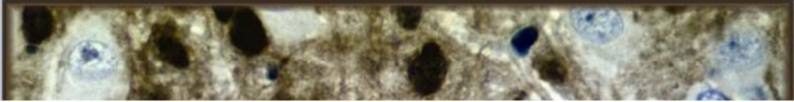
Abstracts of papers presented
at the 2010 meeting on

PTEN PATHWAYS & TARGETS

March 16–March 20, 2010



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Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

Abstracts of papers presented
at the 2010 meeting on

PTEN PATHWAYS & TARGETS

March 16–March 20, 2010

Arranged by

Suzanne Baker, *St. Jude Children's Research Hospital*
Lewis Cantley, *Beth Israel Deaconess Medical Center*
Pier Paolo Pandolfi, *Harvard Medical School*
Ramon Parsons, *Columbia University*

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

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Front Cover: Pten immunohistochemistry in cerebral cortex of *Pten*$^{flox/flox}$;*GFAP-cre* mouse brain, Melissa M. Fraser, PhD.

Back Cover: Art by Antonia Parsons

PTEN PATHWAYS & TARGETS

Tuesday, March 16 – Saturday, March 20, 2010

Tuesday	7:30 pm	**1** Upstream of PTEN
Wednesday	9:00 am	**2** Downstream of PTEN
Wednesday	2:00 pm	**3** Poster Session I
Wednesday	4:30 pm	Wine and Cheese Party
Wednesday	7:30 pm	**4** PTEN Regulation
Thursday	9:00 am	**5** Development and Disease Models
Thursday	2:00 pm	**6** Poster Session II
Thursday	7:30 pm	**7** mTORC1 and Intersecting Pathways
Friday	9:00 am	**8** Therapy and Cancer Models
Friday	6:00 pm 7:00 pm	Concert Banquet
Saturday	9:00 am	**9** Aging, Metabolism and Cancer

Poster sessions are located in *Bush Lecture Hall*

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, March 16—7:30 PM

SESSION 1 UPSTREAM OF PTEN

Chairperson: **R. Parsons,** Columbia University, New York, New York

Examining the PI3K isoform dependence of murine tumors driven by PTEN loss
Thomas Roberts.
Presenter affiliation: Dana-Farber Cancer Institute, Boston, Massachusetts.

Integration of PI3K scaffold and catalytic functions in health and disease
Emilio Hirsch.
Presenter affiliation: University of Torino, Torino, Italy. 1

Insights into the oncogenic effects of *PIK3CA* mutations from the structure of Phosphoinsitide-3-kinase
C.-H. Huang, D. Mandelker, Ignacia Echeverria, B. Vogelstein, Oleg Schmidt-Kittler, S.B. Gabelli, L. M. Amzel.
Presenter affiliation: Johns Hopkins University School of Medicine, Baltimore, Maryland; Johns Hopkins Kimmel Cancer Center, Baltimore, Maryland. 2

Overlapping and distinct oncogenic properties of the E545K and H1047R PIK3CA mutations
Elias E. Stratikopoulos, Matthias Szabolcs, Ramon Parsons, Argiris Efstratiadis.
Presenter affiliation: Columbia University, New York, New York. 3

Context-dependent functions of PI3K signaling in brain
Xiaoyan Zhu, Nader Chalhoub, Lionel M. Chow, Sherri L. Rankin, David W. Ellison, Suzanne J. Baker.
Presenter affiliation: St. Jude Children's Research Hospital, Memphis, Tennessee. 4

WEDNESDAY, March 17—9:00 AM

SESSION 2 DOWNSTREAM OF PTEN

Chairperson: **L. Trotman,** Cold Spring Harbor Laboratory, New York

Akt Isoform-specific signaling in breast cancer cell invasive migration
Alex Toker, Rebecca Y. Chin.
Presenter affiliation: Beth Israel Deaconess Medical Center, Boston, Massachusetts. 5

Transcriptional repressor NFIL3 modulates the PI3K/PTEN/FOXO pathway to attenuate FOXO-mediated gene expression
Megan E. Keniry, Maria M. Pires, Ramon E. Parsons.
Presenter affiliation: Columbia University Medical Center, New York, New York. 6

Coordinated regulation of glucose-dependent survival by FOXO3A and S6K1 downstream of PTEN/AKT
Preeti Tandon, Shikha Khatri, Jennifer F. Barger, Catherine A. Gallo, Ashley Allemang, David R. Plas.
Presenter affiliation: University of Cincinnati, Cincinnati, Ohio. 7

DNA damage-dependent function of AKT is maintained independently of PI3K signaling elements in *C. ELEGANS*
Andrew J. Perrin, W. Brent Derry.
Presenter affiliation: The Hospital for Sick Children, Toronto, Canada. 8

PHLiPPing the switch in lipid second messenger signaling
Alexandra C. Newton.
Presenter affiliation: University of California, San Diego, La Jolla, California. 9

The role of PTEN and PHLPP in prostate cancer metastasis
Muhan Chen, Christopher Pratt, Martha Zeeman, Barry S. Taylor, Danielle M. Grace, Audrey O'Neill, Kato Mamoru, Michael Zhang, Carlos CordonCardo, Charles L. Sawyers, William Gerald, Alexandra C. Newton, Brett S. Carver, Lloyd C. Trotman.
Presenter affiliation: Cold Spring Harbor Laboratory, New York. 10

Tumour suppression by PTEN requires the activation of the PKR-eIF2a phosphorylation pathway.
Zineb Mounir, Jothi Latha Krishnamoorthy, Robertson P. Gavin, Randal J. Kaufman, Maria-Magdalena Georgescu, Antonis E. Koromilas.
Presenter affiliation: Lady Davis Institute for Medical Research and McGill University, Montreal, Canada. 11

WEDNESDAY, March 17—2:00 PM

SESSION 3 POSTER SESSION I

The PTEN-Daxx complex is regulated by acetylation and phosphorylation
Jorge A. Benitez, Webster K. Cavenee, Frank B. Furnari.
Presenter affiliation: Ludwig Institute for Cancer Research, University of California-San Diego, La Jolla, California. 12

Disruption of an inhibitory interface with p85 is responsible for the oncogenic potential of p110ß
Hashem A. Dbouk, Jonathan M. Backer.
Presenter affiliation: Albert Einstein College of Medicine, Bronx, New York. 13

mTORC2 is regulated through multi-site phosphorylation of a regulatory domain within Rictor
Christian C. Dibble, John M. Asara, Brendan D. Manning.
Presenter affiliation: Harvard School of Public Health, Boston, Massachusetts. 14

Separation of two forms of clathrin light chain B that differently affect a type 1-like protein phosphatase from microtubules
Akira Hiraga.
Presenter affiliation: Institute of Development, Aging and Cancer, Tohoku, Sendai, Japan. 15

The role of P-Rex2a in tumorigenesis through inhibition of PTEN
Cindy Hodakoski, Barry Fine, Susan Koujak, Benjamin Hopkins, Tao Su, Ramon Parsons.
Presenter affiliation: Columbia University Medical Center, New York, New York. 16

PTEN plays a pivotal role in retinal neurogenesis by supporting Notch signaling
Hong Seok Jo, Kyung Hwa Kang, Jaeho Lee, Cheol O. Joe, Greg Lemke, Jin Woo Kim.
Presenter affiliation: Korea Advanced Institute of Science and Technology, Daejeon, South Korea. 17

Role of a novel interaction between PTEN and drebrin at central nervous system synapses
Patricia Kreis, Michiel Van Diepen, Maddy Parsons, Nick Leslie, Britta Eickholt.
Presenter affiliation: King's College London, London, United Kingdom. 18

Coordinated regulation of dictyostelium AGC kinases AKT and PKBR1 by PI3K/PTEN, TORC2 and PDK1—Lessons from ancient organism
Xin-Hua Liao, Jonathan Buggey, Alan R. Kimmel.
Presenter affiliation: NIDDK, National Institutes of Health, Bethesda, Maryland. 19

Regulation of RUNX3 by PI3K/AKT in colorectal cancer
Anthony C. Lim, Kosei Ito, Yoshiaki Ito.
Presenter affiliation: Cancer Science Institute of Singapore, Singapore, Singapore. 20

Phosphatidylinositol 3' kinase (PI3K) oncogenic mutations increase invasiveness and drug resistance in breast epithelial cells
Fabiana C. Morales, Yiling Lu, Gordon B. Mills.
Presenter affiliation: University of Texas MD Anderson Cancer Center, Houston, Texas. 21

CD95-induced signaling in glioma downstream of PI3K
Marcin Teodorczyk, Ignacio Sancho-Martinez, Sachin Kumar, Ana Martin-Villalba.
Presenter affiliation: German Cancer Research Centre, Heidelberg, Germany. 22

Evidence for the significance of PTEN's protein phosphatase activity in tumor suppression
Laura Spinelli, Priyanka Tibarewal, Lindsay Davidson, Helene Maccario, Nevin M. Perera, Charles P. Downes, Nick R. Leslie.
Presenter affiliation: University of Dundee, Dundee, United Kingdom. 23

PTEN in whole chromosome instability
Janine H. van Ree, Karthik B. Jeganathan, Jan M. van Deursen.
Presenter affiliation: Mayo Clinic College of Medicine, Rochester, Minnesota. 24

Stimulation of its negative regulator PHLPP provides a feedback loop on Akt that is preferentially lost in glioblastoma
Noel Warfel, Matt Niederst, Alexandra C. Newton.
Presenter affiliation: University of California, San Diego, La Jolla, California. 25

Recurrent interstitial genomic deletion of the human *PTEN* gene are facilitated by flanking microhomologies
Maisa Yoshimoto, Andrew Evans, Kanishka Sircar, Tarek Bismar, Julia Williams, Paulo Nuin, Jeremy A. Squire.
Presenter affiliation: Queen's University, Kingston, Canada. 26

FoxO1 is essential for the regulation of pluripotency in human embryonic stem cells
Xin Zhang, Safak Yalcin, Marion Kennedy, Rani Sellers, Markus Landthaler, Thomas Tuschl, Gordon Keller, Saghi Ghaffari.
Presenter affiliation: Mount Sinai School of Medicine, New York, New York. 27

Functional analysis of the protein phosphatase activity of PTEN
Xiaoqun C. Zhang.
Presenter affiliation: Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 28

WEDNESDAY, March 17—4:30 PM

Wine and Cheese Party

WEDNESDAY, March 17—7:30 PM

SESSION 4 PTEN REGULATION

Chairperson: **P. Devreotes,** Johns Hopkins University School of Medicine, Baltimore, Maryland

The cell's compass—Signaling networks that mediate chemotaxis in eucaryotic cells
Peter N. Devreotes, Yulia Artemenko, Jane Borleis, Huaqing Cai, Jonathan Franca-Koh, Yoichiro Kamimura, Yu Long, Meghdad Rahdar, Kristen Swaney, Michelle Tang.
Presenter affiliation: Johns Hopkins University School of Medicine, Baltimore, Maryland. 29

MyosinV-dependent transport of PTEN regulates PI3K signalling and neuronal morphogenesis
Britta J. Eickholt, Tomas Perez, Michiel T. van Diepen, Maddy Parsons, Nick Leslie.
Presenter affiliation: King's College London, London, United Kingdom. 30

The regulation and mechanisms of action of the PTEN enzyme
Nick R. Leslie.
Presenter affiliation: University of Dundee, Dundee, United Kingdom. 31

Dynamic nuclear localization of Pten in zebrafish
Petra W. van Duijn, Suma Choorapoikayil, Jeroen den Hertog.
Presenter affiliation: Hubrecht Institute, Utrecht, Netherlands. 32

Alteration of PTEN signaling as a driving force for cancer
Ramon Parsons.
Presenter affiliation: Columbia University, New York, New York. 33

THURSDAY, March 18—9:00 AM

SESSION 5 DEVELOPMENT AND DISEASE MODELS

Chairperson: **H. Wu,** University of California, Los Angeles

Mouse models as translational tools to discover treatments for autism spectrum disorders—Focus on rapamycin
Luis F. Parada.
Presenter affiliation: University of Texas Southwestern Medical Center, Dallas, Texas. 34

Tuberous sclerosis—Recent results on genes, pathways, human disease, and mouse models
David J. Kwiatkowski.
Presenter affiliation: Brigham and Women's Hospital, Boston, Massachusetts. 35

Akt2 is required for specific glycolytic isozyme expression and cancer progression in PTEN-deficient mouse liver steatohepatitis
Ganna Panasyuk, Catherine Espeillac, Céline Chauvin, Ivan Nemazanyy, Morris J. Birnbaum, Jean-Ehrland Ricci, Mario Pende.
Presenter affiliation: INSERM, Paris, France; Université Paris Descartes, Paris, France. 36

Pten pathway alterations are associated with bladder tumor progression
Carlos Cordon-Cardo.
Presenter affiliation: Columbia University, New York, New York. 37

PTEN, stem cells and tumorigenesis
Hong Wu, Wei Guo, Suzanne Schubbert.
Presenter affiliation: UCLA, Los Angeles, California. 38

Function and regulation of the PTEN tumor suppressor gene
Pier-Paolo Pandolfi.
Presenter affiliation: Harvard Medical School, Boston, Massachusetts.

SESSION 6 POSTER SESSION II

Lovastatin inhibits EGFR dimerization and AKT activation in squamous cell carcinoma cells
Jim Dimitroulakos, Tong T. Zhao.
Presenter affiliation: Ottawa Hospital Research Institute, Ottawa, Canada. 39

Biallelic inactivation of TSC1 in radial Glia results in mTOR pathway activation
David M. Feliciano, Tiffany Su, Angelique Bordey.
Presenter affiliation: Yale University, New Haven, Connecticut. 40

An important role for PTEN tyrosine phosphorylation in glioblastoma pathogenesis and responses to treatment
Tim R. Fenton, Daisuke Kuga, Akio Iwanami, Claudio Ponte de Albuquerque, Robert M. Bachoo, Ronald A. Depinho, C David James, Huilin Zhou, Suely K. Marie, Paul S. Mischel, Webster K. Cavenee, Frank B. Furnari.
Presenter affiliation: Ludwig Insitute for Cancer Research, La Jolla, California. 41

PTEN and osteoblastogenesis
Anyonya R. Guntur, Martina I. Reinhold, Michael C. Naski.
Presenter affiliation: University of Texas Health Sciences Center, San Antonio, Texas. 42

Genetic and preclinical interrogation of a genetically engineered mouse model of small cell lung carcinoma
Patricia Hamilton, Jason Long, Anthony Lima, Rafael Molina, Hani Bou-Reslan, Tim Cao, Michelle Nannini, Richard Carano, Li Li, William Forrest, Suzana Couto, Leisa Johnson.
Presenter affiliation: Genentech, South San Francisco, California. 43

The dual PI3K/mTOR inhibitor NVP-BEZ235 induces cell death via the extrinsic apoptosis pathway
Irmgard Hofmann, Saskia M. Brachmann, Christian Schnell, Heidi Lane, Christine Fritsch, Carlos Garcia-Echeverria, Michel Maira.
Presenter affiliation: NIBR, Basel, Switzerland. 44

***Pten* and *P53* control self-renewal ability and differentiation potential of prostate epithelial stem/progenitor cells**
Paul G. Hynes, Wassim G. Abou-Kheir, Philip L. Martin, Kathleen Kelly.
Presenter affiliation: NCI, National Institutes of Health, Bethesda, Maryland. 45

Phospho-proteomics and mass-action modeling identify optimal drug combinations in signaling networks
Sergio Iadevaia, Yiling Lu, Fabiana C. Morales, Gordon B. Mills, Prahlad T. Ram.
Presenter affiliation: MD Anderson Cancer Center, Houston, Texas. 46

The PI3K pathway in a mouse model for invasive lobular carcinoma of the breast
Sjoerd Klarenbeek, Tanya Braumuller, Ingrid van der Heijden, Gilles Doumont, Jos Jonkers.
Presenter affiliation: The Netherlands Cancer Institute, Amsterdam, Netherlands. 47

Acinar cell neoplasia following conditional inactivation of Apc and Pten in the mouse salivary gland—Activation of mTOR signaling in human acinic cell carcinoma
Charlotta Lindvall, Cassandra Diegel, Nicole Evans, Adel El-Naggar, Kathleen R. Cho, Bart O. Williams.
Presenter affiliation: Van Andel Research Institute, Grand Rapids, Michigan. 48

The role of *Pten* in murine prostate development
Isabel Lokody, Amanda Swain.
Presenter affiliation: Institute of Cancer Research, London, United Kingdom. 49

Pro-senescence therapy for cancer and The Co-Clinical Trial Project
Caterina Nardella, Andrea Alimonti, Hui-Kuan Lin, Pier Paolo Pandolfi.
Presenter affiliation: Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts. 50

The core protein of HCV 3a triggers PTEN downregulation in hepatocytes—Role in HCV-mediated steatosis and insulin resistance.
Marion Peyrou, Sophie Clément, Manlio Vinciguerra, Andrea Sanchez, David Suter, Kevin Guilloux, Stephanie Pascarella, Karl-Heinz Krause, Laura Rubbia-Brandt, Francesco Negro, Michelangelo Foti.
Presenter affiliation: University of Geneva, Geneva, Switzerland. 51

Combined deletion of Pten, Trp53 and Rb1 induced astrocytomas within and outside of proliferative niches in the mature brain
Sherri L. Rankin, Lionel M. Chow, Raelene Endersby, Xiaoyan Zhu, Junyuan Zhang, Chunxu Qu, David W. Ellison, Suzanne J. Baker.
Presenter affiliation: St Jude Children's Research Hospital, Memphis, Tennessee. 52

Expansion of hepatic tumor progenitor cells in *Pten* deficient mice requires liver injury
Vivian Galicia, Lina He, Gary Kanel, Christopher Vandryes, Ni Zeng, Jennifer-Ann Bayan, C. Bart Rountree, Kasper Wang, Morris Birnbaum, Bangyan Stiles.
Presenter affiliation: University of Southern California, Los Angeles, California. 53

Bioluminescence imaging captures the expression of *p21*$^{Waf1/Cip1}$ in living mice
Kelsey L. Tinkum, Lynn White, Jinwu Sun, David Piwnica-Worms, Helen Piwnica-Worms.
Presenter affiliation: Washington University School of Medicine, St. Louis, Missouri. 54

Oxidative stress-mediated amplification of AKT/mTOR signaling pathway leads to myeloproliferative syndrome in FoxO3-null mice—A role for Lnk adaptor protein
Safak Yalcin, Xin Zhang, Dragan Marinkovic, Wei Tong, Sathish K. Mungamuri, Saghi Ghaffari.
Presenter affiliation: Mount Sinai School of Medicine, New York, New York. 55

Mammalian target of rapamycin regulates cell differentiation through the STAT3-p63-Jagged-Notch cascade
Jianhui Ma, Huangxuan Shen, Xinxin Chen, Haiyong Peng, Qian Sun, Xiaojun Zha, Fang Wang, Ying Wang, Shu Zhang, Lizi Wu, David J. Kwiatkowski, Hongbing Zhang.
Presenter affiliation: Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China. 56

THURSDAY, March 18—7:30 PM

SESSION 7 mTORC1 AND INTERSECTING PATHWAYS

Chairperson: **B. Manning,** Harvard School of Public Health, Boston, Massachusetts

The LKB1/AMPK tumor suppressor pathway coordinates cell growth and metabolism
Reuben J. Shaw.
Presenter affiliation: Salk Institute for Biological Studies, La Jolla, California. 57

Activation of a metabolic gene regulatory network downstream of mTORC1
Katrin Duvel, Jessica L. Yecies, Suchithra Menon, Clary B. Clish, Leon O. Murphy, Brendan D. Manning.
Presenter affiliation: Harvard School of Public Health, Boston, Massachusetts. 58

Integration of amino acid and glucose signaling in mTOR complex 1 (mTORC1)-dependent regulation of cell growth and survival
John Blenis, Andrew Y. Choo, Sang Gyun Kim, Greg R. Hoffman, Neil Kubica, Sarah J. Mahoney.
Presenter affiliation: Harvard Medical School, Boston, Massachusetts. 59

The late endosome is essential for mTORC1 signaling
Rory J. Flinn, Ying Yan, Sumanta Goswami, Peter J. Parker, Jonathan M. Backer.
Presenter affiliation: Albert Einstein College of Medicine, Bronx, New York. 60

FoxOs serve as an energy stress checkpoint and suppress mTORC1-mediated renal cancer development
Boyi Gan, Carol Lim, Gerald Chu, Sujun Hua, Zhihu Ding, Jian Hu, William G. Kaelin Jr., Sabina Signoretti, Ronald A. DePinho.
Presenter affiliation: Dana-Farber Cancer Institute, Boston, Massachusetts. 61

mTOR and the control of growth
David M. Sabatini.
Presenter affiliation: Whitehead Institute, Cambridge, Massachusetts. 62

FRIDAY, March 19—9:00 AM

SESSION 8 THERAPY AND CANCER MODELS

Chairperson: **J. Engelman,** Massachusetts General Hospital, Boston

Forkhead proteins in insulin action
Domenico Accili.
Presenter affiliation: Columbia University, New York, New York. 63

Systems biology approach to personalized medicine
Gordon B. Mills.
Presenter affiliation: University of Texas M.D. Anderson Cancer Center, Houston, Texas. 64

Feedback and redundancy in PI3K activated pathways in cancer—Therapeutic implications
Neal Rosen.
Presenter affiliation: Memorial Sloan-Kettering Cancer Center, New York, New York.

Using mechansitic insights into PI3K regulation to identify therapeutic strategies to treat cancer
Jeffrey Engelman.
Presenter affiliation: Massachusetts General Hospital, Boston, Massachusetts.

The mTOR pathway in cancer: mechanisms and therapeutic promise
Karen Cichowski.
Presenter affiliation: BWH/Harvard Medical School, Boston, Massachusetts. 65

Preclinical evidence supporting combination partners for NVP-BEZ235 breast cancer trials
Saskia M. Brachmann, Irmgard Hofmann, Susan Wee, Carlos Garcia-Echeverria, Michel S. Maira.
Presenter affiliation: NIBR, Basel, Switzerland. 66

FRIDAY, March 19—6:00 PM

CONCERT

Grace Auditorium

Ran Dank, piano

Pianist Ran Dank was awarded the Sander Buchman Memorial First Prize in the 2008-09 Young Concert Artists International Auditions and makes his debut on December 14 this season in the Young Concert Artists Series at Merkin Concert Hall in New York, sponsored by the Jerome L. Greene Foundation Prize. At the Auditions, he was awarded many special prizes: the John Browning Memorial Prize, the Slomovic Orchestra Soloist Prize, the Albany Symphony Prize (GA), the Embassy Series Prize for a concert at an Embassy in Washington DC, and the Saint Vincent College Bronder Prize for Piano (PA). During the 2009-10 season, Mr. Dank will be heard at the University of Florida, the International Keyboard Institute and Festival at the Mannes College in New York, at the Seattle Chamber Music Festival, for the Patrons for Young Artists series (NY), at the Port Washington Library (NY), for Vanguard Concerts (OH), at the Jewish Community Alliance (FL), and for the Xavier University Piano Series (OH).

Mr. Dank won First Prize in the 2008 Hilton Head International Piano Competition and was awarded the 2006 Gina Bachauer Scholarship at the Juilliard School. In 2007, he won top prizes in the Sydney International Piano Competition in Australia, leading to an extensive Australian tour including a concerto performance with the Sydney Symphony, and the Cleveland International Competition, bringing him an appearance with the Cleveland Orchestra under the baton of Jahja Ling. Mr. Dank has performed as soloist with the Juilliard Orchestra as winner of the Concerto Competition, and in his native Israel with the symphony orchestras of Jerusalem, Rishon Lezion and Raanana. Festival appearances have included the Chopin Festival in Warsaw, the Israel Festival, and return engagements to Finland's Mäntta Festival for Virtuoso Pianists.

Mr. Dank was born in Israel in 1982 and started piano lessons at the age of seven. He received his Bachelor's degree from the Rubin Academy of Music at Tel Aviv University, where he studied with Emanuel Krasovsky. He is the recipient of grants from the America-Israel Cultural Foundation. Mr. Dank has a Master's degree from the Juilliard School where he worked with Emanuel Ax and Joseph Kalichstein (both YCA "Alumni"). Mr. Dank also earned an Artist Diploma from the Juilliard School in 2009, working with Robert McDonald.

FRIDAY, March 19

BANQUET

Cocktails 7:00 PM Dinner 7:45 PM

SATURDAY, March 20—9:00 AM

SESSION 9 AGING, METABOLISM AND CANCER

Chairperson: **A. Brunet,** Stanford University, California

Oncogenic signaling in the class I PI3K pathway
Jonathan R. Hart, Li Zhao, Petra Hillmann, Petra Pavlickova, Minghao Sun, Lynn Ueno, Peter K. Vogt.
Presenter affiliation: The Scripps Research Institute, La Jolla, California. 67

Role of FOXO transcription factors in aging
Anne Brunet.
Presenter affiliation: Stanford University, Stanford, California. 68

CRTC-1—A novel Calcineuin and AMPK target for lifespan extension in *C. elegans*
William Mair, Ianessa Morantte, Reuben Shaw, Andrew Dillin.
Presenter affiliation: Howard Hughes Medical Institute and Glenn Center for Aging Research, The Salk Institute for Biological Studies, La Jolla, California. 69

Protection from metabolic damage and increased longevity by PTEN
Ana Ortega-Molina, Alejo Efeyan, Mulero Francisca, Manuel Serrano.
Presenter affiliation: Fundacion CNIO, Madrid, Spain. 70

PDZ-RhoGEF regulates adipose tissue development via insulin/IGF-1 signaling
Ying-Ju Jang, Lily Zhou, Nicole Liadis, Christine K. Ng, Zenyu Hao, Scott Pownall, Vuk Stambolic, Tak W. Mak.
Presenter affiliation: University of Toronto, Canada. 71

PI3K and cancer metabolism
Lewis Cantley.
Presenter affiliation: Beth Israel Deaconess Medical Center, Boston, Massachusetts.

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INTEGRATION OF PI3K SCAFFOLD AND CATALYTIC FUNCTIONS IN HEALTH AND DISEASE

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Phosphoinositide 3-kinases (PI3K) are key players in receptor-mediated signal transduction and play a role in a large variety of biological processes. PI3K consist of heterodimers of a 110 kD catalytic (p110) as well as a regulatory/adapter subunit and are required for the production of a membrane bound phosphorylated lipid (PIP3) that acts as a secondary messenger molecule. Class I p110s (p110α, β, γ and δ) share significant homology but studies using genetically engineered mice show that they all play non-redundant roles. While these reports recently provided support for PI3K as promising drug targets they also unexpectedly revealed that these proteins not only work as kinases but also as scaffolds for protein-protein interactions. For example, we showed that p110β catalytic activity is required for male fertility and for Erbb2 driven mammary gland cancer development. On the other hand, loss of p110β blocks endocytosis and results in reduced fibroblast proliferation and embryonic lethality. Similarly, p110γ plays a crucial role in the mounting of inflammatory reactions as well as in leukemia but it is also part of a multiprotein complex that controls cAMP levels and cardiac contractility. Indeed, we found that p110γ is a scaffold protein that mediates a reciprocal control between cAMP and PIP3 signaling. These data not only support the view of PI3K as drug targets for ATP binding site competitors but also for more sophisticated protein-protein interaction inhibitors.

INSIGHTS INTO THE ONCOGENIC EFFECTS OF *PIK3CA* MUTATIONS FROM THE STRUCTURE OF PHOSPHOINSITIDE-3-KINASE

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Phosphatidylinositide-3-kinases (PI3K) initiate a number of signaling pathways by recruiting other kinases, such as Akt, to the plasma membrane. One of the isoforms, PI3Kα, is an oncogene frequently mutated in several cancer types. These mutations increase PI3K kinase activity, leading to increased cell survival, cell motility, cell metabolism, and cell cycle progression. The structure of the complex between the catalytic subunit of PI3Kα, p110α, and a portion of its regulatory subunit, p85α reveals that the majority of the oncogenic mutations occur at the interfaces between p110 domains and between p110 and p85 domains. At these positions, mutations disrupt interactions resulting in changes in the kinase domain that may increase enzymatic activity. The structure also suggests that interaction with the membrane is mediated by a region of the heterodimer that includes one of the p85 domains (iSH2). The structure of the most common oncogenic mutation, H1047R, shows differences in p110 with the wild-type that indicate that this mutation may activate the enzyme by increasing its interaction with the cellular membrane. These findings may provide novel structural loci for the design of isoform-specific, mutant-specific new anti-cancer drugs.

OVERLAPPING AND DISTINCT ONCOGENIC PROPERTIES OF THE E545K AND H1047R PIK3CA MUTATIONS.

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One of the signaling pathways that is centrally involved in mammary tumorigenesis is the phosphoinositide 3'-kinase (PI3K) pathway. Importantly, it was recently established that *PIK3CA*, the gene encoding the catalytic subunit of PI3K, often harbors heterozygous gain-of-function missense mutations in human malignancies, including ~30% of breast cancer cases. The majority of the mutations in *PIK3CA* cluster in two regions, within the helical and kinase domains (HD & KD). The codons most frequently affected are E545K and H1047R ("hot-spots") in exons 9 and 20, respectively. Both types of these mutations increase the kinase activity of the enzyme, upregulate the downstream AKT pathway, and stimulate cell transformation and tumorigenesis. Despite the similarities in the effects of the two hotspot mutations, there is growing evidence that they are not equivalent. To analyze the action of *PIK3CA* hotspot mutations in mammary tumorigenesis we have generated mouse models carrying either one of these two gain-of-function "knock-in" mutations on conditionally-expressed alleles. Both mutations are embryonic lethal but when they are activated postnatally either globally or specifically in mammary epithelial cells display differential physiological functions and oncogenic properties. PIK3CA mutations cooperate with c-Myc overexpression, leading to rapid formation of multifocal mammary tumors which show increased protease activity and metastasize to the lung soon after their establishment.

CONTEXT-DEPENDENT FUNCTIONS OF PI3K SIGNALING IN BRAIN

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PTEN plays an essential role in development, homeostatic maintenance and tumor suppression in the mammalian brain. Although the effectors in the PI3K/PTEN pathway are ubiquitously expressed, there are cell-type and context-dependent variations in pathway regulation that likely underlie differences in how abnormal PI3K signaling contributes to pathological conditions arising from different cell types. Conditional deletion of key effectors in the PI3K pathway including *Pten* and *Pdk1* revealed cell type-specific differences in regulation of PI3K signaling in brain. Loss of function mutations of *PTEN* and oncogenic mutations of *PIK3CA* both occur in glioblastoma, the most common and most aggressive malignant brain tumor. To determine the physiological consequences of activating the PI3K pathway through these different effectors, we used *in vivo* conditional induction of *Pten* deletion or *Pik3ca* E545K mutation to define their roles in normal and tumorigenic growth regulation in brain.

AKT ISOFORM-SPECIFIC SIGNALING IN BREAST CANCER CELL INVASIVE MIGRATION

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Akt/PKB (protein kinase B) is a proto-oncogene that plays critical roles in cell survival, proliferation and metabolism. There are three mammalian Akt isoforms: Akt1, Akt2 and Akt3. They share a high degree of amino acid similarity and are activated by similar mechanisms. The current paradigm is that all three Akt isoforms promote cancer cell survival and growth. Interestingly, recent studies from our laboratory have shown that Akt1 inhibits breast cancer cell migration and invasion. Conversely, Akt2 has been shown to promote breast cancer cell migration. We therefore seek to test the hypothesis that Akt isoforms regulate breast cancer cell invasive migration in a distinct manner, and investigate the mechanistic basis for this selectivity. Current experiments are undertaken to identify novel substrates of Akt1 and Akt2, which modulate cell motility, with a combination of shRNA and phospho-proteomic approaches. shRNAs specific to Akt1 or Akt2 have been generated and delivered into human breast cancer cells by lentiviral vectors. Using an antibody that recognizes phosphorylated serine/threonine residues contained within a consensus Akt motif (RXRXXS/T), we showed that palladin, a component of actin-containing microfilaments, is phosphorylated at serine 507 by Akt1 but not Akt2. Downregulation of palladin using RNA interference enhances migration and invasion of breast cancer cells and induces abnormal branching morphogenesis in 3D cultures, indicative of an anti-migratory function for palladin. Importantly, palladin is required for Akt1-mediated inhibition of breast cancer cell migration, whereby the migratory effect induced by palladin silencing is reversed by wild-type but not a S507A mutant. Finally, palladin phosphorylation by Akt1 is functionally required for bundling F-actin and maintaining an organized actin cytoskeleton network in cells. These findings identify palladin as an Akt1-specific substrate that regulates cell motility and further provide a molecular mechanism that accounts for the functional distinction between Akt isoforms in breast cancer cell migration.

TRANSCRIPTIONAL REPRESSOR NFIL3 MODULATES THE PI3K/PTEN/FOXO PATHWAY TO ATTENUATE FOXO-MEDIATED GENE EXPRESSION

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PI3K/PTEN signaling is frequently altered in cancer. Unraveling PI3K/PTEN pathway architecture will facilitate the identification of novel biomarkers and drug targets to better treat cancer. We isolated the pro-survival NFIL3 transcription factor in a reporter screen designed to identify novel pathway regulators. We found that NFIL3 integrated into PI3K/PTEN signaling at the transcriptional level by regulating a broad repertoire of FOXO-induced genes. NFIL3 associated with the promoters of FOXO-induced genes and co-repressed transcription with HDAC2. Loss and gain of function experiments revealed that NFIL3 is a critical regulator of apoptosis in cancer cells. NFIL3 and HDAC2 were found to be highly expressed in cancers that confer poor prognosis, suggesting that HDAC inhibition may be an effective therapy for these cancers. NFIL3 expression was associated with poor prognosis in breast cancer. Thus, NFIL3 is a new transcriptional modulator of PI3K/PTEN/FOXO signaling that could potentially serve as a novel pathway biomarker/drug target.

COORDINATED REGULATION OF GLUCOSE-DEPENDENT SURVIVAL BY FOXO3A AND S6K1 DOWNSTREAM OF PTEN/AKT

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PTEN inactivation triggers Akt-dependent apoptosis resistance that contributes to both oncogenesis and resistance to chemotherapeutics. We have shown that Akt-induced glucose metabolism is a required element for apoptosis resistance in PTEN-deficient cells. The downstream signaling elements that mediate Akt-dependent increases in glycolysis are not fully characterized. Our results show that both the FOXO-family transcription factors and the TSC-mTORC1-S6K1 pathways are critical for glucose-dependent cell survival mediated by Akt. FOXO3a knockdown is sufficient to induce glycolysis associated with cell survival. Increased glycolysis in FOXO3a-deficient cells is due to decreased transcription of TSC1, leading to elevated S6K1 activity. Reexpression of TSC1 or rapamycin treatment are sufficient to reestablish basal rates of glycolysis in FOXO3a-knockdown cells. In PTEN-deficient cells, S6K1-knockdown reduces glycolysis and enforces apoptosis sensitivity. Restoring glycolysis by expression of HIF-1α is sufficient to reactivate apoptosis resistance in S6K1-deficient cells. Together the data reveal that crosstalk between the FOXO and TSC-mTORC1-S6K1 pathways downstream of Akt is critical for establishing the glycolytic metabolic program that supports Akt-dependent survival.

DNA DAMAGE-DEPENDENT FUNCTION OF AKT IS MAINTAINED INDEPENDENTLY OF PI3K SIGNALING ELEMENTS IN *CAENORHABDITIS ELEGANS*

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The roundworm *Caenorhabditis elegans* possesses a single, conserved phosphatidylinositol 3-kinase (PI3K) signaling pathway that regulates somatic developmental decisions and lifespan through the Insulin-like receptor tyrosine kinase (RTK) DAF-2, the class I PI3K AGE-1 and the PDK1 homologue PDK-1. This pathway ultimately controls the action of Akt homologues on the forkhead transcription factor DAF-16. We recently demonstrated that the *C. elegans Akt* orthologue *akt-1* also negatively regulates the DNA damage-dependent apoptosis of worm germ cells by interfering with activation of the key transcription factor CEP-1, a sole homologue of p53 in the worm. Because upstream regulation by RTK/PI3K signaling is known to couple with downstream Akt kinase activity, we hypothesized that the worm *daf-2*/*age-1*/*pdk-1* pathway would function upstream of *akt-1*/*Akt* in response to DNA damage. Surprisingly, this was not the case. Instead, loss-of-function (lf) mutants in *daf-2*/RTK, *age-1*/PI3K, and *pdk-1*/*PDK1* were all extremely resistant to DNA damage, while a gain-of-function (gf) mutant in *pdk-1* was hypersensitive, confirming that, unlike *akt-1*/*Akt*, RTK/PI3K signaling components promote damage-induced germ cell apoptosis in *C. elegans*. Subsequent genetic analysis demonstrated that lf mutations in both *daf-2*/RTK and *pdk-1*/*PDK1* were epistatic to *akt-1*/*Akt*, and thus, *daf-2*/RTK and *pdk-1*/*PDK1* do not function upstream of *akt-1*/*Akt* in response to damage. In addition, while AKT-1/Akt functions upstream of CEP-1/p53 and is able to control its damage-dependent transcriptional function *in vivo*, loss of neither *daf-2*/RTK nor *pdk-1*/*PDK1* hindered transcriptional function of CEP-1/p53 at the *egl-1*/BH3-only target gene promoter. Therefore, *daf-2*/RTK and *pdk-1*/*PDK1* function independently of *akt-1*/*Akt* and *cep-1*/*p53* to promote damage-induced germ cell death. It appears that *daf-2*/RTK, *pdk-1*/*PDK1*, and *cep-1*/*p53* co-operate from mutually exclusive pathways to drive germ cell death since a *pdk-1(gf)* mutation cannot revert the *daf-2* phenotype, yet still requires *cep-1*/*p53* to kill. That RTK/PI3K signaling can be separated from Akt function in the worm suggests that *akt-1*/*Akt* may be regulated in a novel manner in response to DNA damage, and that a single PI3K signaling pathway can be re-arranged to suit a variety of situations *in vivo*. This shuffling of RTK/PI3K signaling modules in *C. elegans* may represent a more general example of how signaling pathways acquire functional resilience in the absence of evolutionary complexity.

PHLiPPing THE SWITCH IN LIPID SECOND MESSENGER SIGNALING

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The Ser/Thr specific-phosphatase PHLPP (PH domain leucine-rich repeat protein phosphatase) provides the brakes to signaling in both the PI3 kinase and diacylglycerol pathways. The two isoforms of this recently discovered family, PHLPP1 and PHLPP2, control the amplitude and duration of signaling by two distinct mechanisms: 1] direct dephosphorylation of Akt and PKC at the hydrophobic phosphorylation motif, a C-terminal phosphorylation switch that controls the activity of these kinases, and 2] control of cellular levels of growth factor receptors by regulation of their mRNA levels. This contribution focuses on the cellular function and regulation of PHLPP.

THE ROLE OF PTEN AND PHLPP IN PROSTATE CANCER METASTASIS

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Modeling partial and complete Pten-loss in mice, has revealed that this pathway controls prostate cancer onset, progression, and outcome. While these findings underscore the role of Pten regulation in cancer, we have recently uncovered an equally critical role for signal termination, as Pp2a-mediated Akt inactivation in the nucleus is essential and sufficient for prevention of colon and prostate cancer, even after partial loss of Pten. To broaden our understanding of signal termination in cancer, we are studying the second class of known phosphatases of Akt, namely the Phlpp genes. To this end, we follow prostate tumorigenesis in cohorts of Phlpp knockout mice, which have a normal or hyperactive PI 3-K pathway by virtue of heterozygosity for Pten.

Our analysis reveals that Phlpp-loss on its own initiates tumorigenesis in prostate and other tissues and that it strongly cooperates with partial loss of Pten. To demonstrate its role in human prostate cancer we will present results from our study on the genome wide analysis of several hundred patient biopsies, banked at Memorial Sloan Kettering Cancer Center.

TUMOUR SUPPRESSION BY PTEN REQUIRES THE ACTIVATION OF THE PKR-EIF2A PHOSPHORYLATION PATHWAY.

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Inhibition of protein synthesis by phosphorylation of the α subunit of eukaryotic translation initiation factor 2 (eIF2) at Ser51 occurs due to the activation of a family of kinases in response to various forms of stress. Although some aspects of the phosphorylation of eIF2α are cytoprotective, phosphorylation of eIF2α by RNA-dependent protein kinase (PKR) is largely proapoptotic and tumor suppressing in nature. Phosphatase and tensin homolog deleted from chromosome 10 (PTEN) is a tumor suppressor protein that is mutated or deleted in various human cancers. The function of PTEN is mediated through phosphatase-dependent and -independent pathways. We demonstrate that the eIF2α phosphorylation pathway acts downstream of PTEN. Inactivation of PTEN in human melanoma cells reduced the extent of phosphorylation of eIF2α, whereas reconstitution of PTEN-null human glioblastoma or prostate cancer cells with either wild type PTEN or phosphatase-defective mutants of PTEN induced the activity of PKR and the phosphorylation of eIF2α. The antiproliferative and proapoptotic effects of PTEN were substantially compromised in mouse embryonic fibroblasts that lacked PKR or contained a phosphorylation-defective allele of eIF2α. Induction of the pathway leading to phosphorylation of eIF2α required the presence of an intact PDZ-binding motif in PTEN. These findings establish a link between tumor suppression by PTEN and inhibition of protein synthesis independently of the inhibition of phosphoinositide 3’-kinase signaling.

THE PTEN-DAXX COMPLEX IS REGULATED BY ACETYLATION AND PHOSPHORYLATION

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Post-translational modifications of PTEN, such as phosphorylation and acetylation, which are associated with its conformational changes ("closed" to "open") and stability, have been associated with PTEN tumor suppressor function. We have previously reported that phosphorylation at threonine 366 (T366) in the C-terminal region of PTEN, regulates cellular proliferation and transformation independently of its lipid phosphatase activity and by physical interaction suppresses the transformation potential of MSP58 (human microspherule protein 1) (Okumura et al., 2005), which is highly expressed in GBM samples and glioma cells (Lin et al., 2008). Because little is known about the tumor suppressor function of T366 phospho-PTEN, we analyzed its interactions with others proteins. We found that one of these PTEN-associated proteins that binds in this region is Daxx (death domain-associated protein). Here, we show that physical interaction with Daxx occurs through PTEN amino acids 186-202, which is the same region involved in its interaction with PCAF (p300/CBP-associated factor), a histone acetyltransferase that regulates the acetylation of Lys 125 and Lys 128 of PTEN. After DNA damage in glioma cells the acetylation of PTEN is increased and the PTEN-Daxx complex is destabilized, suggesting a potential role for Daxx in regulating PTEN-acetylation. In addition, the PTEN- Daxx interaction was T366-phosphorylation dependent, as demonstrated by reduced association with PTEN mutated to alanine at this position. Finally, the PTEN-Daxx interaction in cells occurs even in the absence of p53, but the interaction is stronger when either p53 or pten are mutated. We hypothesize that PTEN phosphorylation and acetylation may be mediating the assembly of diverse complexes and thus playing a regulatory role in its tumor suppressor function.

DISRUPTION OF AN INHIBITORY INTERFACE WITH p85 IS RESPONSIBLE FOR THE ONCOGENIC POTENTIAL OF p110β

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Class I PI3Ks signal downstream of RTKs and GPCRs, and have been implicated in tumorigenesis. In contrast to p110α, whose oncogenic potential requires its mutational activation, other p110 isoforms can induce transformation by over-expression of the wild type enzyme. Sequence alignment reveals that the residue analogous to N345 in p110α is K342 in p110β. This difference makes wild type p110β analogous to an oncogenic mutant p110α (N345K). In p110α, N345 in the C2 domain forms hydrogen bonds with D560 and N564 in the iSH2 domain of p85, and mutations of p110 or p85 that disrupt this interface lead to increased basal activity and transformation. We now show that p110β exhibits a higher basal activity with wild type p85 than p110α, and is not further activated by a mutation of p85 that disrupts the C2-iSH2 domain interface. Similar results were seen in soft agar transformation assays, where p110β was similar to p110α N345K in transformation potential. The regulation of p110β by p85 was restored by a K342N mutation of p110β, which showed decreased activity in vitro, and decreased Akt activation and transformation in NIH3T3 cells. Moreover, unlike wild type p110β, p110β-K342N is differentially regulated by wild type versus oncogenic p85 mutants. This study shows that the higher basal p110β activity and its transforming potential are due in part to disruption of an inhibitory interface with the iSH2 domain of p85.

MTORC2 IS REGULATED THROUGH MULTI-SITE PHOSPHORYLATION OF A REGULATORY DOMAIN WITHIN RICTOR

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The serine/threonine protein kinase mammalian target of rapamycin (mTOR) acts within two physically and functionally distinct complexes (mTORC1 and mTORC2) to control cell growth, proliferation, survival, and metabolism. While there has been great progress in understanding the regulation of mTORC1, much less is known about the signaling mechanisms that regulate mTORC2. To better understand mTORC2 regulation, we characterized the phosphorylation events on its essential core component Rictor. Interestingly, most of the 21 phosphorylation sites we identified on Rictor are conserved throughout vertebrates and cluster within an approximately 400 a.a. region in the C-terminal half of the protein. We propose that this region has evolved as a regulatory domain that allows multiple signaling pathways to control mTORC2 through post-translational modification of Rictor. We have recently found that deletion of this regulatory domain yields a Rictor mutant that, as part of mTORC2, induces a dramatic increase in phosphorylation of both growth factor-dependent (e.g., Akt-S473) and -independent (e.g., Akt-T450) substrates of mTORC2. These results suggest that this domain of Rictor plays a net negative role in the regulation of mTORC2. We have identified one specific phosphorylation site (T1135) within this region that acts to negatively regulate mTORC2. Rictor-T1135 is a new substrate of S6K1 that is phosphorylated in response to growth factors and amino acids downstream of mTORC1. Expression of a non-phosphorylatable mutant (Rictor-T1135A) in cells moderately increases mTORC2-dependent phosphorylation of Akt-S473 in response to insulin. However, mutation of this site does not affect the in vitro kinase activity of mTORC2. Therefore, phosphorylation of Rictor-T1135 by S6K1 constitutes a new negative feedback mechanism affecting Akt activation. We are currently determining the molecular mechanisms by which the regulatory domain of Rictor controls mTORC2 signaling to its downstream substrates and exploring potential input from PI3K and PTEN. We will report on the most up-to-date findings from these efforts.

SEPARATION OF TWO FORMS OF CLATHRIN LIGHT CHAIN B THAT DIFFERENTLY AFFECT A TYPE 1 LIKE PROTEIN PHOSPHATASE FROM MICROTUBULES

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PTEN controls a variety of intracellular processes, hydrolyzing 3-phosphate of phosphatidylinositol 3,4,5-triphosphate. The class 1 and the class 2 phosphoinositide 3-kinase can antagonize (the latter when activated by clathrin) PTEN and may function in pathways involving clathrin and MT, respectively, through downstream kinases Akt and GSK3 and through the action on clathrin assembly and the linkage of clathrin and MTs. These pathways may be found in NMDA receptor internalization, CLUT4 translocation, neurosecretion and cellular entry and propagation of viruses. We reported that clathrin light chain b (CLb) could affect potently and selectively a type-1-like protein phosphatase (PP) that was cosedimented with brain microtubules (MTs) and thus termed MT-PP1. Since the known binding motif for PP1 is not found in CLb, the interaction between CLb and MT-PP1 may form a novel regulatory mechanism for PP and also contribute to functional linkage of clathrin and MTs. Thus, small but new information below of the interaction may help further analysis of PTEN downstream pathways. The CLb preparation reported previously was purified from the post-MT supernatant containing 25 % glycerol and comprised two forms of CLb of 29 kDa and 32 kDa (hereafter called respectively CLb29 and CLb32). In the absence of glycerol, CLb32 was coisolated with the clathrin fraction but CLb29 remained in the cytosol fraction. CLb29 and CLb32 were each purified using the procedures we reported for purification of 29 kDa and 32 kDa CLb from the post-MT supernatant. CLb29 affected MT-PP1 with an EC50 of about 1 nM. CLb32 also affected MT-PP1 but with an EC50 of about 500 nM. Interestingly, both CLb affected MT-PP1 several-fold more strongly than PP1 molecules purified from skeletal muscle. CLb29 was similarly purified from the cytosol independent of whether the brain extract was incubated at 37 ˚C for MT assembly and whether it contained 0.5 NaCl.

THE ROLE OF P-REX2A IN TUMORIGENESIS THROUGH INHIBITION OF PTEN

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The tumor suppressor PTEN (phosphatase and tensin homologue deleted on chromosome 10) was discovered through the mapping of homozygous deletions on human chromosome 10q23 due to its frequent loss of heterozygosity in human tumors. PTEN, a lipid and protein phosphatase, acts as a tumor suppressor by inhibiting PI3K signaling through the dephosphorylation of PIP3 to PIP2. Regulation of PTEN itself is poorly understood; therefore, we sought to identify new PTEN interacting proteins by GST-pull down. P-Rex2a was one of the PTEN associated proteins identified. P-Rex2a is a guanine-nucleotide exchange factor for Rac and a member of the Rho-GTPase family of proteins. We have shown that P-Rex2a inhibits PTEN phosphatase activity. Furthermore, P-Rex2a expression leads to increased PI3K signaling by upregulating phosphorylated Akt. Expression of P-Rex2a in the non-transformed human mammary epithelial cell line MCF10A causes increased growth under starvation conditions. In addition, P-Rex2a cooperates with mutations in PIK3CA to transform MCF10As and also with NeuT to form tumor xenografts in nude mice. Furthermore, the physical PTEN/P-Rex2a interaction is being mapped and the mechanism of PTEN inhibition is being analyzed.

PTEN PLAYS A PIVOTAL ROLE IN RETINAL NEUROGENESIS BY SUPPORTING NOTCH SIGNALING

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Neurogenic programs guiding the development of a given repertoire of neurons that arise from neural progenitor cells (NPCs) are closely linked to the general cellular phenomena of cell growth, proliferation, and survival. Multiple developmental cues that affect neurogenesis act simultaneously on NPCs, thus intracellular signaling hubs that integrate various external signals and influence general cellular events are required to balance the maintenance of NPCs with their differentiation into neurons. Here, we propose that phosphatase tensin homolog (PTEN) is located at the intracellular signaling hub in retinal progenitor cells (RPCs) and controls the speed of retinal neurogenesis. Mouse RPCs lacking the Pten gene completed neurogenesis earlier than regularly scheduled, resulting in precocious depletion of the RPC population. We found that hyperactivated Akt in Pten-deficient RPCs inhibited the formation of Notch transcription activator complex through phosphorylation of the Notch intracellular domain, and consequently interfered with Notch-supported RPC maintenance. We further demonstrated a coordinated oscillation of Akt activation and phosphorylation-dependent inactivation of Notch during cell cycle progression. Taken together, our results indicate that PTEN plays a pivotal role in retinal neurogenesis by controlling the negative feedback loop of phosphoinositide 3-kinase (PI3K)-Akt signaling to Notch.

ROLE OF A NOVEL INTERACTION BETWEEN PTEN AND DREBRIN AT CENTRAL NERVOUS SYSTEM SYNAPSES

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PTEN (Phosphatase and tensin homolog deleted on chromosome 10) is a lipid phosphatase that mainly antagonises the PI3K signalling pathway by transforming the phosphatidyinositol 3 phosphate lipid (PIP3) into phosphatidyinositol 2 phosphate (PIP2). As a tumour suppressor, PTEN has been largely studied for its role in cancer, however little is known about its function and regulation in the central nervous system.
We have recently identified novel proteins that form complexes with PTEN in brain tissue by mass spectrometry. One brain specific interactor was characterised as drebrin: 5 peptides matched the drebrin entry (Q07266) with a total coverage of 15% and initial characterisation verified the PTEN/drebrin interaction by co-immunoprecipitation from rat brain lysate. Drebrin is an actin binding protein that is largely known for its function in the formation of filopodia. In particular, drebrin governs spine morphogenesis and overexpression of drebrin in hippocampal neurons induces a drastic increase in the length of the dendritic filopodia. In order to test whether PTEN directly interacts with drebrin we analysed the FRET efficiency between PTEN-GFP and drebrin-mCherry by multiphoton FLIM in PC12 cells. This approach showed reproducible hot spots in the reduction in lifetime that indicate sites of direct PTEN/drebrin interactions. Interestingly, these sites of interaction were specifically localized in the periphery of cells that showed highly differentiated morphologies, and never in cells with non-differentiated, flat morphologies. Similarly, direct PTEN/Drebrin interactions were observed in filopodial protrusions in primary hippocampal neurons.
In summary, we have identified the interaction of two proteins that have individually been linked to controlling dendritic spine morphology. We hypothesize that the association of PTEN with the actin binding protein drebrin governs essential processes during re-organisation of the actin cytoskeleton at the synapse and here we will present our data that test the relevance of this interaction in the formation and maintenance of dendritic spines in hippocampal neurons.

COORDINATED REGULATION OF DICTYOSTELIUM AGC KINASES AKT AND PKBR1 BY PI3K/PTEN, TORC2 AND PDK1: LESSONS FROM ANCIENT ORGANISM

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AGC Kinases AKT and PKBR1 play a crucial role in chemotaxis and multicellular development in Dictyostelium. They are both phosphorylated at PDK1 sites by PDK1 kinase and at PDK2/HM sites by TORC2. However, they have different localizing domains. AKT has a PI(3,4,5)P3-regulated PH domain while PKBR1 is myristoylated and persistently located on the membrane independently of PI(3,4,5)P3. Using strains defective for PI(3,4,5)P3-, PDK1-, and TORC2-signaling or that express phospho-site mutants of AKT and PKBR1, we demonstrate that PDK2/HM site phosphorylation is insufficient for kinase activation; the activations absolutely require PDK1 site phosphorylation. However, both PDK1 site phosphorylations are dependent on PDK2/HM site phosphorylations. We also show that Dictyostelium PDK1 is persistently localized on the membrane via a PH domain but independently of PI(3,4,5)P3. Finally, we show that AKT and PKBR1 exhibit substrate selectivity and identify 2 novel lipid-interacting proteins preferentially phosphorylated by AKT. Despite certain similarities, AKT and PKBR1 have distinct regulatory paths that impact activation and effector targeting. These findings from an ancient multicellular organism prove the conservative property of this important pathway, and the PI(3,4,5)P3 independent PKBR1 and PDK1 functions provide a unique chance to re-examine the current knowledge in mammalian systems.

REGULATION OF RUNX3 BY PI3K/AKT IN COLORECTAL CANCER

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RUNX3 is a prominent tumor suppressor in human cancers. This is highlighted by the frequent inactivation of RUNX3 due to promoter hypermethylation or protein mislocalisation in the gastrointestinal tract (GI) epithelium. As a transcription factor, RUNX3 is known to suppress cell growth by inducing the cell cycle inhibitor *p21/CIP1* as well as the proapoptotic gene *Bim* in gastric epithelial cells. We have reported that TGF-/BMP and Wnt signaling pathways regulate RUNX3. To further elucidate the cellular regulation and functions of RUNX3 in the GI tract, we undertook a proteomics approach to identify new interacting partners of RUNX3. We re-introduced RUNX3 by an inducible system into DLD1 cells, a RUNX3-deficient human colorectal cancer line. From the purified RUNX3 protein complexes, we identified protein kinase AKT as a novel RUNX3-binding partner. Binding was validated by *in vitro* binding assays as well as endogenous co-immunoprecipitation. Using bioinformatics and an AKT-substrate-specific antibody, we identified a residue within the conserved Runt domain that interacts with its transcription co-factor CBFβ and is phosphorylated by AKT. We will present new evidence on the role of PI3K/AKT in the regulation of RUNX3 in colorectal carcinogenesis.

PHOSPHATIDYLINOSITOL 3’ KINASE (PI3K) ONCOGENIC MUTATIONS INCREASE INVASIVENESS AND DRUG RESISTANCE IN BREAST EPITHELIAL CELLS.

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In the search for new targets, the PI3K signaling pathway has emerged as a high potential target. The PI3K pathway is mutationally activated in a higher frequency of tumors and in more molecules in the pathway in breast cancer than any other pathway with at least one-half of human breast cancers harboring oncogenic aberrations. Mutation of PIK3CA, the gene encoding the p110a subunit of PI3K, is the most frequently occurring somatic aberration in breast cancer being present in approximately 25% of breast cancer, with most common amino acid mutations found at positions E545K, H1047R, and H1047L. Strikingly, mutation of PI3K, loss of PTEN, amplification of PIK3CA, AKT, PDK1, KRAS and p70S6K as well as increased signaling through cell surface receptors such as HER2 and EGFR result in activation of the PI3K pathway in more than 70% of breast cancers. In addition, upregulation of signaling through the PI3K pathway has been linked to poor prognosis and therapy resistance across the different types of breast cancer.
In the present work we developed a model of breast human epithelial cell line harboring PIK3CA mutations and chose two of the most frequent mutations at positions E545K and H11047R to overexpress in MCF10A cells. Overexpression of the mutants resulted in increased invasion through matrigel and induced epithelial-mesenchymal transition in MCF10A cells. This observation was followed by a decrease expression of epithelial markers such as E-cadherin and an increase expression of mesenchymal markers such as N-cadherin and Vimentin. Consistent with the invasiveness phenotype, we also observed an increase of MMP2 activity and higher levels of invasion markers, including FAK, PAK and Paxilin. In addition, the expression of EGFR and HER2 was also increased in E545K and H1047R expressing cells.
Despite the observation that the expression of both EGFR and HER2 were elevated, the treatment of E545K and H1047R expressing cells with Lapatinib, a receptor tyrosine kinase inhibitor, showed an increased AKT phosphorylation and a sustained HER2 activation when compared to parental and DMSO treated MCF10A cells. Our results suggest that the presence of oncogenic mutation of PIK3CA caused a rapid resistance response to Lapatinib treatment and a bypass mechanism through AKT activation.
Our results indicate that the presence of PIK3CA mutations not only increased cell invasiveness but also induced drug resistance in epithelial breast cells. Therefore the analysis of the mechanism behind the oncogenic effects of PIK3CA will to lead to a more effective treatment choice for breast cancer patients bearing mutations on PI3K.

CD95-INDUCED SIGNALLING IN GLIOMA DOWNSTREAM OF PI3K

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In our lab we have identified CD95 as a factor contributing to non-apoptotic signals in neural cells. We investigated the role of CD95 signalling in glioma cells and used CD95-induced apoptosis-susceptible (LN18) and -resistant (T98G) cell lines as a model system. In T98G cells CD95 stimulation increases invasion. This response is mediated via novel CD95/Src/PI3K signalling pathway. mTOR (mammalian target of rapamycin) is a know downstream target of PI3K. It was originally described as a cell growth regulator but it has also been associated with the inhibition of apoptosis. Accordingly, we observed increased phosphorylation of S6 kinase and S6 ribosomal protein, both mTOR downstream targets, in T98G but not in LN18 upon CD95 stimulation. However, inhibition of mTOR by rapamycin does not increase CD95-induced cell death of T98G. We have also analyzed the transcripts differentially regulated in T98G upon CD95 stimulation both at the total and polysome-bound RNA level. Gene Ontology analysis reveals down-regulation of genes associated with cell adhesion and cell cycle and up-regulation of those responsible for cell motility and metabolism of biopolymers, which correlates with the invasive phenotype induced by CD95L in those cells. Our aim is to fully characterize the differences between both cell lines at the molecular level, information that might be used to modulate the CD95 output signal.

EVIDENCE FOR THE SIGNIFICANCE OF PTEN'S PROTEIN PHOSPHATASE ACTIVITY IN TUMOR SUPPRESSION

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PTEN is a tumor suppressor frequently mutated in human cancers. The PTEN protein is a lipid phosphatase that mediates many of its effects on growth, proliferation and survival through its PtdIns(3,4,5)P3 lipid phosphatase activity by suppressing the PI3-Kinase signalling pathway. PTEN also has protein phosphatase activity in-vitro and there is evidence that some of the processes regulated by PTEN could be attributed to its protein phosphatase activity such as inhibiting cell migration and invasion. To analyze PTEN's protein phosphatase activity, our group has previously systematically developed a PTEN mutant PTEN Y138L, which has lipid phosphatase activity very similar to wild type PTEN but lacks detectable protein phosphatase activity. Experiments with this mutant provide evidence for the interfacial activation of PTEN against lipid substrates. They also indicate that when re-expressed in PTEN null glioblastoma cells at a physiological level and these cells are grown in Matrigel, both the lipid and protein phosphatase activities of PTEN independently contribute towards inhibition of cellular proliferation. However both activities are required together to achieve efficient inhibition of invasion (Davidson et al., 2009).
The only tumour mutant reported in the literature at Tyrosine 138 is a Cysteine mutation in a small cell lung carcinoma. From preliminary experiments it seems that this mutant, PTEN Y138C, like PTEN Y138L, retains activity against PIP3 but not against PolyGluTyr-P and in cultured cells it controls Akt activation as effectively as wild type PTEN. These data support the importance of the protein phosphatase activity of PTEN in tumour suppression as this appears to have been selectively lost in this lung tumor.

PTEN IN WHOLE CHROMOSOME INSTABILITY

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Aneuploidy, an abnormal number of chromosomes, is a hallmark of most human cancers and can drive neoplastic growth. One mechanism through which whole chromosome instability (W-CIN) can promote tumorigenesis is by accelerating loss of tumor suppressor genes. While studying the role of chromosome reshuffling in Pten loss of heterozygosity, we noticed that inactivation of a single Pten allele is sufficient to cause aneuploidy, suggesting a role for Pten in the chromosome segregation process. Live cell imaging revealed that Pten haploinsufficient mouse embryonic fibroblasts (MEFs) are indeed prone to multiple kinds of chromosome segregation errors, including congression failure, chromosome lagging and chromatin bridging. Importantly, these mitotic phenotypes were not accompanied by overt structural chromosome changes, such as chromosome breaks and chromosome-to-chromosome fusions. Next, we exploited a conditional knockout approach to examine whether complete loss of Pten would aggravate the mitotic defects associated with Pten haploinsufficiency. Pten null MEFs indeed showed substantial increases in congression failure, chromosome lagging and chromatin bridging, with 79% of cells showing at least one of these defects versus 59% of Pten haploinsufficient MEFs and 15% of wild-type MEFs. Colcemid challenge assays revealed that Pten insufficient MEFs have compromised mitotic checkpoint activity. Taken together, these data identify Pten as a W-CIN gene required for proper microtubule-kinetochore attachment and mitotic checkpoint signaling. These findings raise the interesting possibility that inactivation of a single Pten allele accelerates the loss of the remaining wild-type allele, and suggest that prevention of whole chromosome instability represents a novel tumor suppressive function of Pten.

STIMULATION OF ITS NEGATIVE REGULATOR PHLPP PROVIDES A FEEDBACK LOOP ON AKT THAT IS PREFERENTIALLY LOST IN GLIOBLASTOMA

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The PH domain Leucine-rich repeat Protein Phosphatase, PHLPP, plays a central role in controlling the amplitude of growth factor signaling by directly dephosphorylating and thereby inactivating Akt. Here we show that the cellular levels of the two splice variants, PHLPP1α and PHLPP1β, but not PHLPP2, are enhanced by their substrate, activated Akt, providing a negative feedback loop to tightly control cellular Akt output. Specifically, we show that the steady-state level of PHLPP1β, but not PHLPP2, is markedly reduced in cells in which Akt has been pharmacologically inhibited or in mTORC2-deficient cells, which lack fully-functional Akt. In these cells, introduction of a constitutively active Akt, but not kinase-inactive Akt, stabilizes PHLPP1β. Consistent with Akt activity controlling PHLPP1β levels, a screen of the NCI 60 cell lines revealed a significant correlation between the levels of basal Akt activity and levels of PHLPP1β protein. Thus, the level of PHLPP1β in the cell is set by the amount of its substrate, phosphorylated, active Akt. Importantly, this correlation was lost in all four glioblastoma cell lines examined, but not in cell lines derived from less aggressive astrocytomas, suggesting loss of the feedback loop is an event that occurs in later stage disease. Consistent with this, levels of PHLPP1β were insensitive to inhibition of Akt activity in the four glioblastoma cell lines, but not in the astrocytoma cell lines. This negative feedback loop is preferentially lost in glioblastoma and may contribute to amplified Akt signaling in this cancer.

RECURRENT INTERSTITIAL GENOMIC DELETION OF THE HUMAN *PTEN* GENE ARE FACILITATED BY FLANKING MICROHOMOLOGIES

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Genomic deletions of several hundred kb centering on the *PTEN* gene are frequent in prostate cancer (CaP), and have been shown to confer a poor clinical outcome. We have shown that hemizygous *PTEN* deletions in CaP are associated with earlier biochemical relapse, and that homozygous deletions of the gene are strongly linked to metastasis and androgen independent progression (Yoshimoto et al, 2007; Sircar, 2009; Squire, 2009). These, and other studies in both human and mouse models of CaP draw attention to the idea that pten protein dosage reduction is a major selective force of both onset and progression in CaP. In this study we have mapped the location of human CaP-specific *PTEN* deletions by a combination of microarray comparative genomic hybridization and FISH methods. Four-color FISH was performed using a cohort of 330 localized and hormone-refractory CaP tissue microarray on and 6 BAC clones spanning both *PTEN* genomic locus and several kb centromeric and telomeric to the gene. *PTEN* genomic deletions were observed in 132 (40%) CaP samples. FISH analyses showed that the most frequent genomic loss (including both hemi- and homozygous deletions) at 10q23 was a recurrent interstitial, restricted to several hundred kb in size that always included the *PTEN* gene. The second most frequent class of deletion was more heterogeneous and involved loss of *PTEN* and the neighboring *FAS* locus. Variations of deletion size suggest that 10q23 genomic region is highly unstable and is prone to CaP–specific DNA rearrangements. Within the most frequently deleted region there is a fragile site (FRA10A) that may be involved in a subset of deletion events. Furthermore, bioinformatics analysis identified at least three clusters of non-redundant regions of microhomology surrounding *PTEN* gene locus by paired intrachromosomal segmental duplications. We propose a model in which defects of homology-dependent DNA recombination and/or repair process in prostatic somatic cells, lead to a propensity for prostate cancer precursors to undergo genomic *PTEN* deletion. The high incidence of deletion in these cells may be facilitated by error-prone recombinational repair events that utilize the segmental duplication and copy-number variations sequences. Collectively these data highlight the role of sequence microhomology clusters surrounding the *PTEN* gene locus on genomic stability in CaP; and suggest that these regions may facilitate deletion events that lead to the decreased gene dosage of pten essential for CaP initiation and clinical progression.

FOXO1 IS ESSENTIAL FOR THE REGULATION OF PLURIPOTENCY IN HUMAN EMBRYONIC STEM CELLS

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Pluripotency of embryonic stem (ES) cells is defined by their capacity to generate all cell types in the body and is established by an interactive network of proteins namely Oct3/4, Nanog and Sox2 and their binding partners. Forkhead Box O (FoxO) of Forkhead family are evolutionary conserved transcription factors downstream of PI3-kinase/AKT signaling pathway whose function is inhibited by AKT phosphorylation. FoxOs are involved in the regulation of longevity, are essential for stress response and exert critical functions in adult stem cells and in many different tissues, however whether they are involved in the regulation of embryonic stem cells is not known. FoxO1, FoxO3, FoxO4 are wildly expressed while FoxO6 is specific to neuronal tissues. Whether FoxOs are redundant in their functions is not clear. Here we show that in undifferentiated self-renewing ES cells and during lineage specification FoxO protein expression is differentially regulated. In addition, using loss and gain of function approaches, we demonstrate that FoxO1, but not other FoxOs, is critical for maintenance of human ES (hES) cell pluripotency. Doxycycline Tet-On inducible shRNA knockdown of FoxO1 delivered by lentiviruses resulted in 90% reduction in expression of FoxO1 protein within 48 hours associated with rapid and significant reduction in expression of Oct4, Sox2 and Nanog pluripotency proteins as measured by QRT-PCR and Western blot, reduction in expression of cell surface markers of pluripotency TRA-1 81 and TRA-1 60 and SSEA4 as shown by immunofluorescent staining and flow cytometry, and inhibition of teratoma formation in mice. Consistent with this, knock down of FoxO1 led to the erroneous induction of mesoderm and endoderm differentiation under undifferentiation self-renewal conditions of hES cells. Four different cell lines generated by two distinct shRNAs targeting FoxO1 produced comparable results in hES cells. The striking impact of FoxO1 knock down on hES cell pluripotency was not due to the loss of a generic function of FoxO1 on cell proliferation, survival or oxidative stress conditions. In addition, ectopic expression of FoxO1 but not FoxO3 (FoxO4 and FoxO6 are not expressed in undifferentiated hES cells) delivered by lentiviruses induced significant upregulation of Oct4 and Sox2 expression in both H1 and in HES2 hES cells. We show that FoxO1 but not FoxO3 regulates the expression of Oct4 and Sox2 by direct binding to, and activation of, Oct4 and Sox2 promoters. These results support a non-redundant function for FoxO1 in the regulation of hES cell fate. In addition, these findings have important implications for stem cells, development and reprogramming.

FUNCTIONAL ANALYSIS OF THE PROTEIN PHOSPHATASE ACTIVITY OF PTEN

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At least in vitro, the tumor suppressor phosphatase PTEN displays intrinsic activity towards both protein and phosphatidylinositol phospholipid substrates. Whereas the lipid phosphatase activity of PTEN is important for its tumor suppressor function, the significance of its protein phosphatase activity is unknown and represents a gap in our understanding of the function of this important regulator of cell signaling. We have developed an in vivo assay of PTEN function, which has revealed an effect of its protein phosphatase activity on the density of neuronal spines in cultured hippocampal organotypic brain slices. The approach utilized two-photon laser scanning microscopy and biolistic gene delivery of GFP-tagged constructs into organotypic slices of the hippocampus. The organotypic slice culture system maintains the tissue architecture of the hippocampus and produces a mosiac that permits analysis of the transfected neuron in a more natural context. We have observed that expression of wild type PTEN in organotypic brain slices led to a decrease in spine density. Strikingly, the lipid phosphatase-deficient, tumor-derived PTEN G129E mutant produced the same reduction in spine density as the wild type protein, whereas catalytically-dead (C124S) and protein phosphatase-deficient (Y138L) mutants were without effect. We tested expression of the isolated C2 domain in our system, but observed no effect on spine density. We also focused on the four C-terminal phosphorylation sites of PTEN and prepared mutants in which they were changed to non-phosphorylatable Ala residues (4A mutants). Introduction of the 4A mutation into wild type PTEN did not affect its ability to induce a decrease in spine density. More interestingly, combining the C124S and 4A mutations now also led to a decrease in spine density, unlike the inactivating C124S mutation alone. These data suggest that there may be a difference in the phosphorylation status of this cluster of sites in WT and C124S mutant PTEN. We used mass spectrometry to determine the phosphorylation status of this segment of PTEN in protein ectopically expressed in 293 cells. In contrast to wild type and G129E mutants, which are both active as protein phosphatase, we detected phosphate on these sites in the inactive C124S PTEN mutant. Interestingly, PTEN was shown initially to favor acidic substrates and these sites are located in an acidic stretch of the protein. Our data suggest that the protein phosphatase activity of PTEN and the phosphorylation of these C-terminal residues are of regulatory significance to the control of spine density. The critical protein substrate for PTEN may be PTEN itself.

THE CELL'S COMPASS: SIGNALING NETWORKS THAT MEDIATE CHEMOTAXIS IN EUCARYOTIC CELLS

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Mechanisms of sensing chemotactic gradients are remarkably similar in *Dictyostelium* amoebae and mammalian leukocytes. The upstream components and reactions in the signaling pathway are uniformly distributed along the membrane while downstream responses such as PIP_3 accumulation and actin polymerization are sharply localized towards the high side of the gradient. Gradients of chemoattractant cause PI3Ks and PTEN to bind to the membrane at the front and the back of the cell, respectively, leading sharp accumulation of PIP_3 at the anterior. A similar PIP_3-based "polarity circuit" plays a key role in cytokinesis where PI3Ks and PTEN move to and function at the poles and furrow, respectively, of the dividing cell. Disruption of PTEN broadens PIP_3 localization and actin polymerization in parallel, interfering with chemotaxis and cytokinesis.

Although human PTEN (hPTEN) can rescue the deficiencies of pten¯ cells, only very low levels of hPTEN are associated with the membrane. Membrane binding is limited by all phosphorylation-dependent intramolecular interaction that maintains hPTEN in a "closed" conformation. The phosphorylated "tail" portion of hPTEN binds tightly to the rest of the protein. Removal of the tail or inhibition of phosphorylation leads to strong membrane association.

We have recently shown that activations of TorC2 and PKBs occur at the leading edge of chemotaxing cells and play a critical role in directed cell migration. Activation of PKBA requires PIP_3 while activation of PKBR1, a myristoylated form, persistently bound to the membrane, is independent of PIP_3, and depends only on TorC2. Using the latter system, we are able to show that RasC activates TorC2 to phosphorylate the hydrophobic motif of PKBR1. The abberent motility caused by expression of constitutively active RasC is suppressed by disruption of the signature TorC2 subunit, PiaA.

MYOSINV-DEPENDENT TRANSPORT OF PTEN REGULATES PI3K SIGNALLING AND NEURONAL MORPHOGENESIS

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We recently demonstrated a requirement for functional actin-based motor proteins in the control of PI3K signalling, a mechanism involving a previously unknown association between PTEN and class V Myosins (van Diepen et al., 2009, Nat Cell Biol 11, 1191-6). FRET measurements revealed that PTEN interacts directly with MyosinV at discreet cellular sites dependent on PTEN phosphorylation. In addition, inactivation of MyosinV-transport function in CNS neurons affects neuronal soma size in vitro and in vivo, which – in line with known attributes of PTEN-loss - required PI3K and mTor.
Here we will present our current work testing the functional significance of the PTEN:MyosinV interaction in regulating PI3K signalling and neuronal morphogenesis in response to growth factors and axon guidance cues.

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THE REGULATION AND MECHANISMS OF ACTION OF THE PTEN ENZYME

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PTEN is a lipid phosphatase that acts as a component of the core phosphoinositide 3-kinase (PI3K) signal transduction pathway. Most tumours display increased activity of components of the PTEN/PI3K signalling pathway. In particular loss of function mutations of PTEN are amongst the most frequent genetic changes in human cancers. Also, several novel candidate tumour suppressors and oncogenes have been identified recently which appear to affect tumour development principally via effects on PTEN.

As part of work to understand how loss of PTEN function and regulation contribute to tumour development, we are investigating PTEN's ability to dephosphorylate both phosphoinositide lipids and peptide substrates. We have approached this through the development of mutants that selectively metabolise either lipid or protein substrates. These mutants were tested in cell based assays of PTEN function using viral re-expression of PTEN at physiological levels in U87MG glioblastoma cells that lack the phosphatase. The data indicate that PTEN's protein phosphatase activity is not required for its control of total cellular levels of its substrate, PIP3, or signaling via the PIP3-regulated protein kinase, Akt. PTEN's lipid phosphatase activity seems to dominate in the regulation of cellular proliferation. However, both lipid and protein phosphatase activities appear to act together to inhibit invasion in 3D matrigel culture. I will present data from this project addressing the relative significance of PTEN's lipid and protein phosphatase activities and also from studies of the regulation of PTEN by ubiquitination.

DYNAMIC NUCLEAR LOCALIZATION OF PTEN IN ZEBRAFISH

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PTEN is an essential tumor suppressor gene and it has a crucial role in embryonic development. The zebrafish is an ideal model to study vertebrate development and we have used it to further investigate the function of Pten in embryonic development. In the zebrafish genome two pten genes, *ptena* and *ptenb* were identified, which were shown to have redundant functions during embryogenesis. Whereas *ptena-/-* or *ptenb-/-* mutants did not show embryonic phenotypes, double mutant embryos died at 5 days post fertilization. An increased level of p-Akt in pten double mutant embryos indicated that the PI3K/Akt pathway was dysregulated in embryos lacking functional Pten. Injecting double mutant embryos at the 1-cell stage with *pten* RNA partially rescued the double mutant phenotype and significantly reduced the p-Akt level in these embryos. Live imaging of developing zebrafish embryos using fluorescently labeled Pten fusion proteins revealed a specific, highly dynamic behavior of the Pten protein. Pten was restricted from the nucleus, but a rapid nuclear influx was observed immediately prior to cell division. Currently, we are analyzing this behavior of Pten, using a panel of Pten mutants. The role of transient nuclear localization of Pten in normal physiological functions and in embryonic development will be discussed.

ALTERATION OF PTEN SIGNALING AS A DRIVING FORCE FOR CANCER

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PTEN (Phosphatase and Tensin Homolog on Chromosome 10) was discovered as a candidate tumor suppressor in 1997. Since that time, much effort by many laboratories has established that PTEN:

1) is altered by mutation or indirect mechanisms in various types of cancer

2) antagonizes PI3K signaling via its lipid phosphatase activity

3) regulates signals that are independent of the PI3K pathway

4) suppresses spontaneous cancer development in mice in many differrent organ systems

5) modulates sensitivity to cancer therapy

6) controls cellular proliferation, size, survival, metabolism, sensecence, migration and stemness

7) resides in different cell compartments (plasma membrane, cytoplasm, nucleus)

8) is regulated by mutiple mechanisms

9) contributes to normal development of different organ systems and has been implicated in non-cancer diseases of the neurological, reproductive, endocrine and inflammatory systems.

Past progress and recent advances will be presented.

MOUSE MODELS AS TRANSLATIONAL TOOLS TO DISCOVER TREATMENTS FOR AUTISM SPECTRUM DISORDERS: FOCUS ON RAPAMYCIN

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The use of mice as genetic tools to study the molecular basis of brain development and function has grown steadily over recent years. Recent human genetic studies have implicated the PI3 Kinase pathway as an underlying cause of a small subset of autism cases that are related to genetically inherited diseases. Using mutant mouse models, we have shown that modulation of the PI3 Kinase pathway can be an indirect source of synaptic modulation. Moreover, mice lacking PI3 Kinase components in mature neurons exhibit stereotypic behavioral abnormalities reminiscent of autism. Because this signaling pathway is also implicated in many human cancers, drugs that inhibit this pathway are in development. We have applied such drugs, including the specific mTORC1 inhibitor rapamycin in Pten mutant mice, with promising outcomes. It is our hope that continued study of these mice will provide insights into the anatomical and cellular basis of at least a subset of autism spectrum disorders.

TUBEROUS SCLEROSIS: RECENT RESULTS ON GENES, PATHWAYS, HUMAN DISEASE, AND MOUSE MODELS

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Tuberous Sclerosis Complex (TSC) is an autosomal dominant disorder due to inactivating mutations in either TSC1 or TSC2, and characterized by development of benign yet proliferative tumors in multiple organs. Cortical tubers associated with a variety of neurological syndromes, and tumor involvement of the kidney (angiomyolipomas) and lung (lymphangioleiomyomatosis) are the greatest clinical problems in this disorder.
In this presentation, I will review human therapeutic trials in this disorder using rapamycin, and recent results on a related neoplasm, PEComa. I will describe the development of a hypomorphic allele of Tsc2 in mice. I will present results on the contribution of Tsc1 loss to lung cancer development in a mutant Kras driven mouse model, and the lack of involvement of TSC1 or TSC2 in human lung cancer. I will discuss recent studies that clarify the role of TSC1 in the development of human bladder cancer. I will present recent results on the development of novel models of TSC brain disease, and the benefit of prenatal treatment of one such model with rapamycin. Finally, I will present results on deep sequencing of cortical tubers for second hit events in TSC1, TSC2, and KRAS.

AKT2 IS REQUIRED FOR SPECIFIC GLYCOLYTIC ISOZYME EXPRESSION AND CANCER PROGRESSION IN PTEN-DEFICIENT MOUSE LIVER STEATOHEPATITIS

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The mammalian Target Of Rapamycin (mTOR) signal transduction is a master regulator of cell size, number and metabolism and is activated in the majority of human cancers. S6K and Akt protein kinases are activated by two distinct branches of mTOR signaling cascade – mTORC1 and mTORC2 respectively.
Inhibition of mTOR kinase has proven efficacy in PTEN-negative mouse models of cancer, though the mTOR complexes (mTORC) and substrates involved in tumorigenesis are under investigation. Despite the recent progress in understanding the components and interplay between them the question how mTOR signaling controls tumorigenesis is still open.
Using mouse model of liver cancer triggered by PTEN deletion we revealed that development of steatohepatitis-associated liver adenocarcinomas specifically requires Akt2, while the Akt1 homologue and S6K1 and S6K2 are dispensable. In this model rapamycin analogues partially inhibit Akt phosphorylation and decrease tumour growth. Akt2 deletion affects a number of metabolic parameters in PTEN-deficient livers, including the hypoglycaemic action of insulin, the PPAR g-dependent program leading to lipid accumulation and the expression of specific glycolytic isozymes such as hexokinase II and M2 pyruvate kinase (PKM2). Akt2 is involved in regulation of PKM2 and HXKII on mRNA and protein level as well as promotes association of HXKII with mitochondria. PTEN negative human hepatocellular carcinoma cell lines also display PKM2 upregulation in an Akt2-dependent manner, providing an advantage for cell proliferation and anchorage independent growth. Our data have implications on the link between the metabolic action of insulin signal transduction and tumorigenesis, identifying Akt2 as a potential therapeutical target in liver malignancies depending on cancer genotype.
The recent developments on the project will be presented.

PTEN PATHWAY ALTERATIONS ARE ASSOCIATED WITH BLADDER TUMOR PROGRESSION

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Approximately 90% of malignant tumors arising in the urinary bladder are of epithelial origin, the majority being transitional cell carcinomas. Early stage bladder tumors have been classified into two groups with distinct behavior and unique molecular profiles: low grade tumors (always papillary and usually superficial), and high-grade tumors (either papillary or non-papillary, and often invasive). Clinically, superficial bladder tumors (stages Ta, Tis, and T1) account for 75% to 85% of neoplasms, while the remaining 15% to 25% are invasive (T2, T3, T4) or metastatic lesions at the time of initial presentation. Metastatic bladder cancer is an important focus of research because, in most cases, cancer-related death is the result of systemic disease rather than the primary tumor. Even with recent improvements in primary therapy and systemic chemotherapy, the median survival for patients with unresectable metastatic bladder cancer is approximately 7-20 months. Since criteria to determine treatment in a particular patient are incompletely defined, new biological determinants are needed for proper selection and monitoring of therapy.

Studies from our group and others have revealed that distinct genotypic and phenotypic patterns are associated with early versus late stages of bladder cancer. Most importantly, early superficial diseases appear to segregate into two main pathways. Briefly, chromosome 9 deletions and mutations of RAS and FGFR3 are observed in most if not all superficial papillary non-invasive tumors (Ta), but only in a small subset of invasive bladder neoplasms. However, deletions of 3p, 5q, 10q (PTEN locus), 11p, 13q (RB locus), 17p (TP53 locus), and 18q (DCC locus) are absent or very rare in the Ta tumors analyzed, but have been frequently identified in invasive bladder carcinomas. Based on these data, a novel model for bladder tumor progression has been proposed in which two separate genetic pathways characterize the evolution of superficial bladder neoplasms. Transgenic models using tissue specific promoters, such as that of uroplakin II, are being utilized to generate genetic proof of the relevance of certain genes in the context of bladder cancer. Working with the group of Dr. Abate-Shen, we established an alternative procedure to achieve gene recombination specifically in bladder epithelium by delivery of an adenovirus expressing Cre recombinase into the bladder lumen. Using this approach, we have shown that combinatorial deletion of *Pten* and *p53* tumor suppressor genes in bladder epithelium results in invasive bladder tumors that resemble their human counterpart. Additionally, we have developed a renal graft model of human bladder cancer and have shown that combinatorial inactivation of *Pten* and *p53* promotes bladder tumorigenesis in this human model of bladder cancer. Furthermore, we have found that deregulated expression of *Pten* and *p53* is associated with poor survival in clinical specimens of invasive bladder cancer in humans. Finally, we have shown that the mechanisms by which inactivation of *Pten* and *p53* promote bladder tumorigenesis are mediated, in part, by activation of mTOR signaling and that inhibition of this signaling pathway with Rapamycin inhibits bladder tumors in a preclinical mouse model. These and other models, along with translational studies, will be presented.

PTEN, STEM CELLS AND TUMORIGENESIS

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Like normal stem cells, cancer stem cells (CSC) have the capacity for indefinite proliferation and generation of new cancerous tissues through self-renewal and differentiation. Among the major intracellular signaling pathways, Wnt, Shh, and Notch are known to be important in regulating normal stem cell activities and their alterations are associated with tumorigenesis. It has become clear recently that phosphatase and tensin homologue (PTEN) is also critical for stem cell maintenance and that PTEN loss can cause the development of CSCs and ultimately tumorigenesis. I will discuss our recent findings on suppression of PTEN null CSCs transformation and cancer development.

LOVASTATIN INHIBITS EGFR DIMERIZATION AND AKT ACTIVATION IN SQUAMOUS CELL CARCINOMA CELLS

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We recently demonstrated the ability of lovastatin, an inhibitor of mevalonate synthesis, to inhibit the function of the epidermal growth factor receptor (EGFR) and its downstream signaling of the PI3K/AKT pathway. Combining lovastatin with gefitinib, a potent EGFR inhibitor, induced synergistic cytotoxicity in various tumour derived cell lines. In this study, lovastatin treatment inhibits ligand-induced EGFR dimerization in squamous cell carcinoma (SCC) cells and its activation of AKT and its downstream targets 4EBP1 and S6K1. This inhibition was associated with global protein translational inhibition demonstrated by a decrease in RNA associated polysome fractions. The effects of lovastatin on EGFR function were reversed by the addition of the mevalonate metabolite geranylgeranyl pyrophosphate that acts as a protein membrane anchor. Lovastatin treatment induced actin cytoskeletal disorganization and the expression of the geranylgeranylated rho family proteins in SCC cells that regulate the actin cytoskeleton, including rhoA. Lovastatin-induced rhoA was inactive as EGF stimulation failed to activate rhoA and inhibition of the rho-associated kinase, a target and mediator of rhoA function, with Y-27632 also showed inhibitory effects on EGFR dimerization. The ability of lovastatin to inhibit EGFR dimerization by targeting rho function is a novel exploitable mechanism regulating this therapeutically relevant target.

BIALLELIC INACTIVATION OF TSC1 IN RADIAL GLIA RESULTS IN MTOR PATHWAY ACTIVATION

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Malformations of cortical development (MCDs) are disproportionately represented in cases of pharmacologically refractory seizures, yet the underlying cellular contributions to epileptogenesis are incompletely understood. MCDs compose a wide variety of neurological disorders with a proportionately diverse histopathology ranging from loss of cortical lamination to subcortical (band) heterotopias to dysplasias with cytomegalic cells. However, elevated mTOR signaling is a common feature of MCDs suggesting that the molecular determinants which lead to epileptogenesis may be conserved. Tuberous sclerosis complex (TSC) is a representative MCD for which inactivating mutations in one of two genes, Tsc1 or Tsc2, have been identified. Importantly, the protein products of Tsc1 and Tsc2 negatively regulate mTOR signaling which is consequently elevated in TSC associated neurological lesions. Here we describe a novel mouse model of TSC for which biallelic inactivation of Tsc1 in radial glia by *in utero* electroporation generates neurological features of TSC. Tsc1 inactivation produces clusters of enlarged ectopic neurons with elevated mTOR signaling as indicated by increased phospho-S6 staining, nodules located either within the corpus callosum or protruding into lateral ventricles and a reduced latency for PTZ induced seizures. Preliminary experiments suggest that *in utero* electroporation of constitutively active mTOR phenocopies removal of Tsc1, however future experiments will be needed to address to what extent these features are reproduced.

AN IMPORTANT ROLE FOR PTEN TYROSINE PHOSPHORYLATION IN GLIOBLASTOMA PATHOGENESIS AND RESPONSES TO TREATMENT

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Glioblastoma multiforme (GBM), the most common intracranial tumour in adults, exhibits a highly lethal and progressive clinical course despite intensive therapeutic intervention. Mutational activation of the Epidermal Growth Factor Receptor (EGFR) is common in GBM and concomitant loss of the PTEN tumor suppressor confers poor clinical response to the EGFR kinase inhibitors, erlotinib or gefitinib. Notably, however, activated EGFR tumors with intact PTEN show only modest treatment responses, pointing to additional resistance mechanisms. We considered the possibility that the marginal clinical responses of EGFR mutant (EGFRvIII) PTEN wild-type GBM cases might relate to functional inactivation of PTEN brought about by post-translational modifications. Using mass spectrometry, we have mapped Src-induced phosphorylation sites on PTEN. Using this information, we were able to raise phospho-specific antibodies against tyrosine 240 in the PTEN C2 domain. Application of these antibodies in immunohistochemistry has allowed us, for the first time, to directly demonstrate tyrosine phosphorylation of PTEN in human tumors. We observe that phosphorylation of PTEN at tyrosine 240 is associated with shorter survival in GBM patients and with resistance to EGFR inhibitor therapy. Using GBM cell lines, we show that blocking PTEN phosphorylation, either by phenylalanine substitution of a single tyrosine residue, Y240, or by the use of the tyrosine kinase inhibitor, dasatinib, can attenuate mutant EGFR signaling and enhance the cellular response to Erlotinib. Thus, in addition to co-activation of multiple RTKs and mutational inactivation of PTEN, Src-mediated functional inactivation of PTEN is a major modifier of an important class of cancer drug in human GBM. These findings inform the design of clinical studies combining Src and EGFR inhibition in PTEN-intact GBM and possibly in diverse solid tumor types driven by EGFR activation.

PTEN AND OSTEOBLASTOGENESIS

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Pten a negative regulator of Phosphatidylinositol 3 kinase is expressed in osteoblasts. But the role of this tumor suppressor gene during bone development is not completely understood. To define a role for Pten during bone development we deleted Pten in mice by crossing Ptenflox/flox and the Dermo1cre deleter mice. Deletion of Pten in osteoprogenitor cells led to an expansion and formation of bone away from the growth plate in the perichondrium. In the Pten conditional knockout perichondrium we observed 1) increase in FGF18, a potent osteoblast mitogen 2) decrease in expression of Sprouty2 a repressor of FGF signaling. Increase in perichondral bone formation observed in the Pten conditional knockout could be explained by repression of Forkhead transcriptional factors by downstream effectors of activated PI3K leading to increased FGF signaling. This implicated unrestricted MAPK signaling as the major mediator of osteoblast differentiation in the absence of Pten. To test if FGF signaling is required for the phenotype we deleted one allele of FGFR2 to attenuate MAPK signaling and saw a partial rescue of the Pten null bone phenotype. To identify the progenitor population in the perichondrium required for the increased bone phenotype, we utilized SP7cre to delete Pten in bone and compared the phenotype in the perichondrium with the Dermo1cre knockout. The SP7cre did not show a perichondral phenotype, so we utilized a number of stem cell markers including NG2 ,N-cadherin and Slug and identified a population of cells in the perichondrium of the Pten/Dermo1cre that were differentially expanded when compared to the Pten/SP7cre. This study identifies an osteoprogenitor population in the perichondrium that leads to increased bone formation in the absence of Pten.

GENETIC AND PRECLINICAL INTERROGATION OF A GENETICALLY ENGINEERED MOUSE MODEL OF SMALL CELL LUNG CARCINOMA.

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Lung cancer is the leading cause of cancer deaths in the United States, with small cell lung carcinoma (SCLC) representing approximately 15% of all cases. Some of the principle molecular alterations that have been associated with human SCLC include loss of the *RB*, *TP53* and *FHIT* tumor suppressor genes as well as amplification or increased expression of the *C-MYC* and *BCL2* proto-oncogenes. We have begun to characterize the molecular events associated with SCLC progression using a genetically engineered mouse model (GEMM) driven by simultaneous loss of the *Rb* and *Tp53* tumor suppressor genes following intranasal delivery of an adenovirus expressing the Cre and FLPe recombinases. Array comparative genomic hybridization (aCGH) analysis revealed frequent & focal copy number changes in *Pten*, *Mycn*, *Mycl*, and *Nfib*. As in mice, human SCLC frequently undergoes genetic copy number changes in the *PTEN/PI3K* pathway. In contrast to mice, frequent amplifications in *PIK3CA* and *C-MYC* were observed. Moreover, there was no apparent correlation between these copy number changes in either mice or humans. Further examination of PTEN expression levels in human SCLC by immunohistochemistry revealed the heterogeneous nature of PTEN expression, with 95% demonstrating altered expression &/or localization. Interestingly, the expression pattern of $pAKT^{Ser473}$ demonstrated that approximately one third of human SCLC samples have nuclear pAKT (when they are positive), with no obvious correlation between nuclear pAKT expression and PTEN loss. Preclinical interrogation of the SCLC GEMM revealed significant, cytotoxic effects on primary lung tumor burden when the pan PI3K inhibitor GDC-0941 was combined with carboplatin and anti-VEGF. These effects were highly durable and resulted in a significant overall survival outcome. Importantly, this triple therapeutic combination resulted in a significant impact on both primary and metastatic disease to the regional lymph nodes and liver, with ~40% of the mice demonstrating no gross disease at necropsy. Human SCLC has a high mitotic rate and patients generally respond well to frontline chemotherapy. However, it is the residual disease that typically mediates therapeutic relapse and patient mortality. Our data with GDC-0941 in combination with anti-VEGF and chemotherapy has important implications towards improving both the extent and duration of frontline chemotherapeutic responses in human SCLC, ultimately extending the time to disease progression and overall survival.

THE DUAL PI3K/MTOR INHIBITOR NVP-BEZ235 INDUCES CELL DEATH VIA THE EXTRINSIC APOPTOSIS PATHWAY

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NVP-BEZ235 is a dual PI3K/mTOR inhibitor currently in phase I clinical trials. We profiled this compound against a panel of breast tumor cell lines to identify potential patient populations who would benefit from such treatment. This approach found breast cancer lines with HER2 amplification and/or PIK3CA mutations to be sensitive towards NVP-BEZ235 treatment as the compound induced strong cell death in these lines. In contrast, breast lines with PTEN loss of function or K-RAS mutations only showed growth arrest. Cell death induction seen with NVP-BEZ235 could as well be observed with other PI3K inhibitors, thus linking cell death to the inhibition of PI3K. Analysis of death markers revealed that cell death induced by NVP-BEZ235 is accompanied by active PARP cleavage, which is indicative of an apoptotic process. This did not seem to be mediated by the intrinsic apoptosis pathway since we could not detect activation of mitochondrial caspases nor could cells be protected by overexpression of Bcl2, an anti-apoptotic component of the intrinsic pathway. Instead, we observed disappearance of the full-length form of the extrinsic caspase 2, suggesting NVP-BEZ235 induces apoptosis via the extrinsic pathway. This study suggests that breast cancer patients with HER2 amplification and/or PIK3CA mutations might respond best to NVP-BEZ235 treatment, whereas patients with PTEN loss of function or K-RAS mutations might benefit less. Overall, this study will provide guidance for patient stratification for forthcoming breast cancer phase II trials for NVP-BEZ235.

PTEN AND *P53* CONTROL SELF-RENEWAL ABILITY AND DIFFERENTIATION POTENTIAL OF PROSTATE EPITHELIAL STEM/PROGENITOR CELLS

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Pten and *P53* tumor suppressors are among the most commonly inactivated genes in prostate cancer. While loss of *P53* alone results in no obvious phenotype, *Pten* deletion in the mouse prostate epithelium results in the development of carcinoma in situ with eventual progression to adenocarcinoma. Combined loss of *PTEN* and *P53* leads to significantly more rapid and extensive prostate tumor development ultimately resulting in death from urinary outflow obstruction. *Pten* deletion alone has been implicated in the expansion of a prostatic stem/progenitor cell population, while *Pten* and *P53* have recently been shown to act concomitantly not independently in the regulation of neural and glioma stem/progenitor cell renewal and differentiation. We hypothesize that *Pten* and *P53* play a crucial role in regulating self-renewal and differentiation ability, two defining hallmarks of stem/progenitor cells. In order to test our hypothesis, we employed clonogenic 2D colony and 3D sphere formation assays aimed at measuring the progenitor and differentiation activity of cells isolated from *Pten-/-P53-/-* adenocarcinomas. Colony and sphere formation activity was monitored over at least 4 generations of serial propagation in vitro. Protospheres generated from *Pten-/-P53-/-* single cell suspensions were approximately 3 times larger than wild type (wt) spheres and demonstrated significant heterogeneity in morphology, displaying less uniform cell shapes. Sphere forming units (SFU) in single cell suspensions from *Pten-/-P53-/-* prostates showed a 50%-80% increase when compared to wt. *Pten-/-P53-/-* SFU activity was maintained upon serial passage of single cell suspensions for 4 generations, while a reduction in SFU activity was observed over the same 4 generations in wt. Similar findings were obtained from analyzing the colony forming ability of cells taken from wt and *Pten-/-P53-/-* spheres. *Pten* and *P53* deletion significantly affects the differentiation profile of colonies and spheres generated from knockout as compared to wt cells. Interestingly, an increased number of luminal, transit-amplifying and neuroendocrine cells with a relative reduction in the number of basal cells were observed in the *Pten-/-P53-/-* spheres and colonies compared to wt. In addition, *Pten-/-P53-/-* progenitor cells demonstrate sensitivity to drugs targeting the mTOR (Rapamycin), AKT (Triciribine) and androgen receptor (Nilutamide and Bicalutamide) pathways. In conclusion, our findings to date confirm the presence of an amplified stem/progenitor cell population in *Pten-/-P53-/-* prostates that generates morphologically abnormal protospheres displaying changes in the relative production of differentiated progeny.

PHOSPHO-PROTEOMICS AND MASS-ACTION MODELING IDENTIFY OPTIMAL DRUG COMBINATIONS IN SIGNALING NETWORKS

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Analyzing biologic signaling networks is greatly facilitated when mathematical modeling is integrated with experimental data. In this study, we measured insulin-like growth factor signaling through receptor tyrosine kinase in the MDA-MB231 breast cancer cell line using reverse phase protein arrays to quantify the dynamic crosstalk between the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways. We developed a computational procedure that integrated mass-action modeling with particle swarm optimization to analyze the data. The mass-action model was trained using transient experimental data measuring phosphorylated protein levels to infer the unknown model parameters. We then used the model to identify regulatory molecules that could restore user-defined signaling network outputs when targeted with combinations of drug inhibitors and small-interfering RNAs. We tested and validated the combined effect of the most influential targeted perturbations predicted by the model using immunoblotting and cell viability assays. Our results showed that using optimal drug combinations led to desired rewiring of the signaling pathways and decreased cell viability. The integrative approach described here is useful for generating experimental intervention strategies that can lead to the rational use of drug combinations and the discovery of novel pharmacologic targets.

THE PI3K PATHWAY IN A MOUSE MODEL FOR INVASIVE LOBULAR CARCINOMA OF THE BREAST

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PI3K pathway alterations are frequent in invasive breast cancer. Invasive lobular carcinoma of the breast (ILC) is characterized by loss of expression of E-cadherin, infiltrative growth and formation of metastases. The majority of ILCs express estrogen receptor. However, besides endocrine therapy, pharmacologic treatment options are limited. This illustrates the need to find more therapeutic opportunities. We have developed a mouse model for ILC with tissue-specific conditional knock-out of E-cadherin and P53. These mice develop mammary tumors with a latency of around 200 days which phenotypically resemble human ILC, including invasive growth and metastatic spread. High expression of phospho-S6 in mILC tumors, determined by immunohistochemistry, indicates activation of the PI3K pathway. The tumors have been transplanted orthotopically in recipient mice in preclinical intervention studies to investigate therapeutic effects of inhibitors of the PTEN pathway. Cell lines have been derived from these tumors and have been shown to maintain high phospho-S6 expression. Several cell lines have been transfected with luciferase and orthotopically transplanted for in vivo monitoring of tumor growth and spread by bioluminescence. In parallel, a mouse cohort with mammary tissue-specific E-cadherin and PTEN knockout has recently been bred, providing a new relevant ILC model for mechanistic and preclinical studies.

ACINAR CELL NEOPLASIA FOLLOWING CONDITIONAL INACTIVATION OF APC AND PTEN IN THE MOUSE SALIVARY GLAND: ACTIVATION OF MTOR SIGNALING IN HUMAN ACINIC CELL CARCINOMA

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Crosstalk between the Wnt and mTOR signaling pathways occurs at multiple levels in the cell and likely contributes to the oncogenic effects of these pathways in human cancer. To gain more insight into the interplay between Wnt and mTOR signaling in salivary gland tumorigenesis, we developed a mouse model in which both pathways are constitutively activated by conditional inactivation of the Apc and Pten tumor suppressor genes. Loss of Apc and Pten results in the formation of salivary gland tumors similar to human acinic cell carcinoma with 100% penetrance and short latency. Our results suggest that activated Wnt signaling deregulates the salivary gland stem cell compartment but does not on its own cause tumor formation. However, activation of mTOR signaling in this setting leads to rapid tumor development. Although activation of both pathways is required for tumor initiation, aberrant mTOR activity appears to play a pivotal role in acinic cell tumorigenesis. Treatment of tumor-bearing mice using a low dose of rapamycin caused the tumors to completely regress. Consistent with these results, we found that acinic cell carcinomas of both the mouse and human salivary gland always express markers of activated mTOR signaling. Given the availability of rapamycin and its analogs for treatment of several other types of human malignancy, rapamycin may also be useful in treating human acinic cell carcinoma of the salivary gland. The current treatment options for metastatic disease are limited because most acinic cell carcinomas do not respond to chemotherapy.

THE ROLE OF *PTEN* IN MURINE PROSTATE DEVELOPMENT

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Prostate cancer is the most common male cancer in Western societies. Few specific molecular pathways and targets involved in prostate cancer initiation and progression have been identified. Since genetic pathways involved in prostate development are frequently reactivated in prostate cancer, understanding the role of genes in development can give insight into their function in tumourigenesis.
Pten (Phosphatase and tensin homologue deleted on chromosome 10) is frequently mutated in prostate cancer. Correspondingly, adult mice with deletions in *Pten* develop prostate cancer. However the role of *Pten* in prostate development has not been elucidated.
We have used the conditional Cre-loxP system to delete *Pten* specifically in the mouse prostate epithelia from 17.5 days of embryonic development. Loss of *Pten* at this stage does not affect early prostate formation or differentiation into luminal and basal cell types. However, at postnatal day 7, prostates from *Pten* mutants exhibit disorganized lumens, increased cellular proliferation, and precocious cytodifferentiation. Furthermore, upregulation of a target downstream of *Pten*, phospho-AKT, was also observed in *Pten* mutant prostates.
These studies suggest that in prostate development, *Pten* is required for the cellular organization of epithelial cells at the stages of lumen formation in prostatic ducts. Future work will focus on investigating specific pathways affected by *Pten* loss. The study of *Pten* in development may therefore yield insight into its associated pathways and, by extension, its role in prostate cancer and other *Pten* relevant malignancies.

PRO-SENESCENCE THERAPY FOR CANCER AND THE CO-CLINICAL TRIAL PROJECT

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Irreversible cell growth arrest, a process termed cellular senescence, is emerging as an intrinsic tumor suppressive mechanism. We have reported that acute loss of Pten in vivo promotes the activation of a potent cellular senescence response, which we have termed PICS (Pten-loss Induced Cellular Senescence) for short. We now show that PICS represents a unique form of cellular senescence, distinct from oncogene-induced senescence (OIS). The key departure of PICS relative to OIS is characterized by a lack of DNA damage response (DDR) and hyper-replication, rendering therapeutic enhancement of this process suitable for cancer treatment. Further, we have found that PICS is associated with increased p53 translation. Consistent with these data, preclinical trials in mice show that p53-stabilizing drugs potentiated PICS and its tumor suppressive potential. Additionally, we demonstrated that pharmacological inhibition of PTEN drives senescence and inhibits tumorigenesis in vivo. We are now evaluating in mice whether the treatment with DDR-inducing agents may cooperate with PICS to further increase p53 levels and in turn enhance the senescence response or redirect p53 signaling to apoptosis. Importantly, the senescence-enhancing strategies are being tested not only in mice but also in a co-clinical manner. This approach, which we have named “The Co-Clinical Project”, will consist in running pre-clinical trials in appropriate, faithful and genetically relevant mouse models in parallel with clinical trials. The clinical, biological and pharmacological information (i.e. somatic mutational background, germline SNP variations, responsiveness to specific regimens, imaging, microarray and proteomics profiles) will be accrued, analyzed in parallel and integrated in order to facilitate the identification of patient subtypes, key genetic determinants, and biomarkers that predict response to specific treatments.

THE CORE PROTEIN OF HCV 3A TRIGGERS PTEN DOWNREGULATION IN HEPATOCYTES: ROLE IN HCV-MEDIATED STEATOSIS AND INSULIN RESISTANCE.

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Hepatic steatosis and insulin resistance are two important mediators of HCV pathogenesis as their clinical impact includes accelerated fibrogenesis and reduced response to antiviral therapy. We previously reported that high levels of circulating free fatty acids, as observed with obesity and the metabolic syndrome, downregulate PTEN expression in hepatocytes via mTOR/NFκB/miR-21-dependent mechanisms. PTEN downregulation in turn leads to both insulin resistance and steatosis development in hepatocytes. Herein, we investigated whether alterations of PTEN expression/activity in the liver may mediate also the metabolic changes observed in HCV infection.
Our data indicated that PTEN expression is downregulated in the liver of patients infected with HCV genotype 3a at the protein level, without significant changes in PTEN mRNA expression, thus suggesting that HCV affects PTEN protein stability or degradation. Interestingly, downregulation of PTEN protein expression was also triggered in vitro by expressing only the HCV core protein of genotype 3a in Huh-7 hepatoma cells. In contrast, expression of HCV core protein of genotype 1b had no effect on PTEN expression. As expected, the expression of genotype 3a core protein (but not 1b) induced the appearance of large lipid droplets, while both core proteins expression led to a significant decrease of IRS-1, a major contributor to insulin resistance mediated by HCV infection. Overexpression of PTEN in Huh-7 cells expressing the genotype 3a core protein restored to physiological levels the IRS-1 expression, while at the same time prevented core 3a-expressing cells from developing steatosis.
Together, our results suggest that alterations of PTEN expression/activity in the context of HCV infection may be involved in the pathogenesis of two major metabolic changes induced by HCV, i.e. insulin resistance and steatosis.

COMBINED DELETION OF PTEN, TRP53 AND RB1 INDUCED ASTROCYTOMAS WITHIN AND OUTSIDE OF PROLIFERATIVE NICHES IN THE MATURE BRAIN.

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Glioblastoma is an aggressive and heterogeneous astrocytic brain tumor characterized by frequent genomic alterations in the PI3K/PTEN, p53 and RB signaling pathways. This tumor affects both adults and children, and is universally associated with a dismal prognosis. Minimal progress has been made towards effective treatments due in part to a lack of clinically relevant models of this disease.
Inducible conditional deletion of *Pten* in mature astrocytes and their progenitors had minimal effects on the activation of downstream effectors of the PI3K signaling pathway and resulted in no abnormalities in cell size or proliferation. In contrast, deletion of *Pten* in combination with other glioma tumor suppressors generated spontaneous high grade glioma with histological and genetic similarity to the human disease. Tumor penetrance and latency depended both on the number of tumor suppressors targeted, as well as the specific tumor suppressors deleted. Conditional knock-out of three tumor suppressor genes, *Pten*, *Trp53* and *Rb1* (TKO) resulted in increased tumor penetrance and decreased median survival relative to conditional double knock-out (DKO) combinations involving *Trp53*. DKO of *Pten* and *Rb1* failed to generate intracranial tumors. Tumors generated in each model showed robust activation of downstream effectors of the PI3K signaling pathway including pAkt and pS6.
CreER expression in this model was driven by the GFAP promoter and its induction in the mature brain targeted primarily mature astrocytes, with some activity in proliferative niches of the adult brain. MRI studies showed that the tumors preferentially arose within proliferative niches but also in additional distinct regions including the cortex, midbrain and cerebellum, suggesting that cells outside of the proliferative niche can be susceptible to transformation. We further investigated the tumor initiating capability of cells harvested from these tumors, by orthotopic transplantation of limiting cell dilutions into immunodeficient mice. As few as 100 implanted cells successfully induced tumor formation that faithfully recapitulated the histopathological features of the original tumor, suggesting that tumor initiating cells represent a substantial portion of the tumor cell population.
The combined deletion of *Pten* and *Trp53* in mature astrocytes and their progenitors was sufficient to induce high grade glioma in mice and gliomagenesis was potentiated by the additional deletion of *Rb1*.Resulting tumors accurately reflected the heterogeneity, histopathology and genetic alterations frequently noted in human disease, and contained a significant proportion of cells with tumor initiating activity.

EXPANSION OF HEPATIC TUMOR PROGENITOR CELLS IN *PTEN* DEFICIENT MICE REQUIRES LIVER INJURY

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Progenitor or cancer stem cells (CSC) are “altered” stem cells with the capacity to form tumors. We used a liver specific *Pten* (phosphatase and tensin homologue deleted on chromosome ten) deletion murine model to investigate the mechanism of hepatic cancer stem cell activation in vivo. We have shown that loss of PTEN induces the transformation of liver progenitor cells to cancer stem cells (CSCs). We hypothesize that hepatocyte cell death resulting from PTEN loss induced liver steatosis presents a selection pressure for mutant progenitor cells to proliferate and replace liver parenchyma. We found that hepatic injury is followed by expansion of hepatic progenitors and demonstrate that elevated expressions of hepatic progenitor cell markers Cytokeratin 19 (CK 19), Epithelial cell adhesion molecule (EpCAM), and α-fetoprotein (AFP). We identified progenitor cells co-expressing dual lineage markers for hepatocytes and cholangiocytes. In addition, tumors developed in the liver specific *Pten*-null mice are also from both lineages: cholangiocellular carcinoma (CC) and hepatocellular carcinoma (HCC). The development of tumor, progenitor cell proliferation and hepatic injury are all reversed when *Akt2*, the downstream effector of PTEN is deleted in the *Pten* mutant mice. Here, loss of AKT2 did not result in more severe cell death (rather the opposite) or reduce progenitor cell proliferation as would be predicted from its pro-survival and pro-proliferation function. Thus, the major effect of AKT2 is likely metabolic regulation and the effect of this metabolic regulation on hepatocyte cell death. Concomitantly, we observed diminished steatosis occurring with AKT2 loss. Together, our data suggest that PTEN loss induces liver cancer development through two processes: inducing the transformation of CSCs and promoting the activation of CSCs by inducing lipotoxicity in hepatocytes.

BIOLUMINESCENCE IMAGING CAPTURES THE EXPRESSION OF *P21*$^{WAF1/CIP1}$ IN LIVING MICE

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The tumor suppressor protein p53 is a key mediator of the cellular response to DNA damage and *TP53* is one of the most frequently mutated genes in human cancers. DNA damage induces the accumulation and activation of p53, leading to gene expression changes and ultimately causing cell death, senesce or cell cycle arrest. p21 is a key effector of the p53/cell cycle arrest pathway. In response to DNA damage, p53 activates *p21* transcription thereby arresting cells in G1 and enforcing S- and G2- cell cycle arrests. p21 expression is regulated by additional signaling pathways including RAS/MAPK, TGFβ, EGF and IFNγ. To monitor p53-dependent and-independent regulation of p21 expression in living animals, knock-in mice were generated that express firefly luciferase (FLuc) from the endogenous *p21*$^{Waf1/Cip1}$ (*p21*) locus. These mice were also bred with p53 null mice in order to monitor p21 promoter activity in the absence of p53. Bioluminescence imaging of reporter mice prior to and after exposure to etoposide or irinotecan revealed robust activation of the p21 promoter and this response was p53-dependent. Experiments performed with MEFs derived from reporter embryos verified that activation of the p21 promoter by DNA damage is p53-dependent. Interesting *p21* expression patterns were observed throughout untreated animals. *p21* expression was found to be highest in the penis, vagina, epididymis, preputial glands, large intestine, small intestine, bladder, testes, skeletal muscle, peritoneal fat, pancreas, and femur (bone marrow). Little to no expression was observed in mammary glands, seminal vesicles, stomach, olfactory bulb, spleen, lungs, liver, kidney, heart, salivary glands and brain. Low light microscopy revealed bioluminescence with cell-type specificity in various organs including discrete regions of the liver, vagina, penis, pancreas, as well as brain. These mice are currently being used to address the contribution made by p21 to animal homeostasis during nutrient deprivation and chemotherapy.

OXIDATIVE STRESS-MEDIATED AMPLIFICATION OF AKT/MTOR SIGNALING PATHWAY LEADS TO MYELOPROLIFERATIVE SYNDROME IN FOXO3-NULL MICE: A ROLE FOR LNK ADAPTOR PROTEIN

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Reactive oxygen species (ROS) participate in normal intercellular signaling including the regulation of PI3-kinase signaling via PTEN and in many diseases including cancer and aging although the associated mechanisms are not fully understood. Balanced regulation of oxidative stress is critical for maintenance of hematopoietic stem cells. Forkhead Box O (FoxO) 3 of FoxO transcription factors is a downstream target of PI3-kinase/AKT signaling pathway that is critical for the regulation of ROS concentrations in many cell types. FoxO3 redox modulation is essential for maintenance of hematopoietic stem cells in both young (Yalcin et al., 2008) and old (Miyamoto et al., 2007) mice. However the role of FoxO in the regulation of hematopoietic progenitors is not clear. Here we show that loss of FoxO3 causes a myeloproliferative syndrome with splenomegaly and increased hematopoietic progenitors that are hypersensitive to cytokines in mice. The expansion of hematopoietic progenitors in FoxO3-/- mice does not appear to be mediated by activation of their hematopoietic stem cell compartment, since FoxO3-/- hematopoietic stem cells are delayed in their cycling at the G2/M phase, and FoxO3-/- hematopoietic stem cells do not generate an increased number of hematopoietic progenitors in vitro (Yalcin et al., 2008). However, analysis of mutant hematopoietic progenitors found that these cells contain increased ROS. Accumulation of ROS in FoxO3-/- mice results in expansion of primitive myeloid progenitor compartment via activation of the AKT/mTOR signaling pathway but not STAT5 and results in relative deficiency of Lnk, a negative regulator of cytokine signaling. Accordingly, In vivo treatment with ROS scavenger N-acetyl-cysteine (NAC) corrects these biochemical abnormalities and relieves the myeloproliferation. In addition, enforced expression of Lnk by retroviral transfer corrects the abnormal expansion of FoxO3-/- hematopoietic progenitors in vivo. Moreover, in vivo administration of the mTOR inhibitor rapamycin results in significant reduction of FoxO3-/--derived primitive myeloid progenitor CFU-Sd12 as compared to controls in lethally irradiated hosts. In agreement with these results, FoxO3 but not other FoxOs is mostly found in the nucleus of primitive myeloid progenitors suggesting that FoxO3 is the principal FoxO operating in these cells. Our combined results show that loss of FoxO3 causes increased ROS accumulation in hematopoietic progenitors. In turn, this inhibits Lnk expression that contributes to exaggerated cytokine responses that lead to myeloproliferation.

MAMMALIAN TARGET OF RAPAMYCIN REGULATES CELL DIFFERENTIATION THROUGH THE STAT3-P63-JAGGED-NOTCH CASCADE

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The receptor tyrosine kinase (RTK)-PI3K-AKT-mammalian target of rapamycin (mTOR) pathway is frequently altered in cancer, but the underlying mechanism leading to tumorigenesis by activated mTOR remains less clear. Here, we have shown that mTOR is a positive regulator of Notch signaling in mouse and human cells, acting through induction of the STAT3-p63-Jagged signaling cascade. Furthermore, in response to differential cues from mTOR, we found that Notch serves as a molecular switch to shift the balance between cell proliferation and differentiation. We determined that hyperactive mTOR signaling impaired cell differentiation of murine embryonic fibroblasts, via potentiation of Notch signaling. Elevated mTOR signaling strongly correlated with enhanced Notch signaling in poorly differentiated but not in well-differentiated human breast cancers. Both human lung lymphangioleiomyomatosis (LAM) and mouse kidney tumors with hyperactive mTOR due to tumor suppressor TSC1 or TSC2 deficiency exhibited enhanced STAT3-p63-Notch signaling. Furthermore, tumorigenic potential of cells with uncontrolled mTOR signaling was suppressed by Notch inhibition. Our data therefore suggest that perturbation of cell differentiation by augmented Notch signaling could be responsible for the under-differentiated phenotype displayed by certain tumors with an aberrantly activated RTK-PI3K-AKT-mTOR pathway. Additionally, the STAT3-p63-Notch axis may be a useful target for the treatment of cancers exhibiting hyperactive mTOR signaling.

THE LKB1/AMPK TUMOR SUPPRESSOR PATHWAY COORDINATES CELL GROWTH AND METABOLISM

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The serine/threonine kinase LKB1 is a tumor suppressor gene mutated in the familial cancer condition Peutz-Jeghers syndrome (PJS), as well as in 30% of sporadic non-small cell lung cancer (NSCLC). One of the critical substrates of LKB1 is the AMP-activated protein kinase (AMPK). AMPK is a highly conserved sensor of cellular energy status found in all eukaryotic cells. Using a proteomic and bioinformatics approach, we identified the mTOR-binding subunit raptor as a critical substrate of AMPK mediating its effects on growth control. AMPK directly phosphorylates the mTOR binding partner raptor on two serine residues that are conserved throughout eukaryotes, and this phosphorylation induces 14-3-3 binding to raptor. Phosphorylation of raptor by AMPK is required for the inhibition of mTORC1 and for cell cycle arrest following energy stress. More recently we have found additional connections between AMPK, mTOR and control of autophagy. Collectively, these studies have uncovered novel conserved effectors of AMPK that mediates its role as a metabolic checkpoint coordinating cell growth with energy status. These findings suggest a possible therapeutic window for treatment of tumors bearing loss of TSC or hyperactivation of mTOR by other genetic lesions with AMPK agonists. In addition, the AMPK-raptor connection reinforces the idea that LKB1-deficient tumors such as those arising in PJS patients and sporadic NSCLC may be uniquely sensitive to rapamycin analogs, which we have recently explored in preclinical trials in LKB1 heterozygous mice. We have found that hamartomas in LKB1+/- mice exhibit functional increases in glucose metabolism as visualized by FDG-PET, which is suppressed by rapamycin treatment. HIF-1a and HIF-1a target genes including GLUT1 and Hexokinase2 are upregulated in LKB1-dependent tumors providing a molecular explanation for the increased FDG-PET and rapamycin sensitivity. More recently in a K-ras, LKB1 mutant mouse model of NSCLC we have also observed deregulation of mTOR and HIF signaling. The connection between LKB1, AMPK, and mTOR signaling further illustrates molecular connections underlying the development of both cancer and metabolic syndrome.

ACTIVATION OF A METABOLIC GENE REGULATORY NETWORK DOWNSTREAM OF MTORC1

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The mammalian target of rapamycin complex 1 (mTORC1) has the ability to sense and integrate signals from a variety of sources, including intracellular nutrients and energy, growth factors, and cellular stresses. Over the past eight years, the collective work of several laboratories has found that mTORC1 senses many of these signals through a small G protein switch involving the tuberous sclerosis complex (TSC) tumor suppressors, TSC1 and TSC2, and the Ras-related small G protein Rheb. Multi-site phosphorylation of the TSC2 protein downstream of distinct signaling pathways appears to account for much of mTORC1's signal-integrating capacity. Importantly, Akt/PKB-dependent phosphorylation and inhibition of TSC2 activates mTORC1, and this represents a major downstream event driving both physiological and pathological responses to PI3K signaling.

Aberrant regulation of the mammalian target of rapamycin complex 1 (mTORC1) is a common molecular event in a large variety of pathological settings, including genetic tumor syndromes and cancer, obesity and type-2 diabetes, and both childhood and aging-related neurological disorders. Many of the genetic and environmental factors contributing to these diseases affect the signaling network that converges on the TSC1-TSC2 complex. However, the downstream consequences of mTORC1 activation remain poorly defined. Here, we take advantage of genetic settings with loss of the TSC1-TSC2 complex to constitutively activate mTORC1 signaling in a manner that isolates it from upstream pathways. Through a combination of unbiased genomic, metabolomic, and bioinformatic approaches, we demonstrate that mTORC1 activation is sufficient to stimulate specific metabolic pathways, including glycolysis, the pentose phosphate pathway, and de novo lipid biosynthesis. This is achieved through the activation of a transcriptional program affecting metabolic gene targets of hypoxia-inducible factor 1 alpha (HIF1α) and sterol regulatory element-binding protein 1 and 2 (SREBP1 and SREBP2). While HIF1α stimulates glycolysis, SREBP1 and 2 induce both the oxidative arm of the pentose phosphate pathway and lipid biosynthesis and are required for the growth factor-independent proliferation of TSC gene-deficient cells downstream of mTORC1. Therefore, we demonstrate that, in addition to promoting protein synthesis, mTORC1 activation is sufficient to promote specific bioenergetic and anabolic processes that are likely to contribute to the pathophysiological properties of a diverse array of diseases.

INTEGRATION OF AMINO ACID AND GLUCOSE SIGNALING IN MTOR COMPLEX 1 (MTORC1)-DEPENDENT REGULATION OF CELL GROWTH AND SURVIVAL.

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Inappropriate activation of mTORC1 signaling pathways contributes to altered cellular metabolism and growth in a variety of human disorders including benign tumor syndromes like LAM and tuberous sclerosis (TSC), most malignancies, diabetes, heart disease and obesity. Mechanisms by which amino acids and glucose regulate the pathway to drive cell growth, and how specific amino acids can also promote cancer cell survival when the pathway is inhibited (drug resistance), will be discussed.

THE LATE ENDOSOME IS ESSENTIAL FOR mTORC1 SIGNALING

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The multi-subunit mTORC1 complex integrates signals from growth factors and nutrients to regulate protein synthesis, cell growth, and autophagy. Significant evidence suggests that TOR is regulated by and/or regulates various aspects in the endocytic system. To examine how endocytic trafficking might be involved in nutrient regulation of mTORC1, we perturbed specific endocytic trafficking pathways and measured mTORC1 activity using S6K1 as a readout. When early/late endosomal conversion was blocked by either overexpression of constitutively active Rab5 (Rab5CA) or knockdown of the Rab7 GEF hVps39, insulin and amino acid stimulated mTORC1/S6K1 activation were inhibited and mTOR localized to hybrid early/late endosomes. Inhibition of other stages of endocytic trafficking had no effect on mTORC1. Overexpression of Rheb, which activates mTOR independently of mTOR localization, rescued mTORC1 signaling in cells expressing Rab5CA, whereas hyperactivation of endogenous Rheb in TSC2-/- MEFs did not. These data suggest that integrity of late endosomes is essential for amino acid- and insulin-stimulated mTORC1 signaling, and that blocking the early/late endosomal conversion prevents mTOR from interacting with Rheb in the late endosomal compartment.

FOXOS SERVE AS AN ENERGY STRESS CHECKPOINT AND SUPPRESS MTORC1-MEDIATED RENAL CANCER DEVELOPMENT

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The mammalian target of rapamycin complex 1 (mTORC1) has recently been identified as an important therapeutic target for renal cell carcinoma (RCC). The effective clinical application of mTORC1 targeted therapy in RCC would be enabled by knowledge of the key downstream effector(s) of mTORC1 activation and the nature and role of mTORC1-mediated negative feedback signaling in RCC. Here, utilizing the conditional TSC1 knockout (KO) mouse as an mTORC1 hyper-activation-driven renal cancer model system, we show that FoxO1 and FoxO3 are robustly activated in TSC1 deficient benign polycystic neoplasias, but consistently extinguished with progression to renal adenomas and carcinomas. In addition, relative to the TSC1-null model, combined deletion of TSC1 and FoxO1/3/4 provoked dramatic exacerbation of the renal tumor phenotypes, establishing activated FoxO signaling as a potent block in TSC1-null renal cancer development. Mechanistically, we show that FoxO activation initiated by mTORC1 hyper-activation functions to induce apoptosis in response to energy stress. In line with these murine data, we found that FoxO activation serves to promote energy stress-induced apoptosis in human renal cancer cells and that FoxOs are extinguished in the majority of human clear cell and papillary renal tumor samples compared with more benign types of RCC including chromophobe and oncocytoma. Further integrated transcriptomic, computational and functional studies identified Myc-Mxi1 signaling as the key downstream effector of FoxOs in the regulation of renal tumorigenesis. Together, our data identify FoxOs as novel tumor suppressors in the progression to renal cancer and analysis of the FoxO-regulated transcriptional network reveals a metabolic stress checkpoint functioning to constrain mTORC1-mediated renal tumorigenesis.

"MTOR AND THE CONTROL OF GROWTH"

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mTOR is the target of the immunosuppressive drug rapamycin and the central component of a nutrient- and hormone-sensitive signaling pathway that regulates cell growth and proliferation. We now appreciate that this pathway becomes deregulated in many human cancers and has an important role in the control of metabolism and aging. We have identified two distinct mTOR-containing proteins complexes, one of which regulates growth through S6K and another that regulates cell survival through Akt. These complexes, mTORC1 and mTORC2, define both rapamycin-sensitive and insensitive branches of the mTOR pathway. I will discuss new results from our lab on the regulation and functions of the mTORC1 and mTORC2 pathways.

FORKHEAD PROTEINS IN INSULIN ACTION

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Regulation of PTEN signaling downstream of Akt is important for metabolic homeostasis at the cellular and organismal levels. Foxo transcription factors are key Akt substrates that mediate growth factor and nutrient signaling to regulate diverse cellular processes, including metabolism, growth, and differentiation. Studies in our laboratory have focused on the mechanism by which Foxo proteins affect metabolism and cellular differentiation. We have shown that Foxo1 is an insulin-regulated transcription factor. Our studies have demonstrated that Foxo1 is a key effector of insulin action in several tissues: liver, where it controls insulin inhibition of glucose production and stimulation of lipid synthesis; pancreatic β-cells, where it controls proliferation and differentiation; pre-adipocytes, where it controls insulin-dependent differentiation; myoblasts, where it controls myotube formation; and brain, where it controls food intake through its ability to regulate expression of peptidases required for neuropeptide processing. Thus, regulation of Foxo1 function can potentially affect insulin sensitivity in vivo by regulating various aspects of fuel metabolism. Foxo1 activity is regulated by post-translational modifications that include, but are not limited to, phosphorylation and acetylation. Using an allelic series of knock-in mice with mutations in the acetylation sites and DNA binding domain of Foxo1, we have begun to systematically dissect how these modifications affect metabolism and cellular responses to altered nutrient availability. The findings reveal a range of novel biochemical and genetic pathways that can be affected by Foxo1 in response to changes in the metabolic status of the organism.

SYSTEMS BIOLOGY APPROACH TO PERSONALIZED MEDICINE.

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The realization of the promise of personalized molecular medicine will require the efficient development and implementation of novel targeted therapeutics. The goal will be to deliver the right drug to the right patient at the right time at the right dose. This effort will require a integration of information from the DNA, RNA and protein level into predictors of which patients are likely to benefit from particular therapies. The overall likelihood of response to particular drugs represents the interaction between predictors of sensitivity with predictors of resistance. This session will use examples with predictors from the DNA RNA and protein level to illustrate some of the opportunities and challenges to the implementation of personalized medicine. It will demonstrate the need to integrate information from multiple technological platforms into visualizable pathways and networks as a key step in the process.

THE MTOR PATHWAY IN CANCER: MECHANISMS AND THERAPEUTIC PROMISE

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Aberrant activation of TORC1 is triggered by the loss of several tumor suppressors, including PTEN, and is thought to be one of the signals that promote cancer. However the ultimate downstream target proteins that mediate tumorigenesis are largely unknown. Moreover it is not currently clear whether we will be able to effectively harness the therapeutic potential of mTOR inhibitors for treating human tumors. Utilizing cell biological, biochemical and mouse modeling approaches we have identified a critical downstream target of the mTOR pathway in our tumor model. Notably, its expression is potently suppressed by mTOR inhibitors and its re-expression accompanies resistance to these agents. However we have also been utilizing mTOR inhibitors as a platform for rationally developing combination therapies and have identified agents that together promote dramatic tumor regression in vivo in genetically engineered mouse models (GEMMs). The implications for these findings and the specific utility of GEMMs in these studies will be discussed in the context of tumor-types driven by hyperactivation of the mTOR pathway.

PRECLINICAL EVIDENCE SUPPORTING COMBINATION PARTNERS FOR NVP-BEZ235 BREAST CANCER TRIALS.

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NVP-BEZ235 is a dual PI3K/mTOR inhibitor currently in phase I clinical trials. In a former study we have shown that NVP-BEZ235 selectively induced cell death in breast cancer cell lines harboring either HER2 amplification and/or PIK3CA mutation, but not in lines with PTEN LOF or K-RAS mutation (poster Irmgard Hofmann). Here, we show that NVP-BEZ235 efficiently shuts down the PI3K pathway (AktS473P / S6P) in all HER2 amplified and/or PIK3CA mutated lines. In contrast, the HER1/2 inhibitor lapatinib could only efficiently inhibit the PI3K pathway in a subset of these lines which were characterized by high HER3 phosphorylation levels causing strong PI3K recruitment to HER2/HER3. Hence, in HER2 amplified tumors anti-HER2/HER3 agents are expected to display synergistic activities with NVP-BEZ235 treatment.
In the HER2 amplified and/or PIK3CA mutated breast cancer cell lines the ribosomal S6 S235/236 sites were more sensitive to NVP-BEZ235 treatment than in lines with PTEN LOF or K-RAS mutation while the ribosomal S6 S240/244 sites were equally responsive across the panel. In addition, the lines with PTEN LOF or K-RAS mutation exhibited higher MAPK phosphorylation than the HER2 amplified and/or PIK3CA mutated lines. Therefore, in breast cancer lines PTEN LOF might result in redundant phosphorylation of the mTORC1 downstream effecter RPS6 by the ERK pathway and hence incomplete pathway shut down by NVP-BEZ235. Therefore, breast cancer patients with loss of PTEN function might benefit from a combination of NVP-BEZ235 with a MEK inhibitor.

ONCOGENIC SIGNALING IN THE CLASS I PI3K PATHWAY

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Most of the cancer-specific mutations in p110α (PIK3CA) occur in hotspots located in the helical and kinase domains. Helical and kinase domain mutations induce gain of function by different molecular mechanisms. The E542K and E545K helical domain mutations result in independence from binding to the regulatory subunit p85 and mimic activation of the enzyme by upstream signaling. To be active, these mutants still require binding to Ras. The kinase domain mutation H1047R can function in the absence of Ras binding. This mutation appears to induce a conformational change that mimics activation of the enzyme by Ras. The dependence of H1047R on p85 binding may be explained by the contacts between residues of the inter-SH2 domain of p85 and the kinase domain of p110α.
The glioblastoma-derived mutants of p85α induce oncogenic cellular transformation as single oncogenes in cultures of avian cells. The efficiencies of transformation vary greatly between different mutants. All these mutant p85α proteins still bind to p110 and induce elevated levels of downstream signaling. Their sensitivities to isoform-specific inhibitors of p110 suggest that most of the oncogenic activity seen with these mutants requires interaction with p110α, and a minor portion relies on interaction with p110β. The p110γ and p110δ isoforms do not participate in the transformation of cultured cells by the glioblastoma-derived mutants of p85α.

This work was supported by grants from the National Cancer Institute.

ROLE OF FOXO TRANSCRIPTION FACTORS IN AGING

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Aging is regulated by modifications in single genes and by simple changes in the environment. The signaling pathway connecting insulin, PI3K, Akt, and FOXO transcription factors integrate environmental stimuli to regulate lifespan. FOXO transcription factors are directly phosphorylated in response to insulin/growth factor signaling by the protein kinase Akt, thereby causing their sequestration in the cytoplasm. In the absence of insulin/growth factors, FOXO factors translocate to the nucleus where they trigger a range of cellular responses, including resistance to oxidative stress, a phenotype highly coupled with lifespan extension. FOXO factors integrate oxidative stress stimuli via phosphorylation and acetylation of specific residues. Oxidative stress stimuli elicit the physical interaction between FOXO and SIRT1 deacetylase, a member of the Sir2 family, which extend longevity in invertebrates. Our recent results indicate that FOXO transcription factors are also regulated in response to nutrient deprivation by the energy-sensing AMP-dependent protein kinase (AMPK) pathway. AMPK phosphorylation enhances FOXO transcriptional activity, leading to the expression of specific target genes involved in stress resistance and changes in energy metabolism. The AMPK pathway plays a crucial role in the ability of a dietary restriction regimen to extend lifespan in worms. Understanding the intricate signaling networks that translate environmental conditions into changes in gene expression that extend lifespan will be of critical importance to identify ways to delay the onset of aging and age-dependent diseases.

CRTC-1 - A NOVEL CALCINEUIN AND AMPK TARGET FOR LIFESPAN EXTENSION IN *C. ELEGANS*

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Many interventions that extend lifespan do so by reducing costly energetic activities such as translation, growth and reproduction. A key example is AMP-activated protein kinase (AMPK), which down-regulates energy requiring processes when energy intake is low. Recent studies have shown that *C. elegans* expressing gain of function (GOF) AMPK transgenes are long-lived, and that AMPK is critical for the longevity of dauer larvae. Conversely, loss of function (LOF) of the protein phosphatase Calcineurin extends C. elegans lifespan.

We show here that AMPK and Calcineurin phosphorylate and dephosphorylate the worm CREB-regulated transcription co-activator (CRTC-1) respectively. Phosphorylation of CRTC-1 results in its deactivation and retention in the cytosol in a 14-3-3 dependent manner. AMPK GOF or Calcineurin LOF both result in CRTC-1 cytosolic translocation. Site-specific mutation of two conserved AMPK target residues on CRTC-1 from serine to alanine renders CRTC-1 constitutively nuclear. Calcineurin LOF robustly extends the lifespan of worms over-expressing wild type CRTC-1 but has no effect when CRTC-1 is constitutively in the nucleus.

In mammals CRTC family members bind to the transcription factor CREB, promoting transcription of its target genes. CREB has critical functions ranging from memory formation to glucose homeostasis. We show that down-regulation of CRTC-1, CREB and a subset of CREB target genes also extend lifespan.

We therefore propose that the lifespan effects of AMPK and Calcineurin are mediated at least in part via reducing the activity of CREB and its target genes via inactivation of the key CREB coactivator, CRTC-1.

PROTECTION FROM METABOLIC DAMAGE AND INCREASED LONGEVITY BY PTEN

Ana Ortega-Molina[1], Alejo Efeyan[2], Mulero Francisca[3], Manuel Serrano[1]

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Our laboratory is interested in the mechanistic connections between cancer and aging. For this, we have generated a number of genetically-modified mice with increase gene dosage of relevant tumor suppressors, such as p53 and Ink4a/Arf. The lipid phosphatase PTEN is another critical tumor suppressor that counteracts the effects of growth factors and hormones by constitutively decreasing the levels of the second-messenger lipid PIP3 thereby inhibiting the metabolic sensor mTOR. Moderate inhibition of the mTOR pathway is beneficial for aging, although the physiological processes implicated remain poorly understood. Here, we have generated transgenic mice with a modest global increase in PTEN activity. PTENtg mice show a remarkable improvement in healthy aging and a significant extension in longevity. We will present a detailed analysis of these mice and WILL PROPOSE NOVEL CONNECTIONS BETWEEN CANCER AND AGING.

PDZ-RHOGEF REGULATES ADIPOSE TISSUE DEVELOPMENT VIA INSULIN/IGF-1 SIGNALING

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Biological outputs of insulin/IGF signaling are regulated through essential mediators, such as IRSs, PI3-kinase, and PKB/Akt. These mediators serve critical roles in signal propagation, feedback, and as junctions for crosstalk with other pathways. Abnormal insulin/IGF signaling results in disease, such as obesity, diabetes, and cancer. Given the vital role of this signaling pathway to human health, unraveling its regulatory mechanisms is crucial. Components of this pathway are highly conserved throughout evolution. PTEN, one of the well-defined regulators of this pathway, functions as a lipid phosphatase that negatively regulates insulin/IGF signaling at the PIP3 level that is upregulated by activated PI3-kinase in both *Drosophila* and mammals. To discover genetic modulators of PTEN in *Drosophila*, we performed a loss-of-function genetic screen seeking the molecules that modify the phenotype elicited by PTEN overexpression in the *Drosophila* eye. From the screen, we identified a member of the Dbl-family, DRhoGEF2, a guanine nucleotide exchange factor, suppresses PTEN-overexpression eye phenotype via its effects on dPKB/dAkt activation. From a genetic rescue, we established that PDZ-RhoGEF, a member of the regulator of G-protein signal (RGS)-like domain containing Rho GEFs (RGS-RhoGEFs) subfamily of Dbl-family GEFs, is the closest mammalian counterpart of DRhoGEF2. PDZ-RhoGEF is essential for cell proliferation and survival through ROCK-dependent activation of IRS/PI3-kinase signaling cascade, which has a major impact on adipose tissue homeostasis. Through an integrative approach, we have demonstrated that DRhoGEF2/PDZ-RhoGEF-dependent signaling has tissue-specific effects on insulin/IGF-signaling throughput in both *Drosophila* and mammals. Taken together, our results implicate PDZ-RhoGEF as a major physiological modulator of insulin/IGF-1 signaling and a key factor in adipose tissue biology. Particularly, the role of PDZ-RhoGEF in diet related pathological outcome provides an alternative therapeutic opportunity in disease intervention.

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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.

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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
US Airways	800-428-4322