – From tan house phones, dial x8870 for assistance

Pay Phones, House Phones

Grace, lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

CSHL's Green Campus

Cold Spring Harbor Laboratory is pledged to operate in an environmentally responsible fashion wherever possible. In the past, we have removed underground oil tanks, remediated asbestos in historic buildings, and taken substantial measures to ensure the pristine quality of the waters of the harbor. Water used for irrigation comes from natural springs and wells on the property itself. Lawns, trees, and planting beds are managed organically whenever possible. And trees are planted to replace those felled for construction projects.

Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a "single stream waste management" system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory's efforts can be improved. This book, for example, is printed on recycled paper.

1-800 Access Numbers

AT&T	**9-1-800-321-0288**
MCI	**9-1-800-674-7000**

Local Interest

Fish Hatchery	631-692-6768
Sagamore Hill	516-922-4447
Whaling Museum	631-367-3418
Heckscher Museum	631-351-3250
CSHL DNA Learning Center	x 5170

New York City

Helpful tip -

Take Syosset Taxi to Syosset Train Station ($8.00 per person, 15 minute ride), then catch Long Island Railroad to Penn Station (33rd Street & 7th Avenue). Train ride about one hour.

TRANSPORTATION

Limo, Taxi

Syosset Limousine	516-364-9681 (**1031**)
Super Shuttle	800-957-4533 (**1033**)
To head west of CSHL - Syosset train station	
Syosset Taxi	516-921-2141 (**1030**)
To head east of CSHL - Huntington Village	
Orange & White Taxi	631-271-3600 (**1032**)
Executive Limo	631-696-8000 (**1047**)

Trains

Long Island Rail Road	822-LIRR
Schedules available from the Meetings & Courses Office.	
Amtrak	800-872-7245
MetroNorth	800-638-7646
New Jersey Transit	201-762-5100

Ferries

Bridgeport / Port Jefferson	631-473-0286 **(1036)**
Orient Point/ New London	631-323-2525 **(1038)**

Car Rentals

Avis	631-271-9300
Enterprise	631-424-8300
Hertz	631-427-6106

Airlines

American	800-433-7300
America West	800-237-9292
British Airways	800-247-9297
Continental	800-525-0280
Delta	800-221-1212
Japan Airlines	800-525-3663
Jet Blue	800-538-2583
KLM	800-374-7747
Lufthansa	800-645-3880
Northwest	800-225-2525
United	800-241-6522
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