Chromothripsis and focal copy number alterations determine poor outcome in malignant melanoma

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Genetic changes during tumorigenesis are usually acquired sequentially. However, a recent study showed that in 2 to 3% of all cancers a single catastrophic event, termed chromothripsis, can lead to massive genomic rearrangements confined to one or a few chromosomes. In order to explore whether the degree of genomic instability and chromothripsis influences prognosis in cancer, we retrospectively applied array comparative genomic hybridization (aCGH) to 20 malignant melanomas (MM) that showed, despite comparable conventional clinical and pathological parameters, a profoundly different clinical course. We compared 10 patients who died of MM 3.7 years (median, range 0.9 to 7.6 years) after diagnosis with 10 patients who had a median disease-free survival of 14.8 years (range 12.5 to 16.7 years; P = 0.00001). We observed a striking association between the degree of chromosomal instability, both numerical and structural, and outcome. MM associated with good prognosis showed only few chromosomal imbalances (mean 1.6 alterations per case), predominantly whole chromosome or chromosome arm gains and losses while MM with poor prognosis harbored significantly more chromosomal aberrations (13.9 per case; P = 0.008). aCGH showed that these aberrations were mostly focal events, which culminated in two cases in a pattern consistent with the phenomenon of chromothripsis, which was confirmed by paired-end sequencing. This is the first description of chromothripsis in primary MM. Our study therefore links focal copy number alterations and chromothripsis with poor outcome in MM patients (P = 0.0002) and provides a genetic approach to predict outcome in MM.

REC8 is aberrantly expressed in melanoma and may act as a driver of genomic instability

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Melanomas include a subpopulation of cells with stem/germ cell-like features. To examine whether the expression of germ cell proteins plays a role in the development of chromosomal instability in melanoma, we studied a protein known to be crucial for meiotic division, REC8. REC8 is a major subunit of the meiotic cohesin complex, and is involved in sister chromatid cohesion and crossover events during meiosis. While one would expect this protein to be limited to germ cells, we found increased levels of REC8 RNA in melanoma tissue compared to normal tissues by qPCR analysis (p=0.03). We also compared protein levels *in vitro* and found increased REC8 expression in multiple melanoma cell lines as compared to normal human melanocytes and fibroblasts. We hypothesized that the aberrant expression of this protein during mitosis would lead to defects in chromosomal segregation. To further explore this hypothesis, we constructed a REC8-GFP fusion protein under the constitutive expression of the ubiquitin promoter. As a control, GFP alone under the ubiquitin promoter was utilized. These recombinant DNAs were co-transfected with histone H2B tagged with mCherry into HEK293T cells. While the control GFP + cells primarily revealed normal appearing nuclei, the cells that were cotransfected with the REC8-GFP fusion protein demonstrated DNA bridging and polyploidy. In summary, we have shown that a meiosis protein known to be involved in chromosomal cohesion is inappropriately expressed in melanoma. Overexpression leads to nuclear abnormalities suggestive of failed normal segregation. Taken together, these results suggest that the aberrant expression of the meiosis protein REC8 during mitosis may act as a driver for chromosomal instability.

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An attempt to assess the expression of desmogleins 2 and 3 with fluorescent *in situ* hybridization and immunohistochemistry in basal cell carcinomas

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The abnormal expression of desmogleins (DSG) in basal cell carcinomas (BCC) is reported. However, there are no data on their simultaneous expression profile at the various levels of biosynthesis. Here, we comparatively examined the expression of DSG2 and DSG3 with fluorescent in situ hybridization (FISH) and immunohistochemistry (IHC) in BCC nests. Altogether, frozen sections from 47 patients with various clinical/histological subtypes of BCC were analyzed using IHC with monoclonal antibodies against human DSG2 and DSG3, and FISH with fluorescent-labeled oligonucleotide probes for the human DSG2 and DSG3 mRNA. The sequences of human DSG2 and DSG3 mRNA were obtained from GenBank. The probes were used: DSG2 (5′-6-FAM/5-TAMRA-AAAgTgTAgCTgCTgTgTTCCTCTCTgTCCAA-3′), DSG3 (5′-6-FAM-AgTTgTTCATAATCTAgAgCCTTCACCACTTTCAggA-3′). IHC data suggested that at the protein level there is a quantitatively significant overexpression of DSG2 and underexpression of DSG3 in BCC nests. Moreover, it appears that with the use of FISH and semiquantitative expression evaluation there are no changes in DSG2 and DSG3 mRNA expression in BCC nests. Thus, it seems that there are differences between the DSG2/DSG3 protein and mRNA expression in BCC nests. This data might provide evidence that the possible mechanism which regulates biosynthesis of DSG2/DSG3 in BCC nests could be induced through local-dependent translational control/locus control element. The discrepancy in the DSG2/DSG3 expression between mRNA and protein in BCC nests could be due to the posttranscriptional/translational modification of DSG2/DSG3 associated with the complex synthesis and transport of DSG (inactive precursors in the cytoplasm/biologically active mature proteins within desmosomes on the cell surface).

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Autophagy controls p38 activation to promote cell survival under UVB stress

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Deregulated cell survival under carcinogen-induced genotoxic stress is vital for cancer development. One of the cellular processes critical for cell survival under metabolic stress and energy starvation is autophagy, a catabolic process involved in capture and delivery of cytoplasmic components to lysosomes for degradation. However, the role of autophagy following carcinogen-induced genotoxic stress remains unclear. Here we show that UVB radiation, a known human skin carcinogen that operates by causing DNA damage, induced autophagy and autophagic flux through AMPK activation. Autophagy deficiency sensitizes cells to UVB-induced apoptosis through increasing p62-dependent activation of the stress-activated protein kinase p38. As compared with normal human skin, autophagy was activated in human squamous cell carcinomas, in association with decreased phosphorylation of p38, and increased phosphorylation of ATR and formation of gamma-H2AX, two markers of DNA damage response. Our results demonstrate that autophagy promotes cell survival through suppressing p62-mediated p38 activation and thus may facilitate tumor development under genotoxic stress. These findings suggest that autophagy plays an oncogenic role in epithelial carcinogenesis by promoting cell survival.

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Targeting PI3K/mTOR signaling in cutaneous angiosarcoma

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Angiosarcoma is a rare and aggressive malignant neoplasm of endothelial cells. Recent data have shown that the mTOR pathway is also aberrantly activated in cutaneous angiosarcoma. Since the prognosis of this disease is still poor, new therapeutic strategies are required. We investigated the sensitivity of inhibitors for PI3K/AKT/mTOR pathway in ISOS-1 and ISO-HAS cutaneous angiosarcoma cell lines. In both cells, PI3K inhibitor and mTOR inhibitor, not AKT inhibitor demonstrated the growth inhibition in a dose-dependent manner. Interestingly, PI3K inhibitor more effectively caused cell cycle arrest at the G1 phase with downregulation of cyclinD1 expression than mTOR inhibitor in ISOS-1 cells. Taken together, PI3K inhibition rather than mTORC1 inhibition might be the appropriate strategy to treat cutaneous angiosarcoma, and therefore PI3K inhibitor is promising for the therapy.

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UAB30, a novel synthetic RXR retinoid, is an effective chemopreventive agent against UVB-induced nonmelanoma skin cancers

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Systemic retinoids are useful for the chemoprophylaxis of basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs) in high risk populations, but significant toxicities currently limit their long-term use. UAB30 is a unique synthetic retinoid with low toxicity due to its high selectivity for activating retinoid X receptors (RXR), while having no significant retinoic acid receptor (RAR)-binding activity. Our aim was to investigate whether UAB30 is effective at inhibiting UVB-induced skin carcinogenesis in a relevant murine model. We used genetically engineered Ptch$^{+/-}$/SKH-1 hairless mice because they recapitulate the pathogenesis of human nonmelanoma skin cancer development following chronic ultraviolet B (UVB) exposure. The animals were divided in half, with one group receiving UAB30 through oral gavage, while another group received a vehicle control. Both groups were chronically irradiated with UVB (180 mJ/cm^2; twice per week) for 30 weeks. Compared to controls, the UAB30 treated group showed a 48% reduction in tumor number and a 78% reduction in tumor volume. The number of papillomas, BCCs, and SCCs were reduced by 33%, 74%, and 76%, respectively. In addition, treatment with UAB30 diminished UVB-induced skin hyperplasia, and enhanced differentiation of suprabasal epidermal cells. UAB30 also decreased proliferation and increased apoptosis of tumor cells, as demonstrated by increased expression of pro-apoptotic proteins and decreased expression of cell cycle regulatory proteins. Moreover, target genes of the sonic hedgehog signaling pathway, which is constitutively activated in >90% of BCCs, were downregulated in the UAB30 treatment group. In summary, UAB30 is a potent chemopreventive agent for blocking skin photocarcinogenesis, and holds great promise in the chemoprevention of nonmelanoma skin cancers among high risk populations, such as in solid organ transplant recipients and individuals with chronic actinic damage.

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GLI2 induces genomic instability in human keratinocytes by inhibiting apoptosis

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Abnormal Sonic Hedgehog (SHH) signalling leads to increased transcriptional activation of its downstream effector, GLI2, which is implicated in the pathogenesis of a variety of human cancers. However, the mechanisms underlying the tumorigenic role of GLI2 remain elusive. We demonstrate that overexpression of GLI2ΔN in human keratinocytes, is sufficient to induce numerical and structural chromosomal aberrations, such as tetraploidy/aneuploidy and chromosomal translocations, respectively. This is coupled with suppression of cell-cycle regulators 14-3-3σ and p21WAF1/CIP1, and strong induction of the anti-apoptotic signaling which reduces the ability to eliminate genomically unstable cells. We demonstrate that, forced overexpression of GLI2ΔN renders human keratinocytes resistant to apoptosis mediated by ultraviolet (UV) B, one of the most important etiological factors in BCC formation, while inhibition of the anti-apoptotic BCL-2 protein restores endogenous (genomic instability) and exogenous (UVB) DNA damage-induced apoptosis. This study reveals a novel role and associated mechanisms of GLI2 in the generation of genomic instability, a hallmark of human tumors, and may provide new opportunities in designing novel forms of cancer therapeutic strategies by targeting GLI2 itself or relevant mechanisms.

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TLR4 is a negative regulator of keratinocyte proliferation

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Our current study investigates the role of TLR4 in the proliferation capacity of normal keratinocytes. Our preliminary results using a blocking monoclonal antibody (HTA125) against TLR4 showed an unexpected, pronounced proliferation of keratinocytes, assessed by BrDU proliferation assay. In addition, we abrogated the interaction between TLR4 and its accessory protein MDII using a specific blocking peptide for MDII and we detected an induction of proliferation. We observed that with the subsequent growing of normal primary keratinocytes and keratinocytes derived from patients with SCC up to full confluence and differentiation, the expression of TLR4 increased significantly. This correlates with the differential TLR4 expression within the layers in normal skin and skin from patients with SCC In addition, we detected that the blocking HTA125 antibody induces the phosphorylation of SAPK/JNK and ERK1/2 in primary keratinocytes. Furthermore, we found that the tumor SCC13 cell line, stably expressing TLR4 showed lower proliferation capacity and higher motility. Our results show that TLR4 is a negative regulator of keratinocyte proliferation and may be associated with the progression of SCC of the skin. Better understanding of the regulatory role for TLR4 is the basis for a later use in a therapeutic setting to stop keratinocyte proliferation such as in squamous cell carcinoma of the skin and to induce keratinocyte proliferation such as in wound healing.

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Langerhans cell contribution to UV-induced DNA damage and carcinogenesis

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Resident epidermal dendritic cells, called Langerhans cells (LC), were recently shown to facilitate DMBA/TPA-induced chemical carcinogenesis. LC contribution to UV-induced carcinogenesis, however, remains to be elucidated. In this study, we used genetically engineered hairless LC-deficient and LC-intact mice to characterize early and late effects of DNA damage in epidermis following exposure to UVB light. We find that LC density in LC-intact mice remains unchanged at 1hr and 24hr post 100 J/m2 UVB (0hr: 861.4±68.4, 1hr: 894.2±126.4, 24hr: 848.2±101.7 /mm2). Thus, LC are poised to affect the surrounding epidermal cells following acute UVB exposure. Using confocal microscopy, LC-intact mice showed >2x (8556±3561 vs 4255±1320; P<0.0098) the area occupied by keratinocytes containing mutated p53, a tumor suppressor protein highly associated with squamous cell carcinoma, after 9 weeks of UVB. Since mutant p53 arises from unrepaired DNA damage, we examined early DNA damage following UVB exposure. At 1hr post 100 J/m2 UVB exposure, LC-intact mice showed significantly greater keratinocytes containing γH2AX (130.6±15.4 vs 75.7±7.1 /mm2, P=0.0030), a phosphorylated histone indicative of non-specific DNA damage; therefore, LC impart substantial influences on UVB induced keratinocyte DNA-damage. However, when we quantified cyclobutane pyrimidine dimers, the pathognomonic direct UV-induced DNA lesions, LC-intact and LC-deficient skin showed no differences at 1 and 24 hours following 100 J/m2 UVB exposure. Taken together, these results suggest that LC influence UVB-induced DNA-damage and clonal expansion in adjacent keratinocytes, supporting an hypothesis of "cooperative carcinogenesis." Collectively, these data give new insight into the previously unknown role of LC in UV-induced carcinogenesis, and may facilitate the development of novel preventive and therapeutic targets against skin cancer.

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Intercellular contact-dependent induction mechanism of podoplanin in squamous cell carcinoma

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Podoplanin (PDPN), a transmembranous glycoprotein, is expressed in various human tumors including cutaneous neoplasms, and plays an important role in tumor invasion and metastasis. Recent reports show that PDPN-inhibition using neutralizing antibodies and specific inhibitory chemicals prevents tumor progression in animal models. However, detailed regulatory mechanism of PDPN-expression has not been fully elucidated. Here, we investigated an intercellular contact-mediated regulatory mechanism of PDPN-expression and the related signaling pathway in human squamous cell carcinoma (SCC) cell lines. PDPN-expression was upregulated along with increased cell density in highly EGFR-expressing SCC cell-lines, such as A431, HSC2 and HSC4, but not in a less EGFR-expressing cell-line, HSQ89. This cell density-dependent PDPN-induction was accompanied by increased cell motility, which was reversed by PDPN-silencing using shRNA, suggesting an essential role of PDPN in the enhanced cell motility. In the confluent culture condition, phospho-EGFR and -STAT3 was also upregulated and an EGFR-specific inhibitor, AG1478 and STAT3-silencing using shRNA abolished the cell density-dependent PDPN-induction. In addition, exogenous EGF or soluble EGF ligand did not induce PDPN expression, and then EGFR activation due to increased cell-cell contact was thought to be important for PDPN induction. In human SCC lesions, PDPN was co-localized with phosphor-EGFR and phosphor-STAT3 at invading front, suggesting a crucial role of EGFR-STAT3 signaling pathway in the PDPN-induction mechanism both in vitro and in vivo. The novel cell density-dependent PDPN-induction might significantly be involved in progression process of SCC and could be a possible target for SCC treatment.

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Activated p-AKT, but not MDM2, drives malignant progression in Ras/Fos/PTEN^{null} skin carcinogenesis via p53/p21 loss and cyclin D1/E2 over-expression

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Tumour progression depends on a complex combination of the genetic mutation milieu pitted against the sentinel systems that have evolved to resist carcinogenesis at each specific stage. To investigate tumour progression mechanism in transgenic mouse skin carcinogenesis, inducible PTEN ablation [Δ5PTEN] was introduced into the epidermis of mice expressing activated ras^{Ha}/fos oncogenes. RU486-treated HK1.ras/fos-Δ5PTEN mice exhibited accelerated papillomatogenesis but malignant conversion was delayed due to compensatory p53/p21 expression. Following p53 loss, malignant progression was limited to well-differentiated squamous cell carcinoma via persistent p21 expression and down regulation of cyclin E2. Analysis of AKT activity during papillomatogenesis showed reduced p-AKT expression, associated with fos/PTEN feedback, which returned following p53 loss to circumvent/antagonise p21 expression; co-operate with MAPK signalling [i.e. elevated ERK1/2 expression]; and accelerate tumour progression via increased cyclin D1 and E2 expression. In contrast elevated, suprabasal MDM2 expression in p53-positive papillomas was lost and paralleled that of p53; hence sustained MDM2-mediated p53 ubiquination does not appear to influence this progression mechanism. These data suggest p53/p21 counter deregulated MAPK signalling during papillomatogenesis and help minimise consequences of PTEN loss via p-AKT inhibition. Stepwise p53/p21 loss subsequently facilitates ras/MAPK/fos co-operation with PTEN/AKT activities to accelerate malignant progression via major failures in cell cycle control. The interplay between these common mutations thus create unique tumours contexts and insight for therapies geared to reactivating p53/p21 functions or that target ras/MAPK/fos and PTEN/AKT signalling pathways.

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Inducible ROCK 2 activation increases primary keratinocyte differentiation but co-operates with ras^{Ha} activation in transgenic mouse skin carcinogenesis

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To investigate ROCK 2 signalling in epidermal differentiation and co-operation with ras^{Ha} activation in squamous cell carcinogenesis [SCCs], transgenic mice expressing a conditionally active, 4-hydroxytamoxifen (4HT)-regulated, human ROCK2 transgene from a keratin 14 promoter [K14.ROCKer] were crossed with mice expressing activated ras^{Ha} exclusively in epidermal transit amplifying keratinocytes [HK1.ras]. 4HT-treatment of K14.ROCKer alone cohorts for 12 weeks induced epidermal and follicular hyperplasia but no papillomas. Western analysis of primary K14.ROCKer keratinocyte differentiation showed elevated K1 expression following calcium challenge in vitro, consistent with a role in differentiation, whereas little K6 expression was detectable in 4-HT treated K14.ROCKer hyperplasia in vivo; an unusual result for epidermal hyperplasia. In addition hyperplastic anagen follicles expressed elevated collagen deposits in the hair bulb/IRS regions, a result also observed in bi-genic K14.ROCKer/HK1.ras papillomas. Here, 4-HT treatment for 12-14 weeks had little apparent effect on HK1.ras papillomatogenesis, with tumours appearing by approx 8-10 wks in treated and untreated cohorts. However K14.ROCKer/HK1.ras histotype was quite different, with increased collagen deposits appearing in the connective tissue of papillomas, which now expressed K6 but at lower levels and in an atypical, supra-basal fashion compared to untreated, bi-genic controls. Further, the basal layer architecture was also disturbed and now 4-HT treated K14.ROCKer/HK1.ras papillomas taken at 12-14 weeks displayed areas of malignant conversion with both carcinoma in situ and overt SCC. These interesting data demonstrate significant roles for ROCK 2 in epidermal differentiation and identify unique epidermal responses to its deregulated signalling and novel early-stage co-operation with HK1.ras in skin carcinogenesis.

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Cytokine-induced senescence arrests cancers by activating the p16Ink4a/Rb pathway

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Survival of metastatic melanoma after immunotherapy is associated with a stable growth arrest and not with deletion of metastases. Dysfunctional cell cycle regulation frequently caused by impaired p16Ink4a/Rb regulation is a key feature of cancers. RIP1-Tag2 mice (RT2) selectively express the SV40 large T antigen (Tag) under the control of the rat insulin promoter (RIP). Tag inactivates p53 function and partially inhibits the p16Ink4a/Rb pathway what leads to neuroendocrine cancers. Treatment with Tag-specific T helper 1 cells (Th1) inhibits tumor cell proliferation in vivo and prolongs the lifetime of RT2 mice. Here we found that Th1 treatment causes strong induction of p16Ink4a expression in cancer cells. As MHC class II deficient cancers can not be directly recognized by Th1 cells, the in vivo data suggests that the Th1 cytokines IFNγ and TNF may restore the p16Ink4a/Rb mediated cell cycle arrest in cancers. Indeed, in vivo treatment with IFNγ and TNF arrested the β cancer cells in the G0/G1 phase of the cell cycle as determined by flow cytometry. Simultaneously, the two cytokines increased the p16Ink4a protein levels as shown by Western Blot analysis of p16Ink4a. To determine whether the cytokine-induced p16-protein is relevant for cell cycle arrest, we silenced p16Ink4a and p19Arf via transduction of cancer cells with Mscv vectors containing p16Ink4a/p19Arf- or control-shRNA. Control-shRNA did not affect the induction of a stable growth arrest by IFNγ and TNF. In sharp contrast, IFNγ and TNF failed to induce growth arrest in cancer cells where p16Ink4a/p19Arf was silenced. These data show that IFNγ and TNF induced growth arrest in neuroendocrine cancers trough cytokine-mediated activation of the p16Ink4a/Rb senescence pathway. This may be of general validity, as modern immunotherapies of melanomas may arrest melanoma growth through induction of melanoma senescence.

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Drug-resistant basal cell carcinomas maintain hedgehog pathway output by activating aPKC signaling

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Basal cell carcinomas (BCC) are dependent on high levels of Hedgehog (Hh) signaling for tumor growth. Smoothened (Smo) inhibitors effectively treat BCCs, although 20% of patients develop drug-resistant tumors indicating a need for additional therapeutic targets downstream of Smo. Previously, we identified atypical Protein Kinase C iota/lambda (aPKC) as a novel BCC oncogene essential for Hh signaling. How aPKC mediates its effects on the pathway and its role in tumorigenesis remains unclear. Here we show aPKC operates downstream of Smo to phosphorylate and activate Gli1, resulting in high affinity DNA binding and Hh activation. aPKC and Gli1 form a complex as recombinant aPKC and Gli coimmunoprecipitate. Purified aPKC directly phosphorylates Gli1 in vitro and in vivo with the majority of the phosphorylation occurring in the zinc finger DNA binding region of Gli1. We performed a mutagenesis screen of the DNA binding domain to determine the site of aPKC phosphorylation and found that two residues in the DNA binding domain appear to mediate aPKC effects. Phospho-mimetic Gli1 bound to target DNA just as well as wild-type phosphorylated Gli1 and possesses reduced sensitivity to PSI in BCC cells, suggesting these sites are functional aPKC sites. Flag:Gli1 ChIP in BCC cells show that PSI-treated tumor cells left Gli1 nuclear protein levels unchanged but reduced the association with chromatin. Application of a topical aPKC inhibitor suppresses Hh signaling and tumor growth in naïve primary murine BCC tumors, suggesting BCCs are dependent on aPKC pathway activation. Consistent with this notion, we demonstrate that patients harboring Smo antagonist-resistant BCCs overexpress active aPKC and pharmacological inhibition of aPKC in drug-resistant BCC cells suppresses proliferation. These results demonstrate aPKC pathway activation is critical for Hh-dependent processes and suggest aPKC may be a new therapeutic target for the treatment of naïve and resistant BCCs.

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MicroRNA-9 causes expansion of cancer stem cells in skin squamous cell carcinoma metastasis

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Epidermal stem cells of the hair follicle bulge are capable of tumor initiation but an unresolved question is if they retain tumorigenic pluripotency and contribute to metastasis. We show that by targeting mouse K15+ epidermal stem cells with $Kras^{G12D}$ and Smad4 mutations, two frequent mutations in human squamous cell carcinoma (SCC), resulted in multi-lineage tumors including metastatic SCC. In this mouse model, SCC metastasis was associated with increased microRNA-9 (miR-9) expression that correlated with the expansion of side-population (SP+) but not SP-/CD34+/CD49+ cancer stem cells. Transplanting SCC cells overexpressing miR-9 *in vivo* resulted in SCC lung metastases in mice. Conversely, knockdown of miR-9 inhibited metastasis *in vivo*. We found that miR-9 targeted the adherence junction protein α-catenin, facilitating β-catenin nuclear translocation as well as β-catenin-mediated expression of ATP binding cassette (ABC) transporters (a molecular determinant of the SP+ phenotype). Inhibition of miR9 or β-catenin abrogated both murine ABC transporter gene expression and multidrug resistance protein 1 (MDR1) (a protein encoded by an ABC transporter gene). In a human SCC tissue array, we detected miR-9 in metastatic SCC lesions but rarely in primary SCC. Additionally, in human SCC, miR-9 expression correlated with loss of membrane α-catenin as well as increased expression of MDR1. Our data reveal that the combination of $Kras^{G12D}$ mutation and Smad4 deletion converts K15+ bulge stem cells to cancer stem cells promoting SCC metastasis by a mechanism involving miR9.

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Identification of a distinct microRNAome and tumor suppressive role of microRNA-125b in cutaneous squamous cell carcinoma

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MicroRNAs (miRNA) are ~22 nt single-stranded noncoding RNAs, which play important roles in gene regulation. The role of miRNAs in skin cancers is not well understood. To identify tumor suppressor and oncogenic miRNAs involved in the pathogenesis of skin cancers, we performed miRNA expression profiling in two most common skin cancers, squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), as well as healthy skin by TaqMan MicroRNA Low Density Array. We found that the miRNAome of SCC and BCC differs from that of healthy skin and from each other. In SCC, 4 miRNAs were found to be up-regulated compared with healthy skin (miR-31, miR-135b, miR-21 and miR-223) and 54 miRNAs were down-regulated, such as miR-375, miR-125a/b family, let-7a/b/c/d/f/g family, miR-99a/b/100 family, many of which have been implicated in various cancers. Moreover, we found that SCC and BCC display distinct miRNA profiles: 15 up-regulated and 10 down-regulated miRNAs were identified in SCC compared with BCC. MiR-125b is one of the top down-regulated miRNAs in SCC compared with healthy skin or BCC; therefore, its function was further studied in SCC. We demonstrated that overexpression of miR-125b suppresses proliferation, migration and invasion capacity of human SCC cells. Moreover, we identified matrix metallopeptidase 13 (MMP13) as a direct target gene suppressed by miR-125b. Knockdown of MMP13 expression in SCC cells phenocopied the effects of miR-125b over-expression. Together our data indicate that miRNAs are deregulated in SCC and BCC, and these tumors have disctinct miRNAomes. Moreover, our study identified miR-125b as a potential tumor suppressor in SCC.

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Stat1-signaling in cancer cells critically controls senescence and metastases spreading

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Immunotherapy with tumor-specific Th1 cells reduces tumor burden in humans with melanoma and in mice with neuroendocrine cancers. In RIP1-Tag2 (RT2) mice, expression of oncogenic large T antigen 2 of the Simian virus 40 under the rat insulin promoter 1 leads to the development of neuroendocrine cancers. Previous data showed that cancer control by Th1 cells strictly depends on interferon-γ (IFNγ), as in the presence of anti-IFNγ mAb, Th1 cells paradoxically promote multistage carcinogenesis. To investigate the mechanisms underlying IFNγ-mediated cancer control, we generated RT2 mice lacking Stat1, the signal transducers and activator of transcription of IFNs. While Th1 cells were capable of doubling life span of RT2 mice, Th1 cells failed to prolong the survival of RT2xStat1.ko double transgenic mice. Histology revealed that RT2xStat1.ko mice developed the same number of islet carcinomas, and the blood glucose decreased as rapidly as compared with RT2 control mice. Importantly, RT2 mice never develop metastases (<1%). In sharp contrast, 50% of RT2xStat1.ko mice developed metastasis into the mesenteric lymph nodes within 12 weeks. As the data suggested that STAT1 deficient cancer cells have an increased potential to metastasize, we generated β-cancer cells generated from RT2 and RT2xStat1.ko mice and injected the cancer into NOD-SCIDxIL2Rcγ.ko mice. Such RT2 cancer cells grew rapidly and increased the serum insulin levels. Importantly, senescent RT2 cancer cells from STAT1-competent mice failed to grow when injected into NOD-SCIDxIL2Rcγ.ko mice. Whereas, RT2xStat1.ko cancer cells did not undergo senescence, neither upon *in vivo* treatment with Th1 cells, nor after *in vitro* treatment with IFNγ and TNF, and grew rapidly upon transfer into immune deficient hosts. Thus, intact IFNγ and STAT1 signaling is a key event required for tumor growth arrest and the control of metastatic spreading of cancer.

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Role of survivin in the development and progression of squamous cell carcinoma: *In vivo* models

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Survivin is a bifunctional protein, as it inhibits apoptosis and regulates the cell cycle. Survivin is overexpressed in most tumors, while it identifies keratinocyte stem cells (KSC) in humans. Because epithelial tumors originate from KSC, we wanted to evaluate the role of survivin in the development and progression of squamous cell carcinoma (SCC). We first showed that survivin is highly expressed in poorly differentiated SCC, and survivin overexpressing keratinocytes display a more pronounced ability to migrate. Moreover, survivin silencing in SCC keratinocytes results in a decreased proliferation and reduced ability to form colonies. To test whether survivin is involved in SCC development, we separated a subpopulation of neoplastic keratinocytes based on their ability to adhere to a type IV collagen through b1-integrin. When seeded in a silicone chamber grafted onto the back of NOD SCID mice, rapidly adhering (RAD) cells, expressing high levels of survivin, formed tumor 2-4 fold bigger than those derived from NON RAD cells. To better clarify if survivin is associated with tumor initiation, we induced carcinogenesis in SCID mice by injecting human keratinocytes retrovirally transduced with oncogenic HRas G12V and IkB-alpha, in the presence or absence of survivin. First, HRas/IkB-alpha overexpressing keratinocytes proliferate and migrate to a greater extent, as compared to HRas/IkB-alpha keratinocytes in which survivin is silenced, in vitro. In SCID mice, tumors derived from HRas G12V and IkB-alpha overexpressing keratinocytes were significantly more aggressive than tumors where survivin was silenced (mitotic index: 10,2 +/- 2,28 vs. 5,4 +/- 1,81). In these tumors, vascular endothelial growth factor and hypoxia-inducible factor-1 alpha were overexpressed. On the other hand, these markers were reduced when survivin was silenced. All in all, survivin silencing induced a less aggressive pathologic phenotype. The study suggests that survivin plays a crucial role in the development and invasiveness of SCC.

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Tumor cell associated receptor tyrosine kinase, EphB2 modulates gene expression signatures involved in migration and invasion of tumor cells

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Cutaneous SCC is the second most common cutaneous malignancy in white population. We have studied the role of Eph receptors, the largest family of receptor tyrosine kinases in skin SCC. Specific upregulation of EphB2 was noted in SCC cell lines (n=8) and tumors (n=6) compared with normal human epidermal keratinocytes (n=5) and normal skin (n=7) using Affymetrix-based expression profiling, SOLiD™ whole transcriptome analysis, quantitative RT-PCR, Western blotting and immunofluorescence staining. Immunohistochemistry revealed tumor cell-specific overexpression of EphB2 in Bowen's disease (n=56) and cutaneous SCC (n=68) compared with actinic keratoses (n=69) and normal skin (n=12) ($p<0.001$). Moreover, upregulation of EphB2 expression was noted in DMBA-TPA-induced mouse skin SCCs (n=19) compared with normal skin (n=13) ($p<0.001$). EphB2 knockdown with specific siRNA showed inhibition of proliferation, migration and invasion of skin SCC cells. Microarray analysis identified 2460 differentially expressed genes ($P<0.05$) in SCC cell lines (n=3) following EphB2 knockdown. Gene expression profile after EphB2 knockdown was subjected to Ingenuity Pathway Analysis (FC (log2) >0.75 and $P<0.05$). Over 11% of downregulated genes belonged to peptidases classification. *Invasion of tumor cells* (z-score= -2.099, $p<0.001$) and *migration of tumor cells* (z-score= -2.358, $p<0.001$) were among the top biofunctions significantly decreased after EphB2 knockdown. Together these findings highlight the role of EphB2 in progression of cutaneous SCC and particularly in invasion and migration of SCC cells, suggesting it as a therapeutic target in these invasive and metastatic tumors.

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WITHDRAWN

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Interventions for cutaneous Bowen's disease: A systematic review

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The relative effectiveness of the available treatments for cutaneous Bowen's disease is not known. A systematic review was undertaken to assess which is the most effective therapeutic intervention for cutaneous Bowen's disease with the least side effects. We searched the following databases up to September 2012: the Cochrane Skin Group Specialised Register, CENTRAL, MEDLINE, EMBASE and LILACS including online trials registers. Inclusion criteria were all RCTs assessing interventions used in Bowen's disease, preferably histologically proven. Primary outcome measures were complete clearance of lesions after the first treatment cycle and recurrence rate at 12 months. Secondary outcomes included cosmetic outcome and adverse outcomes as reported by consumer and clinician. Two authors independently carried out study selection and assessment of methodological quality. Nine studies were identified. Clearance was significantly better when methyl aminolevulinate with photodynamic therapy (MAL-PDT) was compared to placebo-PDT or cryotherapy, however there was no significant difference in clearance when compared to 5-fluorouracil (5-FU). One study found significantly greater clearance rates (RR 20.19, 95% CI, 1.29 - 319.17) in favour of imiquimod when compared to placebo. No recurrences were reported in the imiquimod group but at 18 months, 2/16 participants in the placebo group had developed early invasive squamous cell carcinoma. Overall, there has been little good quality research on treatments for Bowen's disease. The lack of quality data for surgery and topical therapies has limited the scope largely to a review of PDT studies; 5-FU appears equivalent in efficacy to PDT and cryotherapy whereas MAL-PDT appears to be more efficacious than cryotherapy. The age group, number and size of lesions and site(s) affected may all influence therapeutic choice.

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The rs2910164 G>C polymorphism in microRNA-146a is associated with the pathogenesis of malignant melanoma

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MicroRNA-146a (miR-146a) is one of the microRNAs (miRNAs) implicated in the pathogenesis of various cancers. Recently, single nucleotide polymorphisms (SNPs) located in miRNAs themselves, so-called MIRSNPs, have attracted attention for their possible involvement in the pathogenesis of various diseases. Such MIRSNPs may have functional roles due to the alteration of the miRNA. In this study, we investigated whether MIRSNP rs2910164 in miR-146a is involved in the pathogenesis of malignant melanoma (MM). DNA samples were collected from 50 patients with MM and 107 controls and genotyped by polymerase chain reaction (PCR)-restriction fragment. In patients with MM, the genotype distributions were 15 CC (30.0%), 35 CG (70.0%) and 0 GG (0.0%). The CG genotype was significantly increased in patient compared with controls ($p=0.02$). The minimum free energy between miR-146a and its complementary strand with the G allele was estimated to be -26.8 kcal/mol, while that of C allele was -24.0 kcal/mol, suggesting that the change from C to G may increase the stability of the miR-146a. However, there was no significant difference between the CC and CG genotypes in terms of the relative expression levels of miR-146a. Human melanoma cell lines with the G allele showed significantly higher proliferation, migration and invasion than those with the C allele. In conclusion, miR-146a may be involved in the pathogenesis of MM, and individuals with the CG genotype have an increased risk of developing MM.

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RSK activation of translation factor eIF4B drives deregulated Laminin γ2 expression during epithelial neoplastic progression

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The basement membrane protein Laminin-332 is essential for normal basal epithelial cell adhesion and also plays a key role in migration and hypermotility. Abnormal expression of its γ2 subunit (Lamγ2) is detectable immunohistochemically in premalignant epidermal and oral dysplasias and invasive squamous cell carcinomas (SCC). We recently we reported that deregulated Lamγ2 expression results from EGFR/MAPK signal pathway hyperactivity. To fully elucidate the molecular mechanism of abnormal Lamγ2 expression, here we have examined archival pathology specimens of oral dysplasia immunohistochemically and have compared normal primary human oral and epidermal keratinocytes with cell lines initiated from premalignant dysplasias and SCCs in culture. We immunostained dysplasias for molecular biomarkers that identify and distinguish between MAPK and mTOR pathway activation states, finding that both become hyperactive at the same time during epithelial neoplastic progression and that Lamγ2 overexpression occurs in a subset of cells within regions of signal pathway hyperactivity. Pharmacological kinase inhibitors revealed that, in cultured cells, expression of Lamγ2, as well as MYC, are limited only by MAPK/RSK-dependent activation of the translation factor eIF4B, independent of PI3K/mTOR pathway kinases and their translation factor targets. Lentiviral-mediated eIF4B shRNA knockdown in SCC cells reduced expression of both Lamγ2 and MYC, consistent with the known function of eIF4B in initiating translation of mRNAs having long, complex 5′ UTR sequences. In reporter assays, translation of a luciferase mRNA containing the Lamγ2 5′UTR was RSK- and eIF4B-sensitive. Our data demonstrate that MAPK-dependent RSK activation of eIF4B, leading to increased Lamγ2 translation, are early events during neoplastic progression to SCC. These results have potential application as prognostic biomarkers of preinvasive stages of SCC and as targets for early intervention therapy.

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The expression of B-defensins, cathelicidin (LL-37) in skin tumors

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Antimicrobial peptides (AMPs), which is a kind of innate immune system, are produced by keratinocytes during differentiation. Among them, B-defensins and LL-37 are the major human antimicrobial peptides. Because B-defensins and LL-37 are critically involved keratinocyte migration and proliferation, we hypothesized that both peptides also have an influence on differentiation and proliferation of skin tumors originated from keratinocytes. We studied the expressions of AMPs by tissue array techniques in benign and malignant skin tumors. The expression of AMPs was examined by immunohistochemical staining of 31 specimens of skin tumors including 4 cases of seborrheic keratosis, 4 cases of keratoacanthomas, 4 cases of basal cell carcinomas (BCC), 16 cases of squamous cell carcinomas (SCC) and 3 cases of malignant melanomas (MM). Immunohistochemical analysis of the skin tumor samples revealed a high expression of B-defensin and cathelicidin (LL-37) in benign skin tumors such as keratoacanthomas and seborrheic keratosis than in malignant tumors such as SCCs, BCCs and MMs. Among malignant tumors, BCCs and MMs showed lower expression fo AMPs than SCCs, in which their expression was shown to be decreased with increased dysplasia of tumor cells. These findings showed that AMPs have a negative association with the malignancies of the skin tumors and suggested the role of AMPs in the protection of loss of differentiation and dysplasia of skin tumors.

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The specific expression of PDGF-B and CD34 in dermatofibrosarcoma protuberans implies the underlying pathogenesis with involvement of its mesenchymal stem cell origin

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Dermatofibrosarcoma protuberans (DFSP) is an intermediate grade malignancy of subcutis with frequent local recurrences. The most characteristic cytogenetic feature of DFSP is the chromosomal translocation, causing a fusion of the platelet derived growth factor beta (PDGF-B) and collagen type 1 (COL1) genes. We previously reported a novel gene fusion between PDGF-B and COL1A2 besides the known COL1A1 gene, and our discovery reaffirmed the essential role of PDGF-B expression in DFSP pathogenesis. DFSP, as well as dermatofibroma, is dermal mesenchymal tumor and the expression of hematopoietic stem cell marker; CD34 in DFSP is reliable diagnostic clue to distinguish them. The DFSP tumor cells are thus proposed to originate from one of mesenchymal stem cells (MSC). PDGF-B, a pathogenic fusion protein in DFSP, acts as a ligand for PDGF-Rα at MSC, and plays an important role in recruitment of MSC. To ascertain the origin of DFSP cells as MSC lineage, we studied 23 DFSP and 20 dermatofibroma samples for their expression of PDGF-B as well as various stem cell or differentiation markers including CD34, PDGF-Rα and, α-SMA and S-100. CD34 was expressed in 96.3% of DFSP cases, but also expressed in only 10% of dermatofibroma. Consistently, we found that 92.6% DFSP tumors expressed PDGF-B, whereas dermatofibroma did not express PDGF-B at all, and the specificity of PDGF-B for DFSP was much higher than CD34 among various mesenchymal tumors. Meanwhile, DFSP showed lesser immunoreactivity to α-SMA and S-100 than dermatofibroma. PDGF-Rβ was cooperatively expressed in all DFSP cases with different degree, which suggested the autocrine or paracrine PDGF-B stimulations might cause the PDGF-Rβ internalization. Our results suggest that DFSP tumor cells exhibit a distinct feature similar to MSC, and to lesser extent for cell differentiation as compared with dermatofibroma.

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Diagnostic markers for Sézary syndrome

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Sézary syndrome (SS) is an aggressive type of cutaneous T-cell lymphoma with a poor prognosis. Differentiation between SS and other conditions presenting with erythroderma, including benign inflammatory dermatoses, may be extremely difficult. Also identification and quantification of tumor cells is often difficult, thereby hampering (early) diagnosis and monitoring of progression. Previous studies reported several biomarkers for Sézary cells but these have not been evaluated in independent studies. In this prospective, multicenter study we evaluate the diagnostic and prognostic value of these potential markers that were previously described in literature. We collected peripheral blood mononuclear cells, cDNA and genomic DNA from SS patients (as defined by WHO criteria, 2008) and patients with benign erythroderma. CD4+ T cells were analyzed for expression of cell surface proteins by flow cytometry. Gene expression and copy number alterations were evaluated by using custom made quantitive PCR platforms. Experimental data are correlated with clinical information at inclusion of the study and after 12, 24, 36, and 48 months follow up. During the first two years of this study, samples from 105 individuals were included and clinical data from 86 patients have been collected. Characteristic alterations in copy number for MYC and MNT were observed in respectively 44% and 65% of the SS patients. Flow cytometry revealed aberrant expression of CD2, CD7, CD26, CD158b and/or CD45RA in >90% of patients. In addition, upregulation of DNM3, EPHA4, PLS3 and TWIST1 and downreguation of STAT4 in 65 to 95% of SS patietns was confirmed by RT-qPCR. Our data demonstrate that Sézary cells can be identified by (combinations of) specific biomarkers using different techniques. The prognostic significance of those markers is currently under investigation with the sample data of all included patients.

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Protein kinase D1 plays a unique role in enhancing tumor promotion in chemically-induced skin carcinogenesis

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Protein kinase D (PKD) isoforms are novel diacylglycerol-regulated serine/threonine protein kinases that can be activated by a multitude of stimuli and are implicated in the regulation of diverse cellular functions. The role of PKDs in skin however, is poorly understood. We and other have previously shown that treatment of keratinocytes with a tumor promoter, 12-Otetradecanoyl phorbol-13-acetate (TPA), induced a rapid phosphorylation/ activation of PKD1 in a protein kinase C (PKC)-dependent manner. However, it is not known how PKD1 contributes to TPA-induced responses including tumor promotion. To investigate the role of PKD1 in normal and stress-induced epidermis, we have used a conditional gene knockout system to specifically delete PKD1 in mouse epidermis (cKO-PKD1). These mice exhibited no abnormalities in epidermal growth and differentiation in vivo, although the proliferation rate of PKD1-null keratinocytes in culture was significantly increased. A single topical application of PKD1-null epidermis with TPA resulted in a significantly reduced epidermal hyperplasia and inflammation when compared to control PKD1-floxed littermates. Moreover, the cKO-PKD1 mice exhibited resistance to tumor formation in two-stage chemically-induced skin carcinogenesis. The resistant to tumor formation was consistent with the suppressed effects of tumor promoter, not a consequence of a general impairment in proliferative responses of PKD1-null keratinocytes . Interestingly, despite higher expression of PKD2 and PKD3 in keratinocytes, they failed to play a compensatory role for PKD1 during TPA induced hyperplasia/ tumor promotion. These data demonstrated a unique role for PKD1 as a critical mediator of PKC signaling in carcinogenesis, and suggests a potential target for skin cancer therapeutics.

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The E3 ubiquitin ligase Trim32 has a functional role in the development and progression of cutaneous squamous cell carcinoma

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Cutaneous squamous cell carcinoma (cSCC) is the second most common type of cancer in the United States with over half a million new cases each year. Although SCC is usually detected at an early stage, surgical excision often causes morbidity, and the presence of metastases at diagnosis is associated with a poor five-year survival. We detected overexpression of the E3 ubiquitin ligase Tripartite Motif protein 32 (Trim32) in a clonal mouse model of cSCC and in human cSCC patient samples. TRIM family scaffold proteins have been associated with cancer, innate immunity, and inborn genetic disorders affecting muscle and other organ sites, suggesting roles in multiple signaling pathways. We have previously shown that Trim32 binds and ubiquitylates the E3 SUMO ligase Piasy, leading to Piasy's degradation via the proteasomal pathway, reversal of Piasy's inhibition of the transcription factor NF-κB, and an increase in epidermal cell survival in response to genotoxic and cytokine stress. Similarly, others have demonstrated that Trim32 can promote cell growth and migration by facilitating the degradation of Abl-interactor 2 (Abi2), a putative tumor suppressor. Together, these studies suggest that Trim32 promotes the development of cancer *in vitro* by down-regulating tumor suppressors. In order to determine if deficiencies in the expression of Trim32 altered tumor development *in vivo*, we performed a two stage chemical carcinogenesis experiment and compared $Trim32^{+/-}$ and $Trim32^{-/-}$ mice to their wild type littermates. We observed a delay in tumor formation and a decrease in tumor multiplicity in the $Trim32^{+/-}$ and $Trim32^{-/-}$ mice. Total tumor burden was also decreased in the $Trim32^{+/-}$ and $Trim32^{-/-}$ mice. These results indicate that ablation of Trim32 inhibits tumor growth and suggests an oncogenic role for Trim32. Investigations are underway to determine whether regulation of oncogenic/tumor suppressor pathways and/or innate immunity pathways underlie Trim32's ability to enhance tumor growth.

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Potential involvement of mitochondrial DNA damage elicited by oxidative stress in arsenic carcinogenesis in skin

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Arsenic causes several human cancers. Arsenic-induced Bowen's disease (As-BD), the most common arsenical cancers, is characterized by increased proliferation,full-layer epidermal dysplasia, and individual cell apoptosis, all of which involve mitochondria. We reported arsenic causes aberrant keratinocyte proliferation via mtTFA-mediated mitochondrial biogenesis in As-BD. Increasing mitochondrial biogenesis render cells undergo oxidative stresses. However, how arsenic induces oxidative stress and causes mitochondrial DNA (mtDNA) damage in arsenical cancers remains largely unknown. Using tissues from As-BD patients and arsenic-treated keratinocytes, we determined the oxidative stress, antioxidant enzymes, DNA repair enzymes, and 8-OHdG level in mtDNA by immunofluorescence, real-time PCR, and Western blot. The results showed that oxidative stress was enhanced in both As-BD and arsenic-treated keratinocytes. Antioxidant enzymes including Mn-SOD and Cu/Zn-SOD and DNA repair enzymes were upregulated concomitantly in tissues and cells. In arsenic-treated keratinocytes, increased mitochondrial oxidative stress and the 8-OHdG level in mtDNA was attenuated by pretreatment with ascorbic acid, a potent antioxidant. Further, we found several consistent somatic mutations in the ND4, ND5, and ND6 genes of mtDNA in lesional but not in perilesional skin from As-BD patients. The results suggest oxidative damage and mutations to mitochondria might be involved in progression of carcinogenesis in arsenical cancers in the context of mitochondrial biogenesis.

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Periostin expression induced by wound healing process promotes metastasis of melanoma cells

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Tissue injury has been reported to stimulate metastasis in a number of cancers. However, it remains unknown how wound healing process induces metastasis. Recently, periostin (POSTN), an extracellular matrix (ECM) protein, which facilitates the re-epithelialization during cutaneous wound healing, has been demonstrated to initiate the colonization of cancer cells. Therefore, we aimed to investigate if ECMs associated with the wound healing promote the metastasis of melanoma to the wound. We experienced a patient with melanoma on the right sole and metastatic lesion on the right heel that appeared one month after trauma. Comparative genome-wide microarray analysis of the patient revealed that mRNA of ECM proteins relevant to wound healing, such as POSTN, fibronectin 1 (FN1) and collagen-I (COL-I) were expressed over 4-fold in metastatic lesion compared with those in the primary lesion. Using human melanoma cell line MeWo and murine melanoma cell line B16BL6, we performed adhesion assay with those ECMs. Compared with FN and COL-I, melanoma cells on POSTN became more rounded shape and significantly attenuated adhesion, suggesting that POSTN reduces the cell binding affinities. Next, cellular motility was evaluated by transwell migration assay. We found that POSTN promotes cell migration significantly higher than FN or COL-1. To examine whether wound healing predisposes to metastasis *in vivo*, we injected B16BL6 cells into footpad of nude mouse and created full-thickness excisional punch wounds on the thigh 3 days after the inoculation. Whereas 4 out of 6 mice with wounds developed metastasis on the wound region in 3 weeks after injection, no metastasis was detected in all 6 mice without wound. Immunohistochemistry revealed POSTN expression was higher in the wounded skin at day 4, 7, and 10 than the intact skin. These data suggest that POSTN induced by tissue injury is involved in promotion of melanoma metastasis to the wound.

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Dissecting T-helper-cell-mediated anti-angiogenesis from T cell-induced cancer cell senescence

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Anti-tumoral effects of T helper 1 lymphocytes critically depend on the simultaneous signaling of two cytokines: interferon-gamma (IFN-γ) and tumor-necrosis-factor (TNF). In RIP1-Tag2 mice, adoptive transfer of tumor-specific Th1 cells prevent multistage carcinogenesis, significantly reduce tumor size, tumor cell proliferation and doubles survival. This T cell-induced tumor dormancy in beta-cell cancers is associated with a pronounced reduction of blood vessel density within the solid Langerhans islet tumors and a strong induction of anti-angiogenic chmokines CXCL9 and CXCL10. Inhibiting IFN-γ by the monoclonal antibody XMG1.2 in vivo abrogates the expression of the anti-angiogenic chemokines. Mice treated with Th1 cells even develop significantly more vessels than sham-treated mice. Besides the pronounced anti-angiogenic effect, the combined action of IFN-γ and TNF also arrest cancer growth by inducing senescence through the upregulation of the tumor suppressor protein p16Ink4a. This raised the question whether Th1 cells arrested cancer growth primarily by reducing angiogenesis. To dissect the anti-angiogenic effects from the senescence-inducing effects we isolated cancer cells from the pancreas and downregulated p16INK4a by short hairpin RNA (shRNA). Following the treatment with IFN- γ and TNF we analyzed senescence in β-cancer cells and quantified CXCL9 and CXCL10 proteins in the supernatant. p16ink4a shRNA completely blocked the capacity of IFN-γ and TNF to induce senescence while it did not abrogate the capacity of beta cancer cells to produce high amounts of CXCL9 and CXCL10. Importantly, following transplantation into immunocompromized NOD-SCID mice, senescent cancer cells remained growth arrested even in the absence of anti-angiogenic chemokines while β-cancer cells showed rapid metastatic growth.

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The development of cutaneous squamous lesions in patients treated with vemurafenib: A possible role for human papillomavirus in addition to activated RAS

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Approximately half of metastatic melanomas have an activating mutation in the BRAF oncogene. The BRAF inhibitor vemurafenib is associated with the de novo development of benign and malignant squamoproliferative lesions in up to 25% of patients. The putative mechanism involves paradoxical increased MAPK signalling by BRAF inhibitors in the context of mutated or activated RAS. However, cutaneous SCC (cSCC) have been reported to develop in association with wart-like lesions and upregulation of the MAPK pathway is known to facilitate human papillomavirus (HPV) replication, suggesting a possible role for HPV in the pathogenesis of these lesions. We have examined RAS mutational status and presence of HPV DNA in 30 skin biopsies from 4 patients receiving vemurafenib (20 benign squamoproliferative lesions; 7 SCC; 3 normal skin samples). A high proportion (90%; 27 /30) were positive for beta (EV-associated) HPV; a subset (15%; 4/27) were also positive for cutaneous alpha HPV. HPV DNA was detected at high levels in only a minority (19%; 5/27) of positive lesions, all of which were identified clinicopathologically as viral warts. HRAS mutations were detected in 8/20 (40%) samples (6/16 benign squamoproliferative lesions and 2/3 cSCC). No mutations were identified in the NRAS or KRAS genes. Five of 8 (63%) HRAS mutations were in codon 61, a mutational hotspot previously reported as characteristic of vemurafenib-associated cSCC. The high frequency of HRAS mutations in our sample series together with the rapid timeframe of lesion development support the hypothesis that these mutations are pre-existing and confer a selective advantage in the context of vemurafenib therapy. The role of HPV in these lesions merits further investigation.

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ABCB5 identifies a chemorefractory cell population in Merkel cell carcinoma

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Merkel cell carcinoma (MCC) is a rare and highly aggressive cutaneous neuroendocrine carcinoma that, on a case-by-case basis, is more deadly than melanoma. Responses to systemic therapy are often not durable, and patients experience disease relapse, usually with fatal outcomes. Thus, elucidating the mechanisms of MCC therapeutic resistance is critical for improving patient survival. Here we show that ATP–binding cassette member B5 (ABCB5) identifies a subset of MCC cells that preferentially resists first-line chemotherapy with carboplatin and etoposide. PCR analysis revealed ABCB5 mRNA expression in established MCC lines, MKL-1, MKL-2, MS-1 and WaGa, as well as in 83 of 87 clinical MCC specimens. ABCB5 protein expression ranged from 1-15% of cells in MCC lines and was also detected in 63 of 72 MCC patient samples. As in other cancers, ABCB5(+) MCC cells exhibited preferential positivity for additional cancer stem cell antigens, including CD271. In vitro treatment of MCC cells with the standard-of-care agents carboplatin or etoposide resulted in >1500- and >100-fold upregulation of ABCB5 expression among surviving cells, respectively. Compared to vehicle-controls, treatment of MCC xenograft-bearing mice with carboplatin or etoposide resulted in markedly increased ratios of ABCB5(+) vs. ABCB5(-) cells when residual disease was detected. Consistent with these findings, analysis of successive, patient-matched MCC biopsies revealed enhanced frequencies of ABCB5(+) MCC cells after treatment with carboplatin and etoposide. Mechanistically, ABCB5 blockade sensitized MCC cells to carboplatin and etoposide-induced killing. Our results establish ABCB5 as a novel chemoresistance mechanism in MCC and identify a potential therapeutic target and biomarker for monitoring treatment responsiveness in this malignancy.

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Potential driver genes in the development and progression of cutaneous T-cell lymphoma

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Mycosis fungoides (MF) and Sézary syndrome (SS), two most common forms of Cutaneous T-cell lymphoma (CTCL), are still mostly incurable. Limited knowledge of tumorigenesis prevents us from developing curable therapies. To identify driver genes in the development and progression of CTCL, we used the Human Cancer Pathway Finder PCR Array. Representative (T1-T4) stage skin lesions were biopsied from 26 newly diagnosed MF/SS patients. T stages were according to the ISCL/EORTC revised criteria. Normal skin (HD) and psoriatic skin (PS) lesions were controls. Total RNA was extracted from the paraffin embedded tissue sections, and cDNAs were synthesized using the RT^2 PreAMP cDNA Synthesis Kit. The Human Cancer Pathway PCR was assayed to profile the expression of 84 genes across 6 biological pathways involved in cancer transformation and tumorigenesis. The gene changes were considered significant with > 2-fold change and a p-value of <0.05. There were 28 genes dysregulated in MF/SS lesions including 6 genes in angiogenesis, 7 genes in invasion & metastasis, 4 genes in cell cycle control and DNA damage repair, 6 in apoptosis and cell senescence, 2 in signal transduction and transcription, and 3 in adhesion pathway. All were up-regulated except SERPINB5 or Maspin, a tumor suppressor gene lost in breast cancer cells, never before implicated in MF/SS. Fourteen genes were up-regulated only in T1 lesions, and 10 genes only in T3 tumors. Expression in 4 genes was abnormal in both T1 and T3 lesions including, SERPINB5, JUN, ITGA3, and TNFRSF10B. Further real-time-PCR analysis for SERPINB5 in all MF/SS lesions confirmed a dramatic down-regulation in late stage lesions (T3, T4) compared to early stage (T1, T2) and controls (HD, PS). The overexpression of Twist1 was seen in T3 lesions ($p<0.01$) confirming our previous work by IHC in MF/SS lesions (*Goswami et al, JCP, 2012*). Our preliminary study identified unique cancer pathway gene sets in early or/and late stage lesions. Further validation of gene expression and their relevance to tumorigenesis of CTCL is in progress.

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Status of autophagy and mTOR associate with mortality and cell survival of angiosarcoma

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Angiosarcomas(AS) are rare sarcomas of endothelial cell origin with a poor prognosis. Recent use of sorafenib and pazopanib, broad-spectrum tyrosine-kinase inhibitors targeting VEGF receptors, have limited efficacy. Autophagy is a homeostatic "self-eating" process, which is regulated through mTOR and digests cytoplasmic components via the lysosomal pathway to render cells nutrient for stress or starvation including lack of growth factor receptor signaling. We hypothesized that the autophagy system is involved in AS cell survival and aimed to examine the status of autophagy and mTOR. Compared with benign hemangioma (4 cases), AS (9 cases) exhibited more intensive expression of phospho-p70 S6 Kinase (p-p70S6K) (9/9) suggesting mTOR signal activation and increase in autophagy-related molecules including microtubule-associated proteins 1A/1B light chain 3 (LC3) (9/9) and ATG12 (9/9). Although no significant difference in lysosome-associated membrane protein (LAMP)-1 and LAMP-2 amounts between hemangioma and AS was observed, we confirmed the co-localization of LC3 and LAMP-2 in AS but not hemangioma, suggesting the autophagy system is constitutively activated in AS tissue. The cases with high p-p70S6K and weak expressions of LC3 before treatment had higher mortality rate, and the expression of these molecules altered after chemotherapy. Using mouse AS endothelial cell lines A221a and MS1, immunoblotting demonstrated increase in p-p70S6K, LC3, and ATG5-ATG12 conjugates under serum starvation *in vitro*. 0.03 to 0.3 μM of mTOR inhibitor pemetrexed and 1 to 10 mM of autophagy inhibitor 3-MA dose-dependently induced apoptosis measured as caspase-3/7 activity to both AS cell line. In contrast, sorafenib showed little effect for survival of both cells, and combination with 3-MA and pemetrexed efficiently induced apoptosis of both cells treated with sorafenib. These suggested that mTOR and autophagy system dictate AS cell survival and mortality of AS individuals and that control of mTOR and autophagy system is a key for the successful treatment of AS with tyrosine kinase inhibitors.

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Acantholysis is the poor prognostic factor in squamous cell carcinoma, which is predictable by the anomalies of cytoskeletal-adhesion molecules

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Squamous cell carcinoma of skin (SCC) shows the wide spectrum of histological characteristics, including the highly keratinized, adenoid, acantholytic or mesenchymal variants even in a same tumor. SCCs usually take rather non-progressive clinical prognosis after surgical excision, however, in some contents, SCC lesions can progress and metastasize rapidly. We analyzed the various characteristics including the histological subtypes and clinical prognosis of the independent 81 SCC cases treated at 2005-08. 26 SCC cases showed acantholytic changes, and 5 cases developed metastasis. Three cases with metastasis and 3 cases with the repeated recurrence harbored the acantholytic changes; meanwhile only 2 cases did metastasize among 55 SCCs without acantholysis. The acantholysis is thus considered as the potent poor prognostic factor, and we further analyzed the building of cytoskeleton and adhesion molecules. Keratin 8 (K8) or K18 were ectopically expressed in 5 SCC cases with acantholysis, in contrast no cases in non-acantholytic SCCs. Besides, vimentin were expressed in 3 cases with acantholysis, whereas no cases in other SCCs, and the expression of vimentin and K8/18 tends to overlap in each tumor cells. Desmoplakin is the other marker of acantholysis, and locates at the cell periphery in non-acantholytic SCCs with two exceptional cases. In contrast, in the examined 11 SCC cases with acantholysis, 8 cases showed the cytoplasmic location of desmoplakin. Acantholysis in SCCs seems to be caused by the several distinct pathogenesis, such as by the epithelial mesenchymal transformation in K18 and vimentin positive SCC or by the debilitated desmosome adhesion in K16 positive SCC. Finding out the cellular markers that involves in the acantholytic phenotypes and thus leads to poor prognosis, enables the more adequate therapeutic strategy.

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Cell surface CD74 expression is a key mediator of melanoma cell survival in response to the cytotoxic effect of IFN-γ

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Intensive analysis of the molecular pathogenesis revealed that tumor cell specific BRAF, NRAS and c-KIT gene mutations activate the ERK1/2 MAPK and AKT signaling pathways to control growth and survival of human melanoma. However, new data indicates that additional drivers in the microenvironment are bypassing these specific mutations. Here, we hypothesize that certain molecules on the tumor cells are likely to respond to local inflammatory cytokines and directly regulate these growth and survival pathways. In this study, we identified cell surface CD74 as of one such molecules, which is upregulated in response to interferon-γ (IFN-γ). Immunohistochemical (IHC) analysis of melanocytic tumor samples in a tissue microarray revealed that expression of CD74 serve as a progression marker of melanocytic tumors. In the analysis of six melanoma cell lines with different subtypes representing the spectrum of currently targeted somatic mutations, five cells showed CD74 cell surface expression and all cells expressed its ligand, macrophage migration inhibitory factor (MIF). The MIF-CD74 interaction functions in cell survival manner by regulating AKT Ser473 phosphorylation, secreting interleukin-6 and -8, and expressing antiapoptotic proteins. Patient sample analysis by IHC staining of CD74 on tumor cells correlated with plasma IFN-γ levels. IFN-γ upregulated the transcription and cell surface expression of CD74 and augmented MIF-CD74 interaction promoting survival. The role of IFN-γ in cancer has been reported to be both pro-and anti-tumorigenic, and its effect depends on the tumor specificity and signaling intensity. The result of the present study provides a plausible explanation of pro-tumorigenic effects of IFN-γ via CD74 which contributes to the selection of more aggressive melanoma cell phenotypes.

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Gene expression analysis in CTCL patients validates the clinical importance of novel oncogenes and tumor suppressor genes

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Cutaneous T-cell Lymphoma (CTCL) is the most common lymphoma of the skin. Unfortunately, the molecular pathogenesis of this disease remains poorly understood. The cancer often presents in early stages and remains indolent in the majority of patients. However, in 10-20% of cases it progresses towards advanced stages, where it exhibits high morbidity and mortality. Previously, we performed a microarray and RT-PCR analyses of lesional skin from a cohort of CTCL patients in order to identify novel molecular prognostic markers. Unfortunately, this initial analysis failed to include a number of genes that were previously reported to play an important role in CTCL pathogenesis. In the current work we test by RT-PCR the expression of 160 additional putative tumor suppressor genes and oncogenes in 60 CTCL patients, 21 benign inflammatory dermatoses and 6 normal skin samples and correlate our findings with 6 years of clinical follow up in CTCL patients. These findings demonstrate that loss of a number of tumor suppressor genes (e.g., BCL7A, DLEU1 and CDKN1C) or upregulation of several oncogenes(e.g., JUNB, TOX1 and TCF3) correlates with disease progression. Furthermore, comparison of expression of inflammatory response genes between CTCL and benign inflammatory dermatoses documents that a number of genes (e.g. CCL 26, CCL18 and IL-22) were upregulated in CTCL, but not in eczema, psoriasis or pityriasis rubra pilaris. In summary, our findings combined with previous reports provide clinical confirmation for the importance of the above described putative oncogenes and tumor suppressor genes in CTCL. Furthermore, our study helps delineate inflammation observed in CTCL from other benign inflammatory responses.

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Stromal fibroblasts impaired in TGFβ signaling support oncogenic transformation of adjacent epithelia

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Skin morphogenesis, maintenance and homeostasis require tightly regulated complex epithelial-mesenchymal interactions involving primarily the keratinocyte and fibroblast, the principle cell type of epidermis and dermis layers respectively. Epithelial cancers arise when the homeostatic interaction between epithelial cells and stromal fibroblasts is perturbed, and the latter can support the development of a tumor microenvironment by providing the necessary oncogenic signals. TGFβ, which signals through the canonical Smad cascade and non-canonical TAK1 cascades, is an important cytokine that regulates tumor-stroma interactions. However, the effects of TAK1 and Smad signaling in the stromal fibroblasts on the oncogenic potential of adjacent epithelial remain unclear. We observed that the expression of TGFβRII, Smad3 and TAK1 are frequently altered in cancer-associated fibroblasts from human squamous cell carcinomas compared with normal fibroblasts. Organotypic cocultures constructed using fibroblasts-deficient in TGFβRII, Smad3 and TAK1 led to increased proliferation of the overlying epithelia. We observed that intracellular reactive oxygen species was increased in the fibroblast knockdown concomitant with increased extracellular H_2O_2. Prolonged exposure of keratinocytes to H_2O_2 was sufficient to induce colony formation in soft agar, i.e. tumor transformation. Furthermore, conditioned media from the various OTCs contained elevated levels of mitogenic factors that were differentially regulated by Smad3 and TAK1. Our study has delineated the tumor-suppressive function of stromal TGFβ signaling that includes modulating the microenvironment's redox state and may aid the identification of novel therapeutic targets to reverse or delay cancer progression.

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Mouse Tumor Biology Database (MTB): A centralized resource on mouse models for human skin and adnexal cancers

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Mouse models of human cancer are significant tools for studying the biological mechanisms and genetic predispositions of cancer and developing new methods of treatment. The scientific community is generating an increasing number of novel models and associated data from these models. The Mouse Tumor Biology Database (MTB) is a public access database that provides tools to identify, access, and analyze these data. MTB captures data on endogenously arising tumors (both spontaneous and induced) in genetically defined mice (inbred, hybrid, mutant, and genetically engineered mice) and provides freely available web access to these data (http://tumor.informatics.jax.org). Data in MTB includes tumor classification, frequency, latency, tumor associated quantitative trait loci (QTL), pathologic descriptions and images, references, genetic data, strain susceptibility data, and much more on mouse models for human cancer. MTB is integrated with the Mouse Genome Informatics database (MGI) and provides links to other related online resources. MTB contains significant amounts of dermatology related pathology data. Data are available on over 2640 skin, hair follicle, and nail neoplasias. MTB also includes immunohistochemistry data on over 410 antibodies that work or do not work on mouse tissues presented with accompanying images of positive control samples and links to the respective vendors. MTB encourages direct submission of mouse tumor data and photomicrographs from the cancer research community and has developed a web-based system to facilitate submission of pathology data.

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Low-dose of ionizing radiation impairs DNA repair system in human primary keratinocytes and human skin 3D model

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The effects of low-doses of ionizing radiation in humans are of growing concern, especially in the context of current radiation techniques such as medical imaging. The biological response of healthy tissue to low dose of 1-10 cGy in vivo is largely unknown. In this project, we propose firstly to study the effects (long and short-term) of low-doses on cell proliferation, apoptosis, and capacity to obtain a cohesive and stratified epidermis after irradiation. Secondly, we will evaluate the carcinogenesis risk by measuring the modulation of the DNA repair/damage systems after low-dose exposure. For short-term radiosensitivity, cell viability was determined by MTT assay after 24, 48 and 72 h post irradiation, we also performed an in vivo colony-forming assay, which measures the radiation toxicity after 2 weeks. DNA repair system and damage was assessed by different techniques available in our laboratory (DNA repair chips, modified comet assay …). Finally, organogenesis potential was determined by the capacity of normal exposed keratinocytes to form a pluristratified epithelium in 3D organotypic cultures. We showed that low-dose of ionizing radiation increases 2 fold the oxidative DNA damage (p=0.01) without any activation of the base excision repair pathway, an important pathway to repair oxidative DNA damage. Moreover, we showed that low-dose affects the organogenesis potential of keratinocytes and impairs the proliferation-differentiation balance in the reconstructed skin. We postulate that when the dose or dose rate is very low the radiation damage sensors (ATM or ATR) are not activated, and the repair machinery is not induced. Hence damage could be accumulated in the genome of a cell until eventually it become malignant.

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Thymocyte selection-associated high mobility group box protein TOX is a novel diagnostic and prognostic marker for mycosis fungoides and Sézary syndrome

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The objective of this study is to test the utility of TOX (an early T cell development regulator) as a diagnostic and prognostic marker for mycosis fungoides (MF) and Sézary syndrome (SS), which do not have reliable molecular markers at present. Skin biopsies were obtained from 116 individuals with MF, 25 with benign inflammatory dermatoses (BID), and 11 with normal skin (NS). Peripheral blood CD4+ T lymphocytes were prepared from 12 SS patients and 27 subjects with BID or NS. TOX mRNA was measured using quantitative polymerase chain reaction, and TOX protein was visualized using immunofluorescence (IF) microscopy. Receiver operating characteristic (ROC) was used to evaluate the diagnostic potential of TOX, whereas Kaplan-Meier method with Log rank test was used to test TOX's utility to predict MF/SS patients' risk of disease progression and mortality. MF skin biopsies expressed 11.3 times more TOX mRNA than non-MF skin biopsies (p=0.00001). Similarly, the peripheral blood CD4+ cells in SS patients contained 4.6 times more TOX mRNA than benign CD4+ T cells (p=0.000008). In IF analysis, TOX protein was specifically detected in the nucleus of CD4+ cells in MF biopsies, but not in the CD4+ T cells of non-MF biopsies. Further, in ROC analyses TOX accurately diagnosed MF subjects (with 100% specificity and 91.7% sensitivity) and SS subjects (with 100% specificity and 66.7% sensitivity). Finally, increased TOX expression strongly correlated with increased risk of disease progression for MF patients (p=0.003), and increased disease specific mortality for both MF (p=0.042) and SS patients (p=0.049). In conclusion, TOX is a novel molecular marker for the malignant CD4+ T cells in MF/SS, the most common forms of cutaneous T cell lymphoma. Not only can it be used to establish MF/SS diagnosis, it can also be used to predict long term clinical outcomes of MF/SS patients.

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Squamous cell carcinoma cell line (DJM-1) inhibits the growth of Merkel cell polyomavirus-positive Merkel cell carcinoma cell line in collagen gel matrix culture

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Merkel cell carcinoma (MCC) is a highly aggressive skin cancer linked to a contributory virus, Merkel cell polyomavirus (MCPyV). MCPyV DNA has been confirmed to be present in approximately 80% of MCCs. We rarely have cases of combined MCC and Squamous cell carcinoma (SCC), although the combined tumors account for a minor portion of MCCs. To our best knowledge, at least over 30 cases, including our cases, of combined MCC and SCC with MCPyV status of the MCC component have been reported, and those all are MCPyV-negative. Thus, we hypothesized that there are differences of the cell characteristics between MCPyV-positive and -negative MCCs in a coexistence environment with SCC. To address the effects of SCC on MCC growth and survival, we examined the growth and apoptosis of MCPyV-positive MCC cell line (MKL-1) and MCPyV-negative cell line (MCC13) in coculture with SCC cell line (DJM-1), using a three-dimensional collagen gel matrix culture with a cutaneous environmental factor, air exposure. The growth was estimated by the uptake of bromodeoxy-uridine (BrdU) for 24 h. The BrdU indices of MKL-1 cells and MCC13 cells in cell-free conditions were 6.57 % ± 1.53 % and 13.3 % ± 1.15 %, respectively, whereas the BrdU index of MKL-1 cells and MCC13 cells in the gel with overlying DJM-1 cells were 2.74 % ± 0.49 % and 11.1 % ± 1.44 %. Cell apoptosis was detected with antibody specifically recognizing active cleaved caspase-3. aIn conclusion, these findings indicated that squamous cell carcinoma cell line (DJM-1) inhibit the growth of MCPyV-positive MCC cell line (MKL-1), but not MCPyV-negative MCC (MCC13).

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Nuak2 amplification coupled with Pten deficiency confers tumorigenicity to melanomas via cdk2

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NUAK2 has a pivotal role in regulating melanoma growth and the survival of patients with melanomas, and inhibition targeting NUAK2 itself and/or its downstream pathway would be promising therapies. In this study, we explored genomic aberrations in addition to NUAK2 amplification and cell cycle regulation by those genomic aberrations, and we characterized an efficient molecular target to suppress melanoma growth. We found that a deficiency of PTEN is coupled with amplification at the genomic area around NUAK2 using array-CGH data and immunohistochemical studies using clinical specimens also verified that p-Akt expression has a significant correlation with NUAK2 expression (P=0.005) in acral melanomas. Inactivation of the PI3K pathway reduced the S-phase population by up-regulating p21 expression. Both knockdown of NUAK2 and inactivation of the PI3K pathway efficiently control CDK2 expression, and inactivation of CDK2 specifically abrogates the growth of both NUAK2-amplified and PTEN-deficient melanoma cells compared to melanoma cell without those genomic aberrations. Immunohistochemical studies also verified those results by a high percentage (81.40%) of CDK2 expression in both NUAK2 and p-Akt expressing melanomas. We further show that an inhibitor targeting CDK2 suppresses the growth of both NUAK2-amplified and PTEN-deficient melanoma cells in vitro and in vivo. These results emphasize the importance of CDK2 in cell cycle regulation of melanoma cells and suggest that CDK inhibitors targeting CDK2 are promising therapies for cutaneous melanomas.

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Akt and intermediate filaments inhibit autophagy and promote tumorigenesis through Beclin 1/14-3-3 sequestration

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Aberrant signaling through the class I phosphatidylinositol 3-kinase (PI3K)-Akt axis is one of the most frequent alterations in human cancers. Here we show that Beclin 1, an essential autophagy and tumor suppressor protein, is a target of Akt. The Akt oncogene inhibits autophagy by promoting phosphorylation of Beclin 1 on S234 and S295. Phosphorylated Beclin forms an inactive complex with 14-3-3 and intermediate filament proteins. The disruption of this complex through depletion of vimentin or keratin 10 in vitro and in vivo results in increased autophagy. Moreover, the disruption of this complex through the expression of a Beclin 1 mutant resistant to phosphorylation by Akt increases autophagy, reduces anchorage-independent growth in vitro, and inhibits Akt-mediated tumorigenesis in vivo. Finally, Beclin 1 colocalizes with vimentin and keratin intermediate filaments. Thus, Akt-mediated phosphorylation of Beclin 1 functions in autophagy inhibition and oncogenesis, and leads to the formation of an autophagy-inhibitory Beclin 1/14-3-3/intermediate filament complex. These findings suggest broader roles for Akt signaling and intermediate filament proteins in the direct regulation of autophagy, cell growth, and cancer.

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Redox-based apoptosis induced by amino endoperoxides in tumors

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Majority of human cancer deaths are due to metastases which are resistant to conventional therapies. Anoikis resistance is a pivotal characteristic of metastatic cancer cells. Tumor cells exhibit excessive and persistent elevation of reactive oxygen species (ROS) and utilize a redox-based mechanism to evade death by anoikis. Recent findings showed that tumor cells maintain a relatively high O2-:H2O2 ratio to confer resistance to anoikis. We hypothesize those therapeutic treatments that alter this redox ratio will minimize or prevent metastasis. Endoperoxides-containing compounds have long been proposed as anti-cancer drugs. However, due to instability and complexity in synthesis procedure, few have been employed clinically. We were able to synthesize novel amino endoperoxides and their derivatives with good yields and stabilities. Our finding showed that our amino-endoperoxide selectively targets high-Nox4 expressing tumor, reducing O2-:H2O2 ratio and increasing hydroxyl radical level. Furthermore, we observed that cancer-associated fibroblasts undergo apoptosis in vitro, suggesting a redox-mediated mechanism is important to sustain their tumor-promoting phenotype.

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Nuclear receptor status of cancer-associated fibroblasts modifies squamous cell carcinoma gene expression signature

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Recent advances in cancer research have highlighted the importance of the tumour stroma in supplying a conducive microenvironmental niche enabling several hallmarks of cancer. Cancer-associated fibroblasts (CAFs) which are featured prominently in the surrounding milieu of a neoplastic lesion have been shown to facilitate cancer progression towards malignancy. CAFs are known to promote tumorigenesis via a heterotypic paracrine fashion involving secreted chemokine and cytokine networks. Yet, the transcriptional control of the pro-tumour phenotype of CAFs remains elusive. Nuclear receptors, which are environmentally-responsive, ligand-activated transcription factors, are expected to play a major role in CAF-mediated cancer progression. We profiled the expression levels of all 48 known nuclear receptors in CAFs relative to normal dermal fibroblasts isolated from human squamous cell carcinoma (SCC) biopsies. We further revealed that the nuclear receptor status of CAFs profoundly impacts the gene expression signature of SCC. Our work suggests that selective targeting of nuclear receptors in CAFs perturbs cancer cell behaviour and thus positions drugs targeting nuclear receptor as potential anticancer agents.

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Diagnostic utility of CD10 immunohistochemical staining in squamous cell carcinoma and precursor lesions: Evaluation using tissue microarray

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Cutaneous squamous cell carcinoma (cSCC) is widely accepted to be the result of a transformation from precancerous lesions. Recent studies have suggested that CD10 expression is associated with tumor progression and metastasis in malignant melanoma. This study examined whether CD10 expression in cSCC is associated with tumor progression and other clinicopathologic data. A total of 25 cSCC cases were retrieved from the archives and a 2-mm tissue core was obtained from the each specimen for the generation of the tissue microarray (TMA) blocks. In addition, 28, 28 and 29 cases of actinic keratosis, Bowenoid actinic keratosis and Bowen's disease, respectively, were immunostained for CD10 for comparison. The total area and intensity of the immunostain were arbitrarily defined as follows: area of staining 0 (<5%), 1 (5~25%), 2 (26%~50%), 3 (51%~75%) and 4 (>75%); and intensity 0 (none), 1 (faint/barely), 2 (weak to moderate) and 3 (strong). The variables were multiplied and classified from 0 to 12 grades. Eight of 25 cases of cSCC (32%) showed CD10 expression, whereas no CD 10 expression was observed in the precursors. There was a positive relationship between CD10 expression and grade of differentiation (P< 0.05). Although the cases with recurrence or metastasis of tumor showed the high CD10 score, this was not statistically significant (p= .093, p= .081, respectively). In conclusion, CD10 expression appears to be a late event in cSCC carcinogenesis and is probably associated with tumor progression in cSCC.

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The cross talk between p53 and NFκB determines the response to UVB in keratinocytes

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The susceptibility towards apoptosis-inducing stimuli depends on the intracellular balance of pro- and anti-apoptotic factors. In response to DNA-damaging agents, this balance is shifted towards up-regulation of pro-apoptotic factors via activation of p53. In contrast, activation of the transcription factor NFκB dislocates the balance towards expression of anti-apoptotic genes. Thus, the cross talk between p53 and NFκB significantly governs the cell fate. Putting the role of NFκB into a different perspective, our recent studies revealed that IL-1-induced NFκB activation enhanced UVB-induced apoptosis. This effect was mediated via NFκB-dependent repression of anti-apoptotic genes and coincided with a prolonged expression of TNF. Secreted TNF subsequently activated TNF-R1 in an autocrine fashion to additively enhance apoptosis. Elevated TNF production was due to persistent NFκB activation resulting from disruption of the negative feedback loop, which involves resynthesis of its inhibitor IκB. Investigateing the role of p53 in UVB responsiveness the p53mut carrying keratinocyte cell line HaCaT and the p53wt carrying cell line SCL-1 were compared. Surprisingly, only HaCaT but not SCL-1 cells responded with apoptosis induction. Additionally, only HaCaT cells showed enhanced apoptosis when co-stimulated with IL-1. These results question the island position of p53 in mediating DNA damage-induced apoptosis. Further evidence was provided by the finding that exchanging the genotype of HaCaT cells into p53-ko via stable knock down and further into p53wt by stable re-expression only feeble changes in short term UVB responses (24 h apoptosis assays) were noted, while no influence on clonogenic outgrowth or colony formation after 3 weeks of cultivation was seen. Strikingly, p53-ko cells were no longer able to conduct IL-1-induced and NFκB-dependent enhancement of apoptosis and TNF production. This strongly indicates that NFκB and p53 closely collaborate in generating a proper apoptotic response to UVB, an important finding regarding the development of new antitumoral treatment strategies.

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RNA trans-splicing molecules for targeted suicide gene delivery into cancer cells

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Patients suffering from the severe form recessive dystrophic EB (RDEB-sev gen), have an increased risk to develop aggressive squamous cell carcinoma (SCC). In this context we are evaluating a gene therapy approach for tumor treatment, by inducing toxin-mediated death of the tumor cells. In this study spliceosome-mediated RNA trans-splicing is used to specifically target tumor marker genes, overexpressed in RDEB-associated tumor cells with the aim to introduce a suicide gene and eliminate the cells by its expression. We chose the solute carrier organic anion transporter family member 1B3 (SLCO1B3), which is strongly upregulated in RDEB tumor cells. We designed and evaluated different RNA trans-splicing molecules (RTMs) in order to improve the specificity of the trans-splicing process and to reduce unwanted side effects including ectopic expression of the toxin. Cell death inducing RTMs contain a specific binding domain for SLCO1B3 and the suicide gene thymidine kinase from herpes simplex virus (HSV-tk). The trans-splicing efficiency of each RTM was evaluated after co-transfection with a SLCO1B3 minigene (SLCO1B3-MG) in HEK293 cells. Accurate trans-splicing leads to the generation of a chimeric SLCO1B3-tk fusion construct exhibiting suicide gene activity after ganciclovir (GCV) treatment. Correct expression of the trans-spliced SLCO1B3-tk mRNA and its protein was detected by semi-quantitative RT-PCR and Western blot analysis, respectively. Furthermore, MTT and TUNEL assays confirmed that the constructed RTMs induce toxin-mediated apoptosis in SLCO1B3-MG expressing cells. RTMs with high trans-splicing specificity and efficiency are needed to successfully induce cell death in tumor cells. To conclude, our inducible cell-death system provides a powerful tool to identify optimal RTMs for suicide gene therapy approaches.

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Diverse roles for laminin 332 subunits in squamous cell carcinoma

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The basement membrane zone (BMZ), present in all epithelia, plays an important role in cancer spread acting not only as a barrier to invasion but also signalling through cell surface receptors. Components of the skin BMZ include collagens IV, VII and XVII as well as laminin 332 (Lam332), which is secreted in copious amounts by cancer cells. Patients with severe epidermolysis bullosa (EB) caused by mutations in BMZ components suffer from an increased incidence of aggressive squamous cell carcinoma (SCC). The role of Lam332 and collagen XVII in tumour development is poorly understood. This study dissects the effect of loss of individual subunits of Lam332 or collagen XVII on SCC. We have generated using lentiviral shRNA, 4 stable cutaneous SCC cell lines each lacking a different BMZ component including collagen XVII and the three constituent parts of Lam322, LamA3, LamB3 and LamC2. Using our cell lines we studied their role in cell attachment, proliferation, motility, in vitro and in vivo invasion, and invadopodia. Loss of ColXVII increased cell attachment whereas loss of any of the Lam332 chains reduced cell attachment. Loss of any of the BM components reduced cell proliferation. The loss of LAMA3 and LAMC2 increased cell motility whereas loss of LAMB3 and ColXVII had no significant effect on motility. We demonstrated increased invasion in both in vitro collagen gels and in vivo xenografts with loss of LAMA3, LAMC2 and ColXVII with distinct patterns of invasion seen in each cell line. An increased number of invadopodia were observed in LAMA3, LAMC2 and ColXVII cell lines as well as more invadopodia positive cells compared to control. LAMC2 invadopodia but not LAMA3 and ColXVII were Src dependent. Finally we have used QPCR and Western blotting to identify changes in signalling pathways known to be involved in cancer progression such as EMT (epithelial to mesenchymal transition), integrin signalling and tight junction formation. In conclusion this study further refines the role of laminin 332 and ColXVII in SCC invasion and proliferation in vitro and in vivo.

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Characterisation of the peritumoral immune infiltrate in human primary cutaneous squamous cell carcinomas

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Defective immunity plays a major role in the pathogenesis of cutaneous squamous cell carcinoma (SCC) as evidenced by the significantly increased incidence of this malignancy (up to 250-fold) in immunosuppressed individuals. In immunocompetent patients, a peritumoral immune cell infiltrate is commonly found in cutaneous SCC but is unable to destroy the tumour. We have previously shown that FOXP3+ regulatory T cells (Tregs) are present in skin SCCs, and that Tregs from SCCs suppress peritumoral effector T cell proliferation in vitro. Here we show, using immunohistochemical quantification of Tregs in cutaneous SCCs (n=36), that increased FOXP3+ Treg numbers are present in primary tumours from patients who subsequently developed metastases compared with tumours that did not metastasise (28.6% versus 11.9% of immune cell infiltrate respectively, p=0.010), further implicating Tregs as pathogenic in SCC. We also demonstrate a deficiency in CD8+ T cell function in skin SCCs, despite there being more CD8+ T cells in the tumour than in the peripheral blood of subjects with skin SCCs (40.0% versus 18.3% of CD3+ population respectively; p=0.001). For example, phytohaemagglutinin-stimulated FACS-isolated CD8+ T cells from SCC proliferated significantly less than CD8+ T cells from peripheral blood (mean cpm 3,215 versus 5,576, n=8, p=0.017), suggesting that CD8+ T cells accumulate in SCC but are dysfunctional. FACS quantification of the inhibitory receptor, PD-1, demonstrated increased PD-1 expression on CD8+ T cells in SCC compared with CD8+ T cells from peripheral blood (16.9% versus 2.9% of CD8+ population, n=4, p=0.004). Overall, our results indicate that a combination of defective immune responses are present in skin SCCs, and suggest that these play a fundamental role in the development of these tumours.

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Targeting Fyn restores cell-cell adhesion in Ras-transformed HaCaT cells and inhibits UV skin tumorigenesis

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Ras oncogene activation is a common feature of human cancers, including cutaneous squamous cell carcinoma (SCC). However, directly targeting Ras activity has not proven to be clinically effective. Active Ras mediates its oncogenic effects via multiple effector pathways. We recently identified PI3K/Akt/Fyn signaling as an important Ras effector pathway involved in the migration and invasion of H-Ras(G12V)-transformed HaCaT cells (HaCaT-Ras). HaCaT-Ras cells selectively over-express the Src family kinase Fyn at the transcriptional level, and have undergone an epithelial-to-mesenchymal transition. We investigated the effects of Dasatinib, an approved Src family kinase inhibitor currently used to treat leukemias, as a potential targeted therapy for SCCs using HaCaT-Ras cells as a model. Although Dasatinib inhibited the growth and proliferation of HaCaT and HaCaT-Ras cells with equal potency, it dramatically enhanced cell-cell adhesion and partially reversed the mesenchymal morphology of HaCaT-Ras cells. Dasatinib has similar effects on the morphology of Fyn-transformed HaCaT cells. The Dasatinib-stimulated increase in HaCaT-Ras cell-cell adhesion was associated with enhanced membrane localization of desmoplakin and N-cadherin, implicating both desmosomes and adherens junctions. Dasatinib also significantly inhibited HaCaT-Ras cell migration, consistent with the role of Fyn in HaCaT-Ras cell migration. To determine if Dasatinib could be effective in treating SCC, we performed a UV skin carcinogenesis experiment in SKH1 mice exposed to broadband UVB three times per week. Mice treated topically with Dasatinib (1 μmole) immediately after each UV exposure had significantly lower overall tumor burden, primarily due to reduced tumor multiplicity. Overall, these results indicate that targeting Fyn activity with small molecule inhibitors, such as Dasatinib, can reverse the migratory phenotype of Ras-transformed cells and is a promising approach for the treatment of SCC.

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Crosstalk between Desmoglein-2 and Hedgehog signaling in skin tumorigenesis: Dsg2 modulates Gli expression in keratinocytes

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The desmosomal cadherin, desmoglein 2 (Dsg2) is upregulated in various cancers, including basal and squamous cell carcinomas (BCC, SCC). Furthermore, we recently demonstrated that Dsg2 enhances cell growth and survival and transgenic (Tg) mice overexpressing Dsg2 in the skin develop a hyperproliferative epidermis and are more susceptible to chemical-induced tumor development. Here we demonstrate that the mechanism by which Dsg2 regulates these phenotypic changes may involve the Hedgehog (Hh) signaling pathway. Canonical Hh signaling is a key developmental pathway characterized by the activation of Gli transcription factors which are regulated by Smoothened (Smo). The Hh signaling cascade is initiated upon the binding of Hh ligands to Patched (Ptc), which alleviates Ptc suppression of Smo. The Hh pathway is aberrantly activated in various skin cancers; and in fact, improper activation of Hh signaling is the underlying cause of virtually all BCC. qPCR analysis of Dsg2-Tg mice reveals a correlation between Dsg2 and the expression of Gli transcriptional targets Gli1 and Ptc1. Additionally, immunohistochemical staining shows a correlation between Dsg2 and Gli1 expression in human BCC. Furthermore, RNAi knockdown of Dsg2 results in a concomitant decrease in Gli expression in keratinocytes. We obtained PtcLacZ reporter mice, in which a portion of the Ptc1 gene has been replaced with LacZ, rendering Ptc non-functional and making them efficient reporters of Hh activity. These heterozygous mice are primed for Hh activation, and thus are an ideal model for cancer studies. To assess the effects of deregulating both Dsg2 and Hh signaling in vivo, we crossed the Dsg2-Tg mice with the PtcLacZ reporter mice. As expected, mice overexpressing Dsg2 exhibit a hyperplastic epidermis and abnormal differentiation; furthermore, these phenotypes are enhanced on the Ptc heterozygous background. Taken together, this data supports a role for Dsg2 in enhancing Hedgehog signaling, potentially through the regulation of Gli expression.

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Determination of genes involved in the resistance of squamous cell carcinoma to photodynamic therapy

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Photodynamic therapy (PDT) is widely used to treat actinic keratosis and in situ squamous cell carcinoma (SCC). However, the main problem about PDT, as well as other current treatments against cancer, is the appearance of resistant population cells that would be responsible for relapses and metastatic processes. By the use of comparative genomic hybridization (CGH) we have investigated genomic alterations that might be implicated in resistance of the SCC-13 cell line to methyl derivative of δ-aminolevulinic acid (MAL-PDT). SCC-13 original cell population (Parental) and resistant SCC populations obtained by repeated treatment with MAL-PDT (5th and 10th PDT-generations) were analyzed performing an array CGH (aCGH) to determine the potential genomic differences between the three cells types. The differential selected genes were validated in extracts of Parental and resistant SCC-13 cells using western blot and in histological sections of tumors induced by injecting the SCC-13 cells in immunodeficient mice. The results obtained indicated that all three cell types, Parental, 5th and 10th PDT-resistant cells, showed amplicons in 3p12.1 CADM2, 7p11.2 EFGR and 11q13.3 CCND1 genes. However, 5th and 10th PDT-resistant cells showed an amplicon in 5q11.2 MAP3K1 and genomic losses in 5q14q23, 15q and 21q among others. As a first approach, the changes detected by aCGH on CCND1, EFGR and MAP3K1 were evaluated by immunohistochemistry in the tumors induced by inoculation in mice of Parental, 5th or 10th PDT-resistant cells and in human biopsies obtained from persistent tumors after being subjected to MAL-PDT. An increase in the expression of Cyclin D1, EFGR and MAP3K was detected. In conclusion genomic imbalances related with CCND1, EFGR and MAP3K1 seems to be involved in the development of resistance to PDT of SCC.

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Conditional Cyld mutation induces epidermal cell lineage-specific abnormalities due to deregulation of NF-kB and c-Myc gene regulators

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Genetic mutations in Cyld are linked to multiple pathological conditions including skin cancer. The molecular mechanisms underlying the pathogenesis are still unclear. Here, we generated a conditional CYLD mutant (CYLDm) knock-in mouse model through K14-Cre-mediated deletion of cyld exon 9 (hereafter refer to Cylde9/9). Cylde9/9 mice were born alive but developed a coarse hair phenotype accompanied with sebaceous gland hyperplasia, elongated incisor teeth and toe nails. Upon challenge with DMBA/TPA, these animals displayed reduced growth of papilloma but increased growth of sebaceous nevi and basaloid tumor nodules. Molecular analysis revealed that CYLDm sustained NF-kB activity which in turn maintains CYLDm-gene expression. In addition, CYLDm resulted in an elevated level of c-Myc and c-Myc-K63-ubiquitination in keratinocytes. Topical treatment of Cylde9/9 mice with pharmacological inhibitors of NF-kB and c-Myc significantly increased sebaceous gland cell apoptosis. Taken together, these results indicate that endogenous CYLDm forms a positive feedback loop with NF-kB, and activates c-Myc to promote epidermal cell lineage-specific defects. Thus, our findings identify NF-kB and c-Myc as potential therapeutic targets for pathological conditions associated with Cyld mutation.

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Gene expression profiling of primary fibroblasts isolated from multiple self-healing squamous epithelioma

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We have recently identified disease-specific spectrum of mutations in TGFBR1 gene to be basis of the genetic skin cancer disorder called multiple self-healing squamous epithelioma (MSSE). MSSE, also historically known as Ferguson-Smith disease, is characterized by multiple well-differentiated keratoacanthoma-like tumours, which appear and grow rapidly, mainly on sun-exposed body sites. The onset of this disease varies greatly, from 8-80 years of age. These tumours undergo spontaneous regression and involution, leaving considerable scarring. The reason for such tumour behaviour remains unclear. To understand the contribution of the microenvironment to the tumour progression and regression, global gene expression analysis was performed using Affymetrix Human Genome U133 2.0 Plus Array on total RNA isolated from primary fibroblasts. The fibroblasts were derived from the unaffected skin, peri-tumour and tumour tissues of a Danish MSSE patient. The control fibroblasts were isolated from a healthy Danish family member. Preliminary data from gene expression analysis revealed 119 differentially expressed genes with functions related to skin, cancer development and TGF-β signalling pathway.

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The newly developed vinegar "Izumi" inhibits the proliferation of human squamous cell carcinoma cells by inducing programmed cell necrosis

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Kurozu (Japanese black vinegar), a traditional product made from unpolished rice, contains beneficial organic materials and minerals. The new vinegar that contains large amounts of such constituents was produced by the improvement in the manufacturing process and named "Izumi". Since antioxidative effect of Kurozu is well elucidated, we examined the anticancer activity of Izumi on human squamous cell carcinoma (SCC) cell line, HSC-5. HSC-5 cells were treated with Izumi or the ordinary grain vinegar, which were adjusted to 4.2% acidicity. MTT assay and the trypan blue dye exclusion test showed that Izumi significantly inhibited the proliferation of HSC-5 cells compared to ordinary grain vinegar. Propidium iodide (PI) flow cytometry and annexin V/PI staining revealed that among cells treated or untreated with Izumi or ordinary grain vinegar there was no difference in the number of apoptotic cells. Recently, a new form of necrosis, programmed necrosis or necroptosis, has been proposed. Programmed necrosis is mediated by the receptor-interacting serine-threonine kinase 3 (RIPK3) as a key signaling molecule and results in the release of cellular danger-associated molecular patterns (DAMPs). When HSC-5 cells were treated with Izumi, the cellular level of RIPK3 protein and the amount of high-mobility group protein B1 (HMGB1), one of the DAMPs, released into culture media were remarkably increased. These findings indicate that Izumi inhibits proliferation of human SCC cells via programmed necrosis.

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TRAIP expression is quantitatively and spatially regulated in epithelial cells

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The TRAF-interacting protein (TRAIP, TRIP) is a RING-type E3 ubiquitin ligase that interacts with the deubiquitinase CYLD, a tumor suppressor whose functional inactivation leads to skin appendage tumors. TRAIP is required for embryonic development since removal of TRAIP either in Drosophila or mice by mutations or knock-out is lethal due to aberrant regulation of cell proliferation and apoptosis. Furthermore, shRNA-mediated knock-down of TRAIP in human epidermal keratinocytes (HEK) repressed cell proliferation and induced a G1/S phase block in the cell cycle. To investigate the cellular localization of TRAIP, lentiviral vectors (LV) were constructed driving expression of a TRAIP-GFP fusion protein (TRAIP-GFP) from the CMV promoter. Transduction of HeLa cells or HEK with TRAIP-GFP followed by immuno-fluorescence analysis demonstrated that TRAIP-GFP is localized to the nucleolus during interphase. TRAIP1-289 or TRAIP289-469 GFP fusion proteins expressed in HeLa cells localized to the cytosol or the nucleus, respectively, indicating that the N-terminal part specifies nucleolar localization whereas the C-terminal region determines nuclear transfer. Analysis of the expression levels of TRAIP-GFP or GFP in stably transduced HeLa cells and HEK revealed that the level of TRAIP protein was significantly lower compared to GFP although their mRNA levels were comparable. Determination of the half-life using cycloheximide showed that TRAIP has a half-life of 3.2+/-1.2 hours (n=5) suggesting that it is an unstable protein. These results indicate that the level and sub-cellular localization of TRAIP protein is highly regulated which might be important for control of cell proliferation.

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Enhanced MET signaling in mouse epidermis activates EGFR and initiates squamous carcinogenesis

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Activation of MET signaling is associated with multiple cancers but its function in the process of carcinogenesis has not been elucidated. The multistage induction of tumors on mouse skin was used to address this question. Double transgenic mice were generated by crossing K5-PKCalpha mice that overexpress PKCalpha in basal keratinocytes and are tumor promotion sensitive with MT1-HGF mice that overexpress the MET ligand HGF under a metallothionein promoter to create MT1-HGF/K5-PKCalpha (DT) mice and their respective controls. DT animals developed six fold more squamous tumors than other genotypes when exposed to a single tumor initiating application of dimethylbenz(a)anthracene followed by six applications of tumor promoting 12-O-tetradecanoyl-13-phorbol acetate (TPA). Both DT and MT1-HGF keratinocytes in vitro displayed MET-mediated autonomous conversion to a morphological and biochemical phenotype characteristic of initiation by oncogenic H-Ras. Enhanced MET signaling activated EGFR through specific upregulation of EGFR ligands, a common pathway downstream from oncogenic Ras initiation. Inhibition of EGFR activity reversed many of the biochemical changes resulting from enhanced MET signaling. In vitro and in vivo, MT1-HGF keratinocytes and mice responded to TPA like wildtype keratinocytes while DT keratinocytes and mice responded to TPA like promotion sensitive K5-PKCalpha keratinocytes. Thus, enhanced MET signaling alone did not influence tumor promotion. In vivo without chemical initiation, DT mice developed multiple squamous papillomas when promoted by TPA while none of the three other genotypes developed tumors. Mutations were not detected in any *Ras* allele in DT tumors. Together these results indicate that enhanced keratinocyte MET activity can initiate skin tumor formation through autocrine activation of EGFR, and the synergistic activity of MET and PKCalpha in epidermal keratinocytes can substitute for the lack of *Ras* mutations during skin carcinogenesis.

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Cutaneous SCC and the RAS controversy

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Cutaneous squamous cell carcinoma (SCC), the second most common cancer worldwide, is increasing by at least 5% per annum in Scotland. SCC is now responsible for 25% of skin cancer-related deaths with a lack of targeted treatments for recurrent/metastatic disease because the molecular drivers of cutaneous SCC are poorly understood. Activating mutation of HRAS is the initiating event in the chemical carcinogenesis model of cutaneous SCC, but the role of RAS mutation in UV-initiated SCC is unclear. The development of keratoacanthoma-like SCC skin tumours in patients treated with BRAF inhibitors and the availability of targeted therapies to MEK and EGFR revisit the question of RAS activation in cutaneous SCC. We performed whole exome sequencing on 10 well differentiated (WD) and 10 poorly differentiated (PD) SCC using the Illumina Hi-Seq platform (EASIH, Cambridge), identifying a single codon 12 KRAS mutation in 1/20 tumours. Targeted sequencing of HRAS, KRAS and NRAS was performed using the Fluidigm / Roche 454 GS-Junior platform on a validation series of 82 SCC plus 20 SCC cell lines. Five codon 13 HRAS mutations were identified in 4 WD-SCC and 1 SCC cell line confirming a 5% mutation rate. Ras activation was measured using the Ras GTPase Chemi ELISA Kit with only 1 in10 of the SCC cell lines displaying any activation of Ras. These data, together with low levels of pERK and pAKT in the majority of cell lines, imply a less central role for Ras in UV-induced SCC compared with vemurafenib-induced skin tumours.

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The role of Retinoblastoma protein (Rb) in cell cycle control by T_{H1}-cytokines in mouse and human cancers

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Regulation of the cell cycle progression via the $p16^{Ink4a}$/Rb-pathway is a central mechanism that is impaired in several types of cancer. RIP1-Tag2 mice expressing the T antigen (Tag) under the control of the rat insulin promoter (RIP) develop β-cell cancers due to a partial inhibition of the $p16^{Ink4a}$/Rb pathway and a complete inactivation of p53. Our previous studies showed that Tag-specific, interferon-γ (IFNγ)- and tumor necrosis factor (TNF)-producing T helper 1 (T_{H1}) cells doubled the survival of RIP1-Tag2 mice and strongly reduced cell proliferation. MHC class II restricted T_{H1} cells are unable to directly interact with β-cancer cells or to kill the cancer cells, as β-cancer cells do express MHC class II neither in vitro nor in vivo. We therefore focused on the effects of the soluble T_{H1} cytokines IFNγ and TNF on β-cancer cells. In vitro, IFNγ and TNF both exerted an anti-proliferative effect on β-cancer cells and arrested them in the G_0/G_1 phase of the cell cycle, as shown by flow cytometry. These effects strongly increased when the two cytokines were combined. We next focused on the effects of TH1-cytokines on the p16Ink4a/Rb-pathway in β-cancer cells, first by Western Blot and immunofluorescence. IFNγ and TNF caused within 48h strong dephosphorylation of Rb, while it did not affect Rb protein in the cytoplasm. Thus, T_{H1}-cytokines caused a stabilization of Rb-mediated cell cycle control. To confirm the functional importance of the $p16^{Ink4a}$/Rb-pathway in cytokine-mediated cell cycle control, we performed a knockdown of Rb in β-cancer cells via lentiviral transfection with shRNA. First experiments already confirmed that silencing of Rb in β-cancer cells decreased the responsiveness to T_{H1}-cytokine treatment. Similarly, IFNγ and TNF caused dephosphorylation of Rb in a human sarcoma. Thus, Rb is a central target of T_{H1}-cytokine induced cell cycle control in cancers of mouse and human origin.

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The detection of novel KIT mutations in mastocytosis

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KIT is a receptor tyrosine kinase (RTK) that consists of a ligand-binding extracellular domain (ECD), a transmembrane domain (TMD), a cytoplasmic juxtamembrane regulatory domain (JMD), and a tyrosine kinase domain (TKD). Activating KIT mutations are associated with mastocytosis. Sequence analysis targeting the KIT mutational hot spots have shown that while most adult-onset patients express the autoactivated KIT^{D816V} mutation in the TKD, most childhood mastocytosis patients have mutations within the ECD; however, not all patients express KIT mutations. We speculate that additional KIT activation results from alternative splicing. Thirty-eight mastocytosis subjects (28 adults and 10 children) were evaluated for KIT transcript variations using a long-range PCR amplification targeting the full-length cDNA and direct DNA sequencing. Seventy-nine percent of adult patients (22 out of 28) had the D816V (T>A) mutation, 18% (4 out of 28) had the M541L (C>A) mutation within the TMD, 14% (4 out of 28) had the V560G (G>T) mutation located in the JMD, but 0% had a mutation in the ECD. Forty percent of children (4 out of 10) had mutations at the 816 locus, 10% expressed the M541L mutation, 70% had mutations in the ECD, but 0% had the V560G mutation. Thus, in our mastocytosis patient group, we have detected a higher percentage (14%) of patients expressing the KIT^{V560G} mutation, which has been previouosly reported to be rare. Additionally, a novel Q515H mutation at the ECD glucosamine linking site was identified in two children, and a novel truncated KIT transcripts was found in two other children. One of the truncated KIT results from a nonsense mutation at Q947, while another results from a deletion of exons 3 to 13. The results of this study demonstrate the importance of full-length KIT DNA sequencing in the identification of novel KIT mutations and transcript variants, which may provide additional information into the pathophysiology and treatment of mastocytosis.

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Epigenetic regulation of malignant peripheral nerve sheath tumor development

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Patients with Neurofibromatosis type I (NF1), a common tumor predisposition genetic disorder of the nervous system, develop multiple malignancies, including the Malignant Peripheral Nerve Sheath Tumors (MPNSTs). Previously, we showed that a population of neural crest-related stem/progenitor cells residing in the dermis termed Skin-Derived Precursors (SKPs) as the cell of origin of NF1-associated dermal neurofibromas and generated a novel mouse model for this complex tumor that recapitulates the progressive nature of its human counterpart. We demonstrated that Nf1-deficient SKPs can give rise to neurofibromas, and further loss of p53 robustly generates MPNSTs in vivo. However, the tumorigenic potential of Nf1-/- p53-/- SKPs pales in comparison to the cells derived from developed MPNSTs, suggesting loss of the tumor suppressors Nf1 and p53 is required but insufficient for MPNST development and pointing to the critical roles of further genetic and/or epigenetic changes for MPNST development. Here we show that upon comparative transcriptome analysis, we identified a profound upregulation of HMTases (histone methyltransferase) in MPNSTs. The emerging role of HMTases in cancer development prompted us to determine their exact roles in MPNST development. We found that inhibiting the levels of HMTase attenuates both MPNST proliferation in vitro and tumorigenesis in vivo. However, we unexpectedly found that HMTase inhibition accelerates malignant transformation of Nf1-/- p53-/- SKPs to MPNSTs in mice, suggesting that HMTase regulates in vivo tumor suppression. These findings suggest a dual role for HMTases at distinct stages of MPNST development. Our studies highlight the utility of novel mouse tumor models that allow dissection of gene function during tumor evolution. More importantly, while epigenetic enzymes are promising therapeutic targets, our findings suggest preclinical evaluation of epigenetic therapies at earlier stages of cancer development as a means to personalize cancer therapy based on the stage of cancer progression.

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Expression of microRNAs in Merkel cell carcinoma

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Merkel cell carcinoma (MCC) is a primary neuroendocrine carcinoma of the skin. Although not as prevalent as other skin cancers, MCC is more aggressive than melanoma and has a higher mortality rate, with reviews citing an overall five-year survival of sixty percent. Unfortunately, while the incidence of MCC increases, our knowledge of these tumors remains limited. In an attempt to expand our understanding, we focus our attention on microRNAs, small single-stranded RNA molecules that participate in the negative regulation of gene expression. MicroRNAs have been implicated in the pathogenesis of various skin cancers, including melanoma, squamous cell carcinoma (SCC), and basal cell carcinoma (BCC). Through their unique role in posttranscriptional regulation, they can function as important regulators of tumor growth and metastasis; however, the differential expression of microRNAs and their role in pathogenesis have yet to be explored in MCC. To identify microRNAs specific to MCC (MCC-miRs), high-throughput sequencing (HTS) of small RNA libraries was performed on several tissue samples: MCC, melanoma, SCC, BCC, normal skin, and normal lymph node. Comparison of the sequencing profiles identified several microRNAs upregulated (≥3-fold) in MCC versus other tissues. To validate some of these candidates, their expression was measured via qRT-PCR in a larger group of MCC samples (n=20) and in a comparison group (n=23) composed of other cutaneous tumors (melanoma, SCC, BCC) and normal skin. Out of those evaluated, five microRNAs were confirmed to be upregulated in MCC: miR-7 (11.3-fold), miR-9 (9.9-fold), miR-340 (6.3-fold), miR-502-3p (4.7-fold), and miR-182 (3.3-fold). Several of these have been implicated in the pathogenesis of other cancers and may play a role in the development, progression, and metastasis of MCC. We present data regarding their functional significance, including potential target genes, derived from initial studies in the MCC13 cell line.

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Genetic background of mastocytosis, a synopsis of cooperative studies conducted by ECNM

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Mastocytosis (MC)is a clonal disorder of the hematopoietic progenitor cells that give rise to mast cells and other myeloid and lymphoid lineages and occurs in two general forms: cutaneous (CM) and systemic (SM). The majority of adult and pediatric MC could be characterized by somatic mutations of genes coding KIT or other receptors with kinase activity. In more aggressive form of SM some specific mutations of oncogenes (JAK2V617F, AML1/ETO, BCR/ABL, FLT3) were found. Our study involving 247 MC patients and 175 healthy individuals indicated that single nucleotide polymorphisms (SNP) detected in genes coding cytokines (IL-13,TNF-α, IL-6, IL-10), their receptors (IL-4R, IL-6R) and other proteins including: TLR-2,FcεRI – β, and TAP-1 were associated with development of the disease. Progression of MC could be correlated with inherited SNPs in cytokine genes (IL-13, TLR-2, IL-6, IL-10), activation of Btk/Lyn, N-RAS and inactivation TET-2 genes. Whole genome expression profiles indicate in SM overexpression in blood and bone marrow of genes coding: α-tryptase, carboxypeptidase A as well as regulators of transcription, proliferation, apoptosis, signal transduction, protein transport and ubiquitin mediated proteolysis. SM patients with a history of IVA showed additional alteration indicating more pronounced mast cells dysfunction in this group. Additional somatic mutations deregulating epigenetic mechanisms were found to be frequent in aggressive SM. Finally, mi-RNA depending mechanisms involved in KIT-dependent mast cells activation, MITF synthesis and may probably influence on tryptase production are newly described pathways in MC.

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ΔNp63α drives mutagen-induced skin tumor initiation and progression to malignancy

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P63 is a transcription factor crucial for ectodermal development and the formation of stratifying epithelia. Unlike its family member p53, the p63 gene is rarely mutated in human cancers, but it is often overexpressed. Squamous Cell Carcinoma (SCC) is a common and treatment-refractory form of human cancer in epithelial tissues were the p63 locus is frequently targeted for genomic amplification, leading to increased levels of the ΔNp63α isoform. To investigate the contribution of ΔNp63α in the initiation, promotion and progression of SCC, we developed K5-Cre/LoxP-controlled ROSA26 promoter-driven ΔNp63α conditional transgenic mice. These mice develop epidermal hyperplasia and hair abnormalities, eventually leading to alopecia. Interestingly, spontaneous benign tumors occur in 25% of the transgenic mice. When subjected to two different models of chemical carcinogenesis (DMBA/TPA and DMBA/DMBA), we found that ΔNp63α transgenic mice were more prone to the onset of tumor formation, and display more tumors compared to control mice and this in a gene dosage-dependent manner. Moreover, progression to malignant carcinoma was only observed in the ΔNp63α transgenic lines. In vitro experiments with primary keratinocytes derived from wild-type and ΔNp63α transgenic mice demonstrate enhanced cell survival upon ΔNp63α overexpression. No differences were observed in genotoxic stress-induced apoptosis, instead ΔNp63α was able to block cellular senescence, marked by lower levels of p16Ink4a. In addition, ectopic transgenic ΔNp63α expression was detected in the bile duct epithelium, leading to severe dilatation of the intrahepatic bile ducts and cholangitis, leading to biliary cyst formation and the development of cholangiocarcinoma (CC). Taken together, we generated a new mouse model for SCC and CC and clearly show a causal relationship between p63 gene amplification and tumor development.

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Expression microarray analysis identifies key processes in the transition from actinic keratosis to cutaneous squamous cell carcinoma

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Cutaneous squamous cell carcinoma (SCC) is one of the most common malignancies in fair skinned populations worldwide and its incidence is increasing by at least 5% per annum in the UK. Despite previous observations of multiple genetic abnormalities in SCC, the oncogenic process remains elusive. The purpose of this study was to investigate the transcriptome of SCC and actinic keratoses (AK), to elucidate key molecular events associated with the progression of precursor AK lesions to invasive carcinoma. We have used laser capture microdissection combined with the Affymetrix HGU133 Plus 2.0 microarrays to examine the transcriptome of 30 SCC and 10 AK. We have identified a core set of 196 genes that are differentially expressed between AK and SCC, and are enriched in processes including epidermal differentiation, cell migration, cell cycle regulation and metabolism. Gene set enrichment analysis highlighted a key role for the MAPK pathway in SCC progression from AK. Furthermore, the histological subtype of the SCC influenced the gene expression profile, which may have implications for drug sensitivity and response in clinical trials. These data indicate that progression to SCC is associated with a complex pattern of molecular changes. We have identified relevant pathways involved in this process, in particular that the MAPK pathway may be pivotal to the transition from AK and may represent a potential therapeutic target.

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Analysis of pdgfrα mutations and survivin expression in merkel cell polyomavirus positive and negative merkel cell carcinomas

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Merkel Cell Carcinoma (MCC) is a rare neuroendocrine and highly aggressive cancer of the skin. Little is known on MCC pathogenesis and oncogenesis, more recently a viral involvement has been questioned. We have analyzed 63 MCC specimens for Merkel Cell Polyomavirus (MCPyV) positivity and load. Out of these, 68% of patients were MCPyV positive with viral load ranging from 0.006 to 943 virus DNA copies/beta globin. Viral DNA was found in patients with and without metastatic disease, however patients with distant metastases presented higher loads. This data might indicate a correlation between strong viral positivity and poorer disease outcome. Further, we have investigated whether mutations in PDGFRα gene and expression of Survivin, previously shown to be highly expressed in MCCs, might be the consequence of viral infection and correlate to disease progression. More than one mutation was detected within PDGFRα exons 10, 12 and 18, whereas at least one mutation in the PDGFRα gene was found in 35% of MCPyV positive and 60% of MCPyV negative tumors. This suggests that the presence of mutations is not correlated with the viral positivity. PDGFRα protein expression was detected in all tumors and 11% of analyzed samples showed low expression of this protein. Interestingly the majority of patients with metastatic disease showed low PDGFRα expression. 73% of the patients displayed Survivin with a strong to moderate expression pattern. No correlation was found between Survivin expression and viral positivity. Yet, cellular localization may be correlated to disease progression being nuclear expression of Survivin a negative prognostic factor. In conclusion, our data indicate that strong viral positivity correlates with poor disease outcome but not with mutations of the PDGFRα gene or with Survivin expression suggesting that these three factors independently contribute to Merkel Cell Carcinoma development.

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Comprehensive analysis of the basal cell carcinoma functional methylome

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Introduction Basal cell carcinoma (BCC) is the most common malignancy. Its genetic basis BCC is well defined. However, its epigenetics have not been fully charted and might reveal additional carcinogenic pathways. We used the latest tools for methylation and transcriptome analysis to obtain a detailed map of the BCC functional methylome with a view to unraveling BCC tumorigenesis. Methods DNA and total RNA were extracted from primary BCC and matched healthy control skin samples. Methylated DNA was MBD2 isolated and used for library preparation. After rRNA depletion, a cDNA library was generated from the total RNA. Both libraries were sequenced on an Illumina Genome Analyzer II. Sequence assembly and bio-informatics analysis were performed with open source tools and in-house developed algorithms. Results Our initial analysis indicates global hypomethylation in BCC compared to healthy skin, previously reported for other malignancies. Some genomic areas were significantly hypermethylated. Interestingly, sun-exposed skin did not share the BCC methylation signature. The methylome and RNA-Seq data were matched to determine whether the observed methylation led to gene silencing (functional methylome). Of note, Hedgehog signaling did not emerge as a primary target from our analysis. We targeted the pathways predicted to be active by our data in a translational trial and obtained a dramatic therapeutic response. A thorough understanding of the BCC functional methylome leads to innovations in BCC treatment. Our results warrant high-throughput analysis of BCC responses to known medical interventions in order to uncover their mechanisms of action.

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***In vivo* role of TPL2 activation in squamous cell carcinoma, keratoacanthoma-type development and progression**

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Skin cancer is the most common cancer in the United States with more than 3.5 million new cases diagnosed every year. Although basal cell carcinoma is more common, squamous cell carcinoma (SCC) with an estimated 700,000 new annual cases result in far more fatalities. In fact, deaths resulting from SCC are only surpassed by melanoma in terms of cancers arising in the skin. Squamous cell carcinoma, keratoacanthoma-type (SCC, KA-type) resembles SCC, but it is unique cancer because of its rapid growth. Currently, we do not know how SCC, KA-type arises, but the occurrence of this type of SCC is clinically important since it is a known side effect of a recently-approved drug (Vemurafenib) for advanced melanoma. Previously, our laboratory made a special mouse in which a cancer-related gene (TPL2) was inserted into the mouse DNA to over-express the gene in all mouse tissues. Surprisingly, these mice developed SCC, KA-type. These findings tell us that TPL2 over-expression may be one of causes underlying SCC development. Therefore, we seek to prove that SCC development in these mice has clinical features similar to those in humans. If so, these mice will be able to serve as a new mouse model system to study SCC in the future. Most importantly, we will be able to take advantage of this mouse model to test new anti-cancer drugs for the treatment of SCC.

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Organ transplant recipients with Merkel cell carcinoma have reduced overall, disease-specific, and progression-free survival independent of stage at presentation

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Background: Merkel cell carcinoma (MCC) is an aggressive cutaneous malignancy. Various forms of immunosuppression have been associated with increased incidence of MCC and decreased MCC-specific survival. We sought to identify whether solid organ transplant recipients (SOTR) with MCC had decreased progression-free, disease-specific, and overall survival compared to immunocompetent (IC) MCC patients. Methods: This is a retrospective cohort study examining 103 cases of MCC diagnosed between 1991 and 2012. Diagnosis and clinical data was collected from medical records. Progression-free, disease-free, and overall survival were visualized with Kaplan-Meier methods. Cox regression models were generated for the outcomes of progression, MCC-specific death, and death from any cause, adjusted for patient sex, age at diagnosis, and stage at presentation. Results: 8 SOTR and 95 IC MCC patients were followed for a median followup time of 24.2 months (range 1.5-152.4). There were 31 deaths from all causes among the IC patients (33.0%) and 5 in the SOTR (62.5%). Median time to death was 6 and 1.7 years in each group, respectively. SOTR had a 9-fold increased hazard for all-cause death (95% confidence interval (CI) 2.7-30.5, p<0.0001) and an 8-fold increased hazard for MCC-specific death (95% CI 1.9-32.7, p=0.004), when adjusted for sex, age, and stage at presentation. SOTR also had a 4-fold increased hazard for progression (95% CI 1.5-10.3, p<0.0001). SOTR had a significant reduction in 1-year overall survival compared to IC patients, 46.9% (95% CI 12.0-76.3) vs. 86.9% (95% CI 77.5-92.5, logrank p=0.0018). 1-year MCC-specific survival was similarly reduced in SOTR, 56.3% (95% CI 14.7-84.2) vs. 95.2% (95% CI 87.6-98.2, logrank p=0.0001). Conclusions: SOTR have a significant reduction in overall survival, MCC-specific survival, and progression-free survival compared to IC patients with MCC, even when adjusted for stage at presentation. More research is needed in order to improve MCC outcomes for SOTR.

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High frequency of *PTEN* mutations in pre-malignant and malignant pigmented lesions in xeroderma pigmentosum

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We examined the relationship between pre-malignant pigmented skin lesions and melanomas in xeroderma pigmentosum (XP) patients who have defective DNA excision repair and 1000-fold increase in melanomas. Unlike freckles, the XP lesions had a lentiginous appearance with markedly increased numbers of melanocytes clinically and histologically. Using laser capture microdissection, we performed DNA sequencing on 18 pre-malignant pigmented lesions (benign and atypical nevi) and 75 melanomas (melanomas *in situ* and invasive melanomas) from 10 XP patients. We found a similar high frequency of *PTEN* mutations in nevi and in melanomas [61% (11/18) vs 53% (39/73)]. Both had a very high proportion of UV type mutations (occurring at adjacent pyrimidines) [91% (10/11) vs 92% (36/39)]. However, fewer of the *PTEN* mutations were missense in the XP nevi than in the melanomas [33%(7/21) vs 66% (41/62) $p<0.05$]. Immunostaining of the lesions using an antibody to phospho-S6 indicated activation of the mTOR pathway in the atypical nevi and melanomas but not in the benign nevi. In contrast to melanomas in the general population, the frequency of *BRAF* mutations (11%, 7/61), *NRAS* mutations (21% 13/62) and *KIT* mutations (21% 6/28) in the XP patients was lower than for *PTEN*, and the *BRAF* V600E mutation hotspot was not detected. Thus the clinical and histological appearances and the molecular pathology of XP nevi and melanomas were different from nevi and melanomas in the general population. The increased frequency of *PTEN* mutations and activation of the mTOR pathway in the XP patients may offer an opportunity for melanoma control by use of *PTEN*/ mTOR pathway inhibitors.

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Soluble adenylyl cyclase alters cellular metabolism to reflect a cancer-like phenotype

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Cancer cells have an altered metabolic state whereby they rely heavily on glycolysis even though they are provided with adequate oxygen levels. This metabolic state is called the Warburg effect and is essential for the growth of cancer. Soluble adenylyl cyclase (sAC) is a novel metabolic sensor that influences many cellular functions, primarily cell growth and proliferation, through its generation of cyclic AMP. Recent publications demonstrate the importance of sAC in hyperproliferative keratinocyte skin diseases such as squamous cell carcinoma. Our lab is interested in studying the role of sAC in cancer. sAC knockout (KO) cells are more easily transformed as compared to wild type (WT) cells likely due to activation of Ras. We wanted to examine whether sAC also influenced the ability of cells to respond to changes in nutrients as this might also influence cellular transformation. sAC KO and WT mouse embryonic fibroblast (MEF) cell lines were grown in different concentrations of glucose over one week and cell number was counted using a Beckman Coulter Counter. At the highest concentrations of glucose, KO cells grew at a faster rate resulting in a 2-fold increase in total cell number. Repeated measures ANOVA revealed a significant difference between KO and WT cell growth ($p = 0.0007$). Below 3 mM glucose, KO cells grew more slowly than WT cells, and over time cell death occurred. These results suggest that KO cells are more sensitive to glucose availability than WT cells. Incubation in 2-deoxyglucose led to a dose-dependent decrease in cellular growth in both KO and WT cells with an identical IC50; however, KO cells had a greater extent of inhibition (20% in WT cells and 75% in KO cells; $p<0.0008$). Increased sensitivity to glucose concentration and inhibitors of glycolysis is a hallmark of cancer cell metabolism and suggests that sAC KO cells exhibit a metabolic state consistent with cancer. Better understanding of the role of sAC in metabolic control may provide a more complete understanding of cancer development and insight into novel drug targets.

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The covert stages of cancer immunoediting provide prevention targets

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The earliest events of multistage cancer development remain a mystery, especially regarding elements of the pre-cancer microenvironment. While a cell-intrinsic understanding has taken shape, several intercellular interactions that precede clinical presentation remain largely uninvestigated. In part, this is due to the difficulty of modeling and observing primary carcinogenesis. One process that precedes the onset of malignancy is immunoediting, whereby effectors of the immune system exert selective pressure on a burgeoning neoplasm. To emerge from this pruning process and proceed to malignancy, a clone(s) of cells must acquire immune-evasive characteristics. We hypothesize that this is a rate-limiting step in carcinogenesis and a barrier against cancer cell heterogeneity in most cancer types. The drastic increase in Squamous Cell Carcinoma (SCC) incidence among immunosuppressed organ transplant recipients suggests that intact immune function can limit skin malignancies. There have also been striking case reports detailing the transfer of malignant melanoma from organ donors to recipients. While there was no clinical evidence of cancer in the donors, the recipients experienced immediate outgrowth of melanomas from transplanted organs upon receiving immunosuppressant drugs. This suggests that the donor immune systems were able to restrict the outgrowth of micrometastatic lesions. Since an understanding of these immunologically restricted neoplasms may provide opportunities for cancer prevention, we have developed an in vivo model of carcinogenesis that allows tracking of pre-malignant clones in immunocompetent microenvironments. Using inducible Cre-lox technology, we are able to activate common initiating mutations of SCC in combination with fluorescent reporters. This enables us to trace the entire process of carcinogenesis via whole animal imaging and intravital microscopy. More importantly, we are able to isolate immune-controlled cells for further expression profiling, assessment of cellular hierarchy, relative heterogeneity measurement, and screening for drug/small molecule sensitivity.

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Suppression of autophagy is associated with enhanced regression of skin tumors in mice

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Autophagy has been implicated in the pathogenesis of cancer, but whether it acts as a repressor or promoter of tumorigenesis in the skin has not yet been investigated. We have thus performed a multistage skin carcinogenesis study in mice deficient of epidermal expression of the essential autophagy-related gene Atg7 (K14-Cre ATG7 F/F) and in control mice (ATG7 F/F). 7, 12-dimethylbenz(a)anthracene was used as carcinogen and 12-O-tetradecanoylphorbol-13-acetate (TPA) was applied as tumor promoter for 25 weeks. Skin papillomas arose with similar kinetics in mice of both genotypes. However, papillomas in the K14-Cre ATG7 F/F mice were significantly smaller than in ATG7 F/F mice ($p=0, 05$). Moreover, deficiency of keratinocyte autophagy was associated with significantly higher incidence of tumor regression at all time points between week 12 and week 45. There was no difference in the rate of conversion from papillomas to carcinomas. Histological analysis of tumors revealed that the suppression of epithelial autophagy led to a substantial accumulation of p62, a multifunctional protein involved in the control of protein turnover and pro-inflammatory signaling. Together, these data suggest that autophagy does not protect from tumor formation but rather supports the survival of established tumors in the epidermis.

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Gene expression profiling demonstrates that the effects of PTCH1 suppression are not fully reversed upon pharmacological inhibition of SMOOTHENED in human keratinocytes

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BCC is predominantly associated with mutational inactivation of the PTCH1 gene product resulting in hyper-activation of the HEDGEHOG developmental pathway. Tumour formation is linked to induction of the GLI (GLI1 and GLI2) transcription factors via a pathway that is thought to require the transmembrane protein SMOOTHENED (SMO); accordingly, SMO is attracting much interest as a therapeutic drug target. Although there has been a certain degree of success in treating BCC with anti-SMO compounds, many tumours are only partially or unresponsive indicating that SMO-independent mechanisms may contribute to tumour viability. To further understand how reduced PTCH1 function contributes to BCC, RNAi was employed to suppress PTCH1 in immortalised NEB-1 human keratinocytes. Compared to control NEB1-shCON cells, NEB1-shPTCH1 cells displayed more compact colony formation and increased GLI1 (but not GLI2) expression; however, whereas GLI1 was suppressed by the SMO antagonists KAAD-Cyclopamine and SANT-1 in NEB1-shCON cells, its increased expression was maintained in NEB1-shPTCH1 cells. Indeed, cDNA microarray profiling revealed that over 80% of transcripts that were differentially expressed in NEB1-shPTCH1 cells (>2-fold, $p<0.01$) remained differentially expressed in the presence of KAAD-CYC and included CXCL10 and CXCL11 which have recently been shown to be expressed at high levels in BCC. These data indicate that many of the effects of PTCH1 loss are SMO-independent (or they are unresponsive to anti-SMO drugs) in cultured human keratinocytes and, therefore, the mechanism(s) leading to BCC formation may be more complex than current dogma purports.

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Romidepsin, but not Pralatrexate, alters expression of a genetic signature in CTCL

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Romidepsin, a histone deacetylase (HDAC) inhibitor, was recently FDA-approved in the US for the treatment of CTCL. HDACs exhibit increased activity in cancer; they both catalyze the removal of acetyl groups from histone lysine residues and deacetylate transcription factors, together producing a modulation of gene expression. HDAC inhibitors thus work to correct altered gene expression. The specific mechanism by which romidepsin changes gene expression in CTCL has not been characterized. To determine the effects of romidepsin on gene expression in CTCL, we isolated peripheral blood mononuclear cells from six Sézary syndrome (SS) patients. PBMCs were cultured overnight, and romidepsin was added at three concentrations. Pralatrexate, a folate analog metabolic inhibitor that competitively inhibits dihydrofolate reductase and halts DNA synthesis by depleting thymidine was used as a control for drug-specific alteration of gene expression. Cells were harvested at 24 and 48 hours following treatment. RNA was isolated, and qRT-PCR was performed on a set of genes identified by our group and others as dysregulated in CTCL. RNA isolated from healthy controls was used as a negative control. To ensure that the observed changes in gene expression were attributable to changes within malignant cells and not the result of decrease in cell numbers due to death during culture with the drugs, we performed flow cytometry on selected samples to confirm that the proportion of live malignant cells in the cultures was constant. Romidepsin induced significant gene expression alterations of many genes, including but not limited to PLS3, TOX, and RUNX3. Many of these changes resulted in normalization of gene expression. PLS3 expression, which can be greater than 1000 times normal in SS was decreased by over 80%. Pralatrexate, however, did not induce changes in expression of these genes. This work provides new insight into the mechanism of action of romidepsin. The observed changes in selected genes may be considered in the future studies for validation as markers predictive of response to romidepsin.

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K-homology (KH)-type splicing regulatory protein (KSRP) protects against inflammation-mediated cutaneous carcinogenesis by destabilization of inflammatory mediators

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Progressive research has enlightened the association of inflammation and the development of cancer. K-homology (KH)-type splicing regulatory protein (KSRP) plays an important role in the control of inflammatory gene expression by modifying the post-transcriptional mechanisms. KSRP triggers rapid degradation of mRNAs for various cytokines, chemokines, and other inflammation-related proteins by interacting with adenylate-uridylate rich element elements (AREs) in their 3′-untranslated regions. To determine the role of KSRP with respect to inflammation mediated tumor development, KSRP knockout (KSRP-/-) and wild type (WT) mice were subjected to a standard two-stage 7,12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) model, for initiation and promotion respectively, in which inflammation plays a central role. The percentage of mice with tumors was much greater ($p<0.05$) in KSRP-/- mice than in WT mice. Similar results were obtained when the data were evaluated as the cumulative number of tumors per group. A significantly high ($p<0.05$) rate of conversion from benign papillomas to malignant carcinomas in KSRP-/- mice was observed compared to the WT mice. Absence of KSRP caused significant up-regulation ($p<0.05$) of pro-inflammatory cytokines like IL-1β, IL-6, and TNF-α in KSRP-/- mice compared to WT mice. Moreover, inflammation induced skin hyperplasia and pro-inflammatory mediators such as, COX-2 and iNOS were substantially upregulated ($p<0.05$) in KSRP-/- mice compared to WT mice. These results demonstrate that KSRP has a vital role in regulation of inflammation. Thus, compounds that can enhance the ability of KSRP to bind to inflammatory mRNAs can be screened and used to develop therapeutic strategies for inflammation-mediated carcinogenesis.

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Laser cancer prevention: Is the ablative laser for rejuvenation preventive against ultraviolet-induced skin cancer?

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Non-melanoma skin cancers, one of the most commonly diagnosed cutaneous malignancies in human, are mainly caused by prolonged ultraviolet(UV) exposure. However, there is no effective prevention other than avoiding sun exposure. Recently, ablative fractional photothermolysis (FP) laser treatment is actively being carried out for facial rejuvenation. We elucidated whether the occurrence of skin tumor caused by exposure to ultraviolet light can be decreased by with multi-sessions of ablative FP with CO2 laser. Two groups of hairless mice were treated with either FP or nothing at 3-weeks interval during the 20 weeks of UV exposure period, simultaneously exposed to 60 mJ/cm2 of UVB and 1.8 J/cm2 of UVA three times per week for first 20 weeks. The other group was treated with only FP without UV exposure. In the following 10 weeks, mice were examined for tumor development every 2 weeks without any treatments. At the 30-week, termination of the experiment, representative tumors were taken to evaluate the type of tumor. In addition, we evaluated the change of skin barrier function, thickness, elasticity at 12 week, 20week. At 30weeks, FL treated group showed significantly lower average size and the number of tumors than UV exposed group. Tumor occurred in FL treated group 2~3 weeks later than UV exposed group. There was no tumor development in the FL-only treated group, and their skin looked more smooth, tightened. All mice of UV exposed group developed skin tumor, but 64.3% mice of FL treated group developed skin tumor. And UV exposed group showed significantly increased in trans-epidermal water loss, than FL-treated group. In conclusion, Ablative FP can be effective for not only the rejuvenation but also the prevention against skin tumors induced by UV.

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Screening for cellular binding partners of the Merkel cell polyomavirus-small t antigen by yeast two-hybrid system

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The purpose of this study was to investigate the role of the Merkel Cell Polyomavirus (MCpyV) Small t antigen (St) in Merkel Cell Carcinoma (MCC) through the determination of its cellular binding partners using the yeast two-hybrid system. MCpyV, which was recently identified as an infectious etiology of MCC, expresses two "early" proteins: the large T (LT) and St antigens. The LT antigen is truncated in MCC, resulting in loss of its helicase activity and putative inactivation of its p53 tumor suppressor binding capacity, suggesting the St antigen has a more significant role in carcinogenesis. To characterize the role of the St antigen, yeast two-hybrid technology was employed using full-length MCPyV St antigen as "bait", which was then introduced to Universal Human and Bone Marrow libraries. The quantitative α-Gal Assay was utilized to determine differences in the relative strength of binding between mutant forms of interacting proteins. The screening experiments using a Universal Human Normalized library revealed six human proteins as binding partners of MCPyV St antigen. Four clones showed cereblon (CRBN) sequences suggesting that CRBN is a primary binding partner for MCPyV St antigen in our experiments. Co-transformation experiment in Y2H Gold yeast cells and the α-Gal assay confirmed the CRBN-St interaction. CRBN may act as a DDB2 competitor component of E3 protein ligase complex and may have a putative role of response to ultraviolet exposure and for subsequent DNA repair. Additionally, the human bone marrow library revealed further notable protein-protein interactions with the St, including PP2A and β-Hemoglobin. The findings of these experiments are currently being confirmed through co-immunoprecipitation and pull-down assays. We expect that the results will identify human proteins affected by St, which will aid in the design of new therapies that can be used to combat the highly malignant MCC.

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A comparative study of Langerhans Cell Histiocytosis (LCH) and non-melanoma skin cancer transcriptional profiles

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LCH is a rare and potentially fatal disorder of unknown aetiology, although recent reports of frequent BRAFV600E mutations are consistent with neoplasia. We hypothesized that comparison of LCH gene expression profiles with basal and squamous cell carcinoma (BCC and SCC) might reveal common processes in cutaneous tumourigenesis, or identify biomarkers of clinical relevance. We profiled gene expression in biopsies of cutaneous LCH and normal epidermal LCs, whereas SCC and BCC data were retrieved from the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo; BCC GSE6520 and SCC GSE2503). A combination of open access and proprietary software, including MetaCore (www.genego.com/metacore.php), were used to identify transcripts discriminating disease and normal compartments for each dataset (fold change thresholds >1.5, $p<0.05$), followed by analysis between disease datasets. 772 genes were shared between LCH and SCC, 407 between LCH and morphoeic BCC, and 93 were common to all datasets. Enrichment analysis revealed significant common pathways, including oxidative phosphorylation (LCH v SCC: p=4.3x10-8; LCH v BCC: p=1.1x10-8) and MAPK-associated RAN regulation pathway (LCH v SCC: 1.6x10-5; LCH v BCC: p=2.8x10-3). We also observed an overall alteration in ECM remodelling, although constituent transcripts varied across disorders. For example, MMP1 expression increased 13 fold in LCH and 74 fold in SCC, but decreased 9 fold in BCC. SPARC expression was up 3 fold in LCH and 20 fold in BCC, with no change in SCC. IL8 expression decreased 11 fold in LCH but increased 51 fold in SCC, with no change in BCC. Histological evaluation of ECM organisation confirmed active collagen and elastic fibre remodelling in the microenvironment of all diseases studied. As the oncogenic BRAFV600E mutation has been linked to suppression of oxidative phosphorylation and ECM remodelling in other disease states, we are currently investigating the relationship between ECM remodelling, mitochondrial dysregulation and BRAF mutation status in LCH.

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Fc gamma receptor II (CD32) as novel regulator of keratinocyte proliferation and differentiation

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During the process of maturation there are critical steps that have to be strictly regulated. Two of them are cell proliferation and differentiation. Alterations or defects in these steps of keratinocyte maturation cause increased proliferation and impaired differentiation leading to cancer development including cutaneous squamous cell carcinoma (SCC). We showed previously, that the Fc gamma receptor II (CD32) that is commonly known to be expressed on immune cells is also present on keratinocytes. We found all CD32 isoforms (A, B and C) expressed on primary human keratinocytes as well as on the immortalized keratinocyte cell line HaCaT and the cutaneous squamous cell carcinoma cell line SCC13. All isoforms were higher expressed on cancer cells than on keratinocytes. Additionally we found a correlation of CD32 expression and differentiation of primary human keratinocytes. Blocking of CD32 by a specific antibody leads to an increase of keratinocyte proliferation while the proliferation of SCC13 cells is not altered. New data provide further evidence that CD32 plays a role in proliferation and differentiation of keratinocytes. We overexpressed CD32 in SCC13 cells and saw decreased proliferation in these cells. Preliminary data show the same also for primary keratinocytes. Since human IgG is the natural ligand of CD32 we challenged primary keratinocytes and SCC13 cells with doses from 1 ng/ml to 1 ug/ml of human IgG. While SCC13 cell did not show any reaction primary keratinocytes showed an increased proliferation at the highest treatment concentration. To address the involved signaling pathways we treated primary human keratinocytes with CD32 blocking antibody and looked for phospho-ERK activation. After 15 minutes we were able to detect activation of ERK. These data provide evidence that CD32 plays a role in the regulation of proliferation and differentiation of keratinocytes via ERK signaling.

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Three siblings with epidermodysplasia verruciformis caused by an aberrant splicing of the TMC8 gene

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Epidermodysplasia verruciformis (EV) is a rare inherited skin disease. Patients with EV develop cutaneous squamous skin cancer (SCC) under the influences of HPV infection and UV irradiation. Majority of patients are carrier of homozygous or compound heterozygous mutations in the TMC6 or TMC8 gene. In consequence of these mutations patients with EV are susceptible to skin infections by particular types of human papilloma viruses (HPV) that are considered to be innocuous for the general population. To date mutations of only 14 families with EV are described in the literature. We investigated a large family with at least three clinically affected children and identified a novel splice site mutation in TMC8, which was homozygously present in all affected children but heterozygously in both parents. Analysis of cDNA revealed an aberrant spliced product in all affected children and both parents;the parents showed the wildtype spliced product additionally. In this report we describe the first splice site mutation within TMC8 leading to EV in all homozygous carrier.

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Loss of collagen VII increases aggressive tumor behavior in Col7al hypomorphic mice

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Recessive dystrophic epidermolysis bullosa (RDEB) is an inherited severe skin fragility disorder that is caused by abnormalities or absence of collagen VII. This collagen is one of the major proteins that stabilize the dermal-epidermal junction in the skin. Its lack is associated with trauma-induced skin blistering and healing with scarring. Patients with RDEB often develop partial or total mitten deformities of the hands and feet and are also at greater risk of developing squamous cell carcinomas (SCC), which are aggressive and often lethal. The molecular events leading to the development of aggressive SCC tumors in RDEB remain elusive. In this study, we investigated the susceptibility of Col7al hypomorphic mice (a mouse model for RDEB with only 10% of functional collagen VII) to induced tumor formation by means of chemical carcinogenesis. Wildtype mice produced benign papillomas, whereas Col7al hypomorphic mice developed aggressive, invading tumors within a 20-week treatment period with DMBA and TPA. Immunofluorescence staining of the tumors revealed the characteristic features associated with high risk tumors such as increased proliferation, elevated epithelial to mesenchymal transition (EMT), high invasion of proliferating keratinocytes, and high inflammatory infiltrates, in the hypomorphic mice, in contrast to the wildtype tumors. Our findings uncovered a molecular mechanism involving TGFβ1 in aggressive behavior of tumors in hypomorphic Col7al mice. Absence of collagen VII increases TGFβ1 and TGFβ1, in turn, increases tissue stiffness by increasing collagen I synthesis and myofibroblast maturation, and thereby activating the FAK-AKT-MMP9 pathway leading to highly invading, aggressive carcinomas. Moreover, our results also suggest that targeting TGFβ signaling may have therapeutic potential and reduce the risk of RDEB-associated aggressive squamous cell carcinomas.

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Distinct mechanisms mediate cellular proliferation by p63 and p73 in human skin squamous cell carcinoma

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Skin squamous cell carcinomas (SCCs) arise on sun-exposed areas and may behave aggressively, rapidly invading the surrounding tissue and resulting in recurrence or metastasis. In this study we investigated the role of the p53 family members p63 and p73 in early stages of SCC development using two cell lines derived from facial SCCs carrying a mutant p53 allele. In head and neck SCC (HNSCC) p63 has been shown to play a crucial role in promoting cell survival, as p63 depleted cells undergo apoptosis in a p73-dependent manner. Here we show that in contrast to HNSCC, in skin SCCs both p63 and p73 depletion induced cell cycle arrest without affecting cell survival, whereas p53 depletion had no effect on cell proliferation. In SCCs the major p63 isoform is the DNp63alpha, which has been reported to act primarily as a transcriptional repressor in the context of cancer cells. Here we show that both DNp63alpha and p73 acted primarily as activators rather than repressors in the context of cutaneous SCCs. Identification of differentially regulated genes in p63 or p73 depleted cells revealed that many early target genes were in common, however a significant fraction was regulated specifically by either p63 or p73, while only very few genes were controlled in the opposite fashion. Among this latter gene category, the cell cycle inhibitor CDKN1A was repressed by p73, while it was modestly induced by p63. Consistently concomitant depletion of both p73 and p21 resulted in cell cycle progression, whereas p21 depletion was unable to rescue cell cycle arrest in p63 depleted cells. p63 positively regulated several ERBB ligands by direct transcriptional activation. Consistently treatment with ERBB ligands was able to rescue cell proliferation in p63 depleted cells, but not in the p73 depleted ones. These data provide the first evidence that p63 and p73 play crucial and partially independent roles in sustaining cell proliferation during early stages of skin tumorigenesis.

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Molecular analysis of primary cutaneous aggressive T-cell lymphomas

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We explore genomic alterations possibly involved in tumorigenesis of aggressive primary cutaneous T-cell lymphomas (CTCL). In particular we focused on 17 cases of CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma (AECTCL), 8 of peripheral T-cell lymphoma, not otherwise specified (PTL-NOS) with CD4+ (4) or CD8+ (4) phenotype, as well as 4 cutaneous gamma-delta lymphoma (CGDL). By array-based comparative genomic hybridization investigations, we found the presence of extensive gains and losses of both large and small chromosomal regions; copy number gains were more frequent than losses. We found some alterations present in all entities such as loss of 9p21.3 and gains of 11q12.3-q13.2 and 17q21.31. Some aberrations seems to be more frequent in CD8+ lymphomas (both AECTCL and PTL-NOS-CD8+) (gains of 3p21.33-p21-2, 7q11.23, 7q21.2-q22.1, 8q24.3, 9q33.3-q34.2, 11pter, 17q25 and trisomy 19) while others were found only in CD4+ lymphomas (losses of 2p24.2-p21, 6q24.2-q27, 10p and 10q). Gains of 7q36.1-q36.3, 16p13.3 and trisomy 22 as well as loss of 8p22 were found only in AECTCLs and should be related to epidermotropism, while loss of 13q were observed only in PTL-NOS (both CD8+ and CD4+). In CGDL, recurrent copy number alterations (observed in 50% to 75% of cases) were gain of 2q13-qter and loss of 6q23.3. Loss of 9p21.3, harbouring CDKN2A and CDKN2B genes, and gain of 17q21.1 (STAT3 and STAT5) regions were imbalanced in all four entities studied and they may be involved in aggressiveness of tumors and poor outcome. Trisomy of chr19 (JAK3) was shared by CD8+ lymphomas suggesting a possible role of JAK3/STATs pathway in the pathogenesis of these malignancies. Although some genetic aberrations detected in our study have been described in tumoral stage of mycosis fungoides, we suggest that the combination of aberrations appear characteristic in these aggressive disorders.

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Mutant p53 in cutaneous squamous cell carcinoma

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p53 mutation results in loss of its normal tumour suppressor activity and in some circumstances can result in tumour-promoting gain of function. Upregulation of mutant p53 protein levels is thought to be required for this gain of function activity. Mutant p53 accumulation is not simply due to intrinsic properties of the mutants but also requires other cellular events. The mutation and accumulation of p53 are early steps in cutaneous squamous cell carcinoma (cSCC) development. We are investigating the potential of targeting mutant p53 for cSCC therapy. Low passage cSCC cell lines were transfected with individual siRNA complementary to different sequences in p53 and the effect on cell growth was assessed. Knockdown of mutant p53 inhibits the proliferation of SCCIC1 cells. Reducing the level of mutant p53 may consequently be a therapeutic approach for cSCC. Mutant p53 protein levels are high in cSCC cell lines compared with wild-type p53 in normal human keratinocytes. To identify therapeutically tractable ways to reduce mutant p53 protein levels we are investigating the mechanism(s) of mutant p53 accumulation in cSCC. We have verified that mutant p53 accumulation in cSCC is due to a reduction in p53 protein degradation. The general deubiquitinating enzyme (DUB) inhibitor PR-619 reduces the levels of p53 in cSCC cell lines. The proteasome inhibitor bortezomib blocks the effect of PR-619 on mutant p53 expression. This is consistent with an involvement of DUBs in the stabilisation of mutant p53 through protection against proteasomal degradation. The role of individual DUBs in mutant p53 stabilisation in cSCC is currently being investigated.

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Immunohistochemical assessment of MRG-binding protein and polo-like kinase 1 over-expression in human cutaneous SCC

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Cutaneous squamous cell carcinoma (cSCC) is the second most common cutaneous neoplasm affecting Caucasian populations and can cause significant morbidity and mortality. We have recently shown MRG-binding protein (MRGBP) and Polo-like Kinase 1 (PLK1) to be up-regulated in cSCC compared with normal and psoriatic skin and that siRNA knockdown induces apoptosis in cSCC keratinocytes with no effect on normal cells. The aim of this study was to assess the extent of protein expression of MRGBP and PLK1 in cSCC by immunohistochemistry using two anti-MRGBP antibodies (raised against the N- and C-termini respectively) and a commercial anti-PLK1 antibody. A total of 43 tumour samples with different histological grades were stained and compared with non-UV exposed normal skin samples. PLK1 was detected in mitotic cells in both cSCC and normal skin samples. MRGBP antibodies were validated previously with Western blotting and overexpression constructs as well as siRNA. MRGBP is expressed in the nucleus of proliferative keratinocytes localized in the basal layer of the normal epidermis and in the non-differentiated areas within cSCC. Nuclear staining was also observed in fibroblasts and endothelial cells in both normal and tumour samples. Both antibodies showed similar nuclear localization but the N-terminal anti-MRGBP antibody also readily bound within the cytoplasm of differentiated keratinocytes in normal skin and regions of cSCC undergoing notable differentiation suggesting that MRGBP localizes to two distinct cellular compartments. In conclusion we demonstrate that MRGBP and PLK1 are overexpressed in cSCC compared with normal skin and that MRGBP localizes to the nucleus in dividing keratinocytes and stromal cells as well as the cytoplasm of differentiating keratinocytes.

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WITHDRAWN

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Depletion of MRG-binding protein invokes TIP60 histone acetyltransferase-dependent apoptosis in cutaneous squamous cell carcinoma cells

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Cutaneous squamous cell carcinoma (cSCC), the most frequent skin neoplasm with malignant potential, is responsible for 1 in 4 skin cancer related deaths. A targeted therapy for cSCC remains an unmet clinical need. C20orf20, encoding the TIP60 histone acetyltransferase (HAT) complex member MRG-binding protein (MRGBP), is overexpressed in cSCC versus normal keratinocytes and RNAi-mediated depletion induces apoptosis in vitro and attenuates tumour growth in vivo. Normal keratinocytes are unaffected. Lactate dehydrogenase release and caspase activation assays reveal a profound induction of cell death following knockdown (up to 3-fold increase in cytotoxicity compared to control siRNA, n=20) in a caspase 3/7 dependent manner (5-fold increase in caspase activity, n=4). A RNAi screen of putative MRGBP interacting partners identified 6 proteins (4 TIP60 related, 2 unrelated) where knockdown invoked a similar response (TRRAP, DMAP1, EPC1, VPS72, RSL1D1 and VAV2). However, depletion of the HAT catalytic subunit, TIP60, had little effect. Interestingly, parallel knockdown of both MRGBP and TIP60 resulted in significantly attenuated cell death compared to siMRGBP alone (151% ± 4.1 vs 227% ± 0.06 increase in cytotoxicity relative to control siRNA, n= 7) indicating a TIP60-dependent mode of death. Finally, gene expression profiling of siMRGBP cells revealed a 3.45-fold increase in expression of ACSL4, the gene responsible for the synthesis of the precursor to acetyl-CoA, the sole source of acetyl group used by HATs. Together these data suggest that a MRGBP-containing complex confers a pro-survival drive in cSCC through regulation of TIP60. Our data show that RNAi depletion of MRGBP unleashes caspase-dependent apoptosis through disruption of the MRGBP/TIP60 balance resulting in pro-death HAT activity. Regulation of MRGBP and the TIP60 complex may therefore provide an effective strategy for future cSCC-specific therapies.

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Targeting Merkel cell carcinoma dependence on Bcl-2 family members promotes efficient cell death *in vitro* and *in vivo*

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Merkel cell carcinoma (MCC), a rare but aggressive cutaneous neoplasm, has a poor prognosis at late stages of disease with no proven chemotherapeutic regimens. Although the Merkel cell polyomavirus (MCPyV) is emerging as a potential key contributor to MCC tumorigenesis, signaling pathways modulating progression and survival have not yet clearly been defined. Here we provide both genetic and pharmacological evidence that the anti-apoptotic Bcl-2 family members (Bcl-2, Bcl-xL and Mcl-1) act as key survival factors that impart a point of vulnerability in MCC that can be exploited for rational drug therapy. Based on immunoblot analysis of 19 tumors, most MCCs expressed high levels of Bcl-2 and Bcl-xL(94% of tumors) and Mcl-1 (79% of tumors). In a panel of 12 novel MCC cell lines we established for functional studies, 9 of which are tumorigenic in nude mice, expression patterns of the Bcl-2 family members mimicked those seen in human tumors. We inactivated individual Bcl-2 family members by short hairpin-driven RNA interference which promoted MCC cell line death, indicating a dependence on these proteins for survival. In parallel, we analyzed the impact of targeted inhibition of Bcl-2/Bcl-xL by the small molecule ABT-263 (Navitoclax). A dose-dependent decrease in cell proliferation was observed in 11 of 12 MCC lines treated with ABT-263. Detailed analysis of four lines indicated that regardless of MCPyV status or Mcl-1 levels, ABT-263 induced p53-independent apoptosis (>83% death, 5μM ABT, 72h), characterized by rapid cleavage of caspase-3 and PARP. Furthermore, ABT-263 treatment of mice with established MCC xenografts (>250mm^3) led to a rapid and sustained halt in tumor growth ($p<0.004$), which was accompanied by a dramatic increase in the number of apoptotic tumor cells. Our results indicate that the use of Bcl-2 family antagonists may provide a valuable addition to MCC treatment regimens by reversing an intrinsic resistance to cell death in these aggressive malignancies.

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UISO cell line is not representative of Merkel cell carcinoma tumors

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When using a cell line to study cancer, phenotypic similarity to the original tumor is paramount. Multiple cell lines have been used to study the cutaneous neuroendocrine tumor Merkel cell carcinoma (MCC), but little has been done to characterize how closely they model native MCC tumors. The cell line UISO has been used extensively in the research of MCC since it was first characterized in 1993. To determine its similarity to MCC tumor samples, we characterized the UISO cell line and two other commonly used MCC cell lines (Mkl-1 and WaGa) with gene expression microarrays. Using whole transcriptome gene expression signatures and a computational bioinformatic approach, we identified significant differences between the UISO cell line and the two other MCC cell lines. Furthermore, we identified significant differences between the UISO cell line and fresh frozen MCC tumors, irrespective of the Merkel cell polyomavirus status in the tumors. In comparison, the Mkl-1 and WaGa cell lines more closely approximated the global transcriptome of MCC tumors. Next, we characterized UISO cells grown as xenografts in immunocompromised mice. Histologically and immunophenotypically UISO tumors did not resemble typical MCC tissue. In contrast, WaGa xenograft tumors showed immunohistological features consistent with MCC. Spectral karyotyping (SKY) and short tandem repeat (STR) analysis of the UISO cell line matched that of the originally described UISO cells, ruling out contamination by another cancer cell line. Our results indicate that the UISO cell line is not representative of MCC tumors, whereas the Mkl-1 and WaGa cell lines more closely model MCC tumors.

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Combination of histone acetylation and dna demethylation is synergistic in ctcl treatment

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Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of malignancies derived from skin-homing T cells. CTCL is a chronic and at times debilitating disease with many open questions regarding its pathogenesis and treatment. The current moderate success of histone deacetylase inhibitors (HDACi) in the clinical practice for CTCL treatment encourages the investigation of combinational therapy in order to increase the response rate. To determine the effect of the combination treatment with Romidepsin and Azacytidine compared to single agent or untreated cells, we performed MTT assays for cell viability and Annexin V staining via flow cytometry for apoptosis. CTCL cell lines (MyLa2973 and SeAx) were cultured in the presence/ or absence of Romidepsin 1.25nM and/ or Azacytidine 2.5uM for 48hrs. The combination treatment showed an increase in apoptosis and necrosis in a time and dose dependent manner compared to single agent treated. Increase in cell cycle regulators such as p15, p16, p21 as well as down-regulation of CDK4 in the combination treatment suggests this combination treatment counteracts the loss of cell cycle control more efficiently in CTCL. This combination triggers an increase in cleaved caspase 9 as well as cleaved caspases 3 and 7 and an increased in the cleavage of PARP1 compared to Romidepsin or Azacytidine single treatment. Analysis of the Cancer Drug Target PCR array of the CTCL cell lines treated demonstrated a significant re-expression of a GTP binding protein from the Rho protein family, RhoB, in the combination treatment. We validated this data by PCR analysis and western blot of RhoB expression at an RNA and protein level. Defective regulation of apoptosis has been considered as a main cause for accumulation of clonal T cells and therefore we hypothesize based on our current results RhoB might have an important role in the sensitization of the tumor cells to DNA damage and subsequent apoptosis. Furthermore, peripheral blood lymphocytes (PBLs) from Sezary Syndrome patients were used to validate the results we attained in vitro.

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IQGAP1 and IQGAP3 are required for invasive epidermal tumorigenesis but are relatively dispensable for normal epidermal homeostasis

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Proteins required for neoplastic transformation, but dispensable for normal tissue function are potential therapeutic targets. In this regard, the IQGAP family of ubiquitously-expressed molecular scaffolds may be ideal. IQGAPs are frequently highly expressed in tumors, and regulate a number of cell processes related to receptor-ligand signaling, cytoskeletal-rearrangement, cell-cell communication, proliferation and invasion. Despite these critical functions, IQGAP1 knockout mice are developmentally normal, suggesting functional redundancy with other IQGAP proteins. IQGAP3 null murine tissue has not yet been generated. We sought to understand the roles of IQGAP1/3 in human epidermal maintenance and tumor progression through shRNA-mediated IQGAP 1/3 antagonism in primary human keratinocytes. Engineered IQGAPi human skin tissue was xenografted on mice with and without concurrent oncogenic Ras signaling sufficient to drive invasive SCC in control grafts. Wild-type levels of both IQGAP1 and IQGAP3 were required for invasive tumor formation, indicating that in the cancer setting, they are not functionally redundant. In contrast, IQGAP3 was dispensable for normal epidermal function, highlighting a tumor-specific requirement. However, IQGAP1 and IQGAP3 are not completely functionally redundant in normal human epidermis, as high efficiency IQGAP1 knockdown alone caused profound proliferation arrest in keratinocytes accompanied by hypophosphorylation of Rb. This result contradicts IQGAP1 dispensability in the murine model, indicating that IQGAP requirements may differ between mouse and human. The IQGAP1 effect was dose dependent, as partial knockdown of IQGAP1 in human keratinocytes was well-tolerated both in vitro and in vivo. Importantly, this partial IQGAP1 knockdown tissue still did not support tumor formation when challenged with active Ras. These findings demonstrate a role for IQGAPs in epithelial tissue maintenance and tumor development, and identify IQGAP1 and 3 as potential therapeutic targets for human tumors.

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Cyclosporine A polarizes T cells toward T22 and induces IL-22 receptor in human SCC cells *in vitro*: A mechanism driving IL-22 induced SCC proliferation

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Solid organ transplant recipients (OTR) often develop hundreds of SCC lesions prone to recurrence and metastasis. Many OTR are immunosuppressed with Cyclosporine A (CsA). Previously, we showed transplant-associated SCC (TSCC) contain a higher percentage of IL-22 producing CD8+ T cells relative to immune competent SCC (SID 2012, Abs. 615). Herein, we stimulated PBMC derived T cells with or without CsA and determined T cell phenotype by intracellular cytokine staining. We also studied the effect of CsA on IL-22 receptor (IL-22R) expression in the SCC cell line A-431 using RT-PCR. Finally, we evaluated the role of IL-22 on SCC proliferation in vitro through Ki67 expression and cell counts. We found that the number of CD4+ and CD8+ IL-22 producing cells remained constant following incubation with CsA (100ng/mL, 24h), while the number of IFN-γ and IL-17 producing cells was dramatically reduced. This resulted in an elevated proportion of T22 cells within the total CD4+ (8.8% vs 26.4%) and CD8+ (5.8% vs 19.2%) T cell population ($p<0.001$). Additionally, CsA upregulated mRNA expression of both subunits of the IL-22R, IL-22RA1 and IL-10R2B, in normal epidermal keratinocytes and A-431 cells. Further, IL-22 treatment of A-431 cells (100ng/mL, 48h, 0.1% FBS) significantly increased SCC cell proliferation, as reflected by Ki67 staining and an 8-fold increase in cell counts ($p<0.001$). CsA thus increased the percentage of T22 cells and induced IL-22R expression on SCC cells, while IL-22 increased proliferation of SCC cells. CsA may therefore drive accelerated SCC growth in transplant patients via polarization toward T22 and induction of IL-22R. Targeting the IL-22 axis may provide a novel means of TSCC therapy with a focused attack on tumor proliferation without jeopardizing allograft survival.

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A mutational storm revealed by Exome sequencing: Dramatic increase of UV signature mutations during development of skin cancer in Xeroderma pigmentosum patients

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Xeroderma pigmentosum (XP) is a rare autosomal recessive disease clinically characterized by photosensitivity, xerosis cutis and a 1000-fold increased risk to develop skin cancer. Patients with XP have defects in a DNA repair system, responsible for removal of bulky helix-distorting DNA damage mainly induced by ultraviolet (UV) radiation. Unrepaired UV-induced DNA damage can give rise to UV-signature mutations such as C to T and CC to TT transitions. This is the first study analyzing the complete exome of humans in this context. Here, we identified somatic mutations in DNA from three patients, suffering from in Germany the most frequent XP complementation group C. Skin samples of non-sun-exposed, sun-exposed and manifest skin tumors at UV-exposed body sites were compared to the respective germline genomes derived from patient blood as well as samples from individuals with normal DNA repair. Exome-sequencing was performed by next-generation sequencing with average exome coverage of >50 reads per base and >90% total target coverage, covering 36Mbp and 30.000 genes. The mutations which were only present in sun exposed or tumor tissue are called differential mutations. The load of differential mutations, especially UV signature mutations, was dramatically increased only in XP patients and not in control groups of elderly people. The highest level of differential mutations with approximately 8000 transitions was observed only in XP tumor tissue and not in other tumors of non XP patients. Interestingly excessive cancer risk in XP patients correlates well with excessive differential mutations in the XP tumors, linking UV induced mutational load and skin cancer. Annotation analysis will reveal relevant pathways with UV signature mutations to carcinogenesis. These findings can help solving the questions of the existence and magnitude of mutational threshold levels sufficient for cancer formation.

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RNAi depletion of PRSS21 in cutaneous squamous cell carcinoma keratinocytes induces maspin dependent apoptosis

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Cutaneous Squamous Cell Carcinoma (cSCC) is the most frequent skin cancer with the propensity to metastasise contributing to 1 in 4 skin cancer deaths in the UK. Currently there are no molecular targets clinically available for cSCC. Gene expression analysis identified Protease, Serine, 21 (PRSS21) to be overexpressed in cSCC in vivo (average fold change of 1.5 over three independent data sets) compared with normal skin and in vitro (7.4 fold, p<0.0005, n=8). Overexpression of PRSS21 was confirmed in a panel of cSCC cell lines (n=7) by qPCR, Western blotting and immunofluorescence. PRSS21 is frequently overexpressed in other malignancies and has been shown to promote tumour progression and metastasis in breast and ovarian cancers. We show that PRSS21 is essential for cSCC cell survival. RNAi knockdown of PRSS21 significantly decreases cSCC cell viability (40%±5% vs. non-targeting control siRNA, P<0.005, n=3), as determined by MTS assay, through increased cytotoxicity (20%±5.1% PRSS21 vs. non-targeting control siRNA, n=4) as measured by LDH release. Normal primary keratinocytes are unaffected (n=3). Cell death is via apoptosis as detected by a 2.3 (SD±0.4) fold increase in cytoplasmic nucleosomes a 25% (SD±5.2) increase in Annexin V/7AAD positive cells (p<0.05, n=2) and a 1.9 fold increase in caspase activity (p<0.001, n=3). PRSS21 has been shown to interact with the serine protease inhibitor maspin, a known tumour suppressor. Using the Proximity Ligation Assay, PRSS21 interacts with maspin in cSCC cells. Additionally, we show that knockdown of PRSS21 resulted in a concurrent 60% increase in expression of maspin and that increased caspase activity induced by PRSS21 depletion is dependent on maspin (P<0.001, n=3). In conclusion, we show that PRSS21 negatively regulates maspin induced caspase activity in cSCC cells and that maspin and PRSS21 are promising molecular targets in cSCC.

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Perifosine, an alkylphospholipid inhibitor of Akt1, a downstream target of the sonic hedgehog (Shh) pathway, suppresses growth of basal cell carcinomas (BCC) cells

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Targeted blockade of Shh pathway components like smoothened (SMO) suppresses the growth of BCCs in patients with basal-cell nevus syndrome (BCNS); however tumors frequently recur following cessation of treatment hence the need to find novel targets. One such target is Akt1 whose dysregulation drives the growth of sporadic and hereditary cancers in human populations. We have shown that Akt1 activation is essential for the growth of BCCs and partial genetic ablation of Akt1 effectively abrogates the formation of BCCs in Ptch1+/-/SKH-1 mice, a murine model of BCNS. Here, we compared the impact of Akt1 activation in primary Ptch1+/-/SKH-1 and Ptch1+/+/SKH-1 keratinocytes, and the efficacy of targeted Akt1 inhibition on BCC growth. Ptch1+/- keratinocytes resist UV-induced apoptosis whereas Ptch1+/+ keratinocytes do not. Akt1 phosphorylation at S473 in Ptch1+/- keratinocytes, blocked apoptosis and was accompanied by inhibitory phosphorylation of pro-apoptotic Bad at S136, an Akt1 substrate. Overexpression of Akt1 (myr-Akt1) in UV-sensitive Ptch1+/+ keratinocytes restored resistance to UV-induced apoptosis. Moreover, sequence analysis of the Ptch1 gene from UV-resistant myr-Akt1-expressing Ptch1+/+ keratinocytes identified frequently repeated mutations (e.g., Phe1315Leu and frame shift deletion 4148C), suggesting that Akt1 activation facilitates the survival of UV-irradiated mutant keratinocytes thereby providing a survival advantage for cells harboring Ptch1 mutations. Screening of various PI3K and Akt inhibitors revealed that perifosine, an alkylphospholipid targeted Akt inhibitor previously shown to be nontoxic and well-tolerated in Phase I/II clinical trials for other human cancers, abrogated S473 Akt1 phosphorylation, cyclin D1 expression, and induced apoptosis in Ptch1+/-/SKH-1 keratinocytes and ASZ001 murine BCC cells. Targeting Akt1 may offer an alternative therapeutic approach for treating BCCs in BCNS patients.

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Loss of p38δ mitogen-activated protein kinase (MAPK) expression reveals a stage-specific regulation of skin inflammation and malignant progression in a two-stage chemical skin carcinogenesis model

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p38δ MAPK expression and/or activity are increased in human cutaneous malignancies including invasive squamous cell carcinoma (SCC) and head and neck SCC, but the role of p38δ in cutaneous carcinogenesis has not been well-defined. We have previously reported that mice with germline loss of *p38δ* exhibit a reduced susceptibility to skin tumor development compared with wild-type mice in the two-stage 7,12-dimethylbenz(*a*)anthracene (DMBA)/12-*O*-tetradecanoylphorbol-13-acetate (TPA) chemical skin carcinogenesis model. Additionally, we have reported that while keratinocyte-specific p38δ loss impairs growth of tumors arising from v-*ras*^{Ha}-transformed keratinocytes in skin orthografts to nude mice, conditional epidermal-specific *p38δ* gene targeting does not result in significant changes in tumor latency, incidence or multiplicity during the promotion stage, revealing a context-dependent regulation of skin carcinogenesis in these two distinct experimental models of tumor induction in mouse skin. We now report that mice with keratinocyte-specific deletion of p38δ show reduced overall tumor incidence during the progression stage of the DMBA/TPA regimen, as well as reduced incidence of clinically defined invasive SCCs, indicating that keratinocyte p38δ contributes to malignant conversion of DMBA/TPA-induced papillomas. Notably, skin of mice with globally deleted p38δ exhibits enhanced inflammation in response to a short-term DMBA/TPA regimen compared with skin of wild-type mice, as assessed by measuring expression of pro-inflammatory cytokines, including IL-1β, IL-6, IL-17, and TNFα, *in vivo*, underscoring a correlation between increased inflammation during the initial phases of the DMBA/TPA treatment and the resistance to DMBA/TPA-induced skin tumor development. Studies examining the molecular mechanisms underlying this regulation are underway.

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FRIZZLED7 and ERBB3 regulate distinct metastatic properties in melanoma

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Melanoma is a highly aggressive cancer. The low 5-year survival of late-stage melanoma patients (~15%) treated with current methods calls for novel interventions. ~70% of melanomas express a consitutively active form of BRAF (BRAF-CA); the remaining ~30% express wild type BRAF. Recently, a BRAF-CA inhibitor, vemurafenib, has provided some initial benefits in patients, but resistance quickly developed. Also, the drug was ineffective in melanomas expressing wild type BRAF, begging for alternative therapeutic strategies. Previously we showed that highly metastatic melanoma cells differentially express a gene signature relative to low metastatic cells, which correlated with poor patient survival. We analyzed two of these genes, FZD7 (WNT receptor) and ERBB3 (receptor tyrosine kinase), in metastasis. The expression of both genes correlated with poor survival in melanoma patients. We found that shRNA knockdown of FZD7 or ERBB3 reduced the metastatic potential of multiple cell lines expressing BRAF-CA irrespective of their sensitivity for vemurafenib, providing preclinical evidence for using FZD7 and ERBB3 as intervention points to treat both vemurafenib-sensitive and -resistant melanomas. Further analyses indicated that, though both FZD7 and ERBB3 affected the number of lung metastases, only FZD7 knockdown affected the tumor initiating frequency, and ERBB3 but not FZD7 was important for the seeding capacity of melanoma cell lines in lung after injected into the tail vein of mice. These results suggested that ERBB3 and FZD7 regulate distinct metastatic steps. We are in the process of examining the signaling pathways mediated by these factors. We hope our work will reveal the mechanisms by which distinct steps of metastasis are regulated and provide the foundation for combinatorial therapies that more effectively treat metastatic melanoma.

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Cutaneous squamous cell carcinoma carries the highest burden of mutation of any human tumor type and harbors prevalent mutations in CARD11 and CREBBP

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To assess genetic mutation in cutaneous squamous cell carcinoma (cSCC) we sequenced 20 tumors with agilent 50Mb whole exome capture and illumina technology. We followed this by a focused PCR amplicon based approach in a further 82 independent fresh frozen cSCC using Roche 454 pyrosequencing. In total we sequenced 102 micro-dissected fresh frozen cSCC of varying histological grade. Specialist sequencing and bioinformatics for the exome study were provided by the EASIH - University of Cambridge supported by NIHR - Cambridge BRC. Our data show that mutation (determined by variation present in tumor and absent in germ line DNA) across 20 cSCC exomes ranged from 0.8 to 3.9 mutations per 30,000 base pairs with an overall average of 2.2. Such a mutation burden is higher than any previously reported tumor type; an order of magnitude greater than Ovarian Cancer (0.06), 9 times greater than Lung SCC (0.24) and 3.5 times greater than Melanoma (0.62 average of 4 published studies). C > T transition base substitutions were by far the most common change (>78%), consistent with UV damage. In addition to confirming known mutation rates in TP53 (57%), NOTCH1 (59%), NOTCH2 (52%) and RAS (5%), we identify significant missense mutation in CARD11 (38%) and CREBBP (35%). cSCC CREBBP variants were frequent within the HAT domain but absent from the bromodomain, the large region reported to interact with the nuclear factor TRERF1 and the Creb binding domain. Mutations were present in all domains of CARD11, including the coiled-coiled domain where variants in Lymphoma are shown to increase NF-kB activation. Here we show the cSCC variant P833L (within the SH3 domain) significantly increases NF-kB activation in the absence of TNF-alpha stimulation compared with wild type, demonstrating prevalent CARD11 mutation in cSCC potentially increases NF-kB activation.

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The Bmi-1 helix-turn and ring-finger domains are required for Bmi-1 antagonism of (-) epigallocatechin-3-gallate suppression of skin cancer cell survival

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The Bmi-1 Polycomb Group (PcG) protein is an important epigenetic regulator of chromatin status. Elevated Bmi-1 expression is observed in skin cancer cells and contributes to cancer cell survival. (-)Epigallocatechin-3-gallate (EGCG), an important green tea-derived cancer prevention agent, reduces Bmi-1 level resulting in reduced skin cancer cell survival. This is associated with increased p21 and p27 expression, reduced cyclin (cyclin D1 and E), and cyclin dependent kinase (cdk2, cdk4) expression, and increased cleavage of apoptotic markers. These EGCG-dependent changes are attenuated by vector-mediated maintenance of Bmi-1 expression. In the present study, we identify Bmi-1 functional domains that are required for this response. We confirm that Bmi-1 expression reverses the EGCG-dependent reduction in SCC-13 cell survival but, in contrast, Bmi-1 mutants lacking the helix-turn-helix-turn-helix-turn (Bmi-1ΔHT) or ring finger (Bmi-1ΔRF) domains do not reverse the action of EGCG. Immunofluorescence studies confirm that the mutant and wild-type Bmi-1 proteins are in the nucleus. We further show that these mutants act in a dominant-negative manner to inhibit Bmi-1 function. Our results suggest that the HT and RF domains are required for Bmi-1 activity and ability to maintain skin cancer cell survival upon challenge with stress agents.

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mTOR, a potential molecular target for the chemoprevention of non-melanoma skin cancer in organ transplant recipients

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Non-melanoma skin cancers (NMSCs) are the most common neoplasm in organ transplant recipients (OTRs). Rapamycin, a specific mTOR inhibitor has been demonstrated to be an effective chemopreventive agent against a variety of cancers both in humans and in experimental animals. Here, we show that both topically and parenterally administered rapamycin reduced UVB-induced p-mTOR, cyclin D1and PCNA in SKH-1 hairless mice. Intraperitoneally administered rapamycin blocks UVB-induced BCCs and SCCs by 60% and 76%, respectively in genetically engineered Ptch+/-/SKH-1 hairless mice. A significant reduction both in number and size of tumors was noted. Topically administered rapamycin lead to regression of UVB-induced SCCs in SKH-1 mice. Rapamycin reduced the expression of proliferation markers, PCNA and cyclin D1, other cell cycle regulatory proteins and increased apoptosis both in Ptch+/-/SKH-1 and SKH-1 mice. As expected, an inhibition in mTOR signaling was common in tumors and tumor-adjacent skin of rapamycin-treated animals. Similarly, a dose-dependent reduction in tumor growth was noted in rapamycin-treated to nu/nu mice carrying A431 xenograft tumors. To understand the mechanism of tumor growth in chronically immuno-suppressed OTRs, mice carrying A431 xenograft tumors were treated with cyclosporine (CsA) alone or in combination with rapamycin. CsA-augmented tumor growth was attenuated by rapamycin. These tumors were less invasive and aggressive as compared to CsA-treated tumors a response similar to that reported in OTRs receiving CsA+rapamycin. Decreased tumor invasiveness was correlated with the reduced expression of mesenchymal markers and increased E-cadherin in residual tumors in rapamycin-treated mice. The exact mechanism by which rapamycin blocks EMT in CsA-treated tumors is unclear, but, reduced MAPK/Akt kinases in rapamycin-treated tumors appear to be involved in this process.

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Bone marrow-derived epithelial cells as well as hair follicle bulge-derived cells contribute to skin tumors and ulcer-associated dysplasia mediated by tumor promotion in the mouse

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A stem cell origin for tumors was postulated nearly 200 years ago; however, a critical role for keratin-15 expressing hair follicle stem cells in skin tumorigenesis has only recently been established. Here, we examined skin-homing, bone marrow-derived cells (BMDCs) in cutaneous papillomas during chemically induced carcinogenesis (a single application of 200 nmol of 7, 12-dimethylbenz[a]anthracene and thrice weekly application of 17 nmol of 12-O-tetradecanoylphorbol-13-acetate) following gender-mismatched allogeneic bone marrow transplantation in mice. First, we detected proliferating green fluorescent protein labeled, keratin immunoreactive BMDCs in the basal epithelium of papillomas in numbers approaching 25% of tumor area in 17 of 45 papillomas. Second, an enhanced contribution of Y-chromosome positive bone marrow-derived epithelial cells was observed in 26 of 49 ulcer-associated skin lesions in numbers approaching 35% of lesional area. Third, keratin expression was induced in a subset of plastic adherent bone marrow cells following co-culture with primary keratinocytes and treatment with bone morphogenetic protein 5 in the absence of cellular contact. These results demonstrate that BMDCs participated as an epithelial cell source in chronically damaged skin lesions including papillomas and ulcers. We conclude that BMDCs as well as bulge-derived cells contribute to the pathogenesis of skin tumors and associated lesions induced by skin tumor promotion.

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EGFR/Ras-induced CCL20 production is a critical factor within the tumor microenvironment

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The activation of the EGFR/Ras signaling pathway in tumor cells induces a distinct chemokine repertoire, which in turn modulates the tumor microenvironment. In particular, we show that tumors facilitate progression by the EGFR/Ras-induced production of CCL20. Enhanced expression of the chemokine CCL20 in tumors correlates with advanced tumor stage ($P<0.001$) and increased lymph node metastasis ($P<0.05$) in several types of cancer, and was associated with decreased survival in breast cancer patients ($P<0.05$). Endothelial cells (EC) abundantly express the specific CCL20 receptor CCR6. Activation of CCR6 signaling in EC induces directional migration and enhanced vessel formation. In vivo, CCL20 promoted vascularization of Matrigel plugs in wild-type mice, but not in CCR6-deficient mice. Furthermore, tumor growth and tumor-associated vascularization in CCR6-deficient mice were decreased compared to wild-type mice. Experiments with bone marrow chimeras indicates that the observed phenotype is dependent on CCR6 deficiency in stromal cells but not within the immune system. Taken together, our findings underscore the importance of tumor-stroma interactions and identify a novel chemokine-driven mechanism that critically modulates the tumor environment and affects survival of cancer patients.

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Phospholipase C-beta 4 regulates proliferation and invasion in cutaneous SCC

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Phospholipase C beta 4 (PLC-β4) belongs to the phospholipase C family of enzymes, known to play a central role in intracellular signalling pathways regulating cell growth, differentiation and migration through activation of protein kinase C (PKC) and intracellular calcium release. We found using gene expression arrays that PLCB4 is highly expressed in SCC cells with knockdown of type VII collagen, modelling recessive dystrophic epidermolysis bullosa (RDEB) known to be associated with aggressive SCC. Our hypothesis is that PLC-beta4 may play a regulatory role in SCC tumourigenesis. Immunostaining of cutaneous SCC samples demonstrated nuclear staining of PLC-β4 in moderately-differentiated tumours and RDEB SCC while well-differentiated (grade I) tumours exhibited cytoplasmic localisation. Using RNAi technology, we achieved transient and lentiviral stable knockdown (KD) of PLC-β4 (siPLC-β4 and shPLC-β4) in cutaneous SCC cell lines. SCC cells with KD of PLC-β4 had decreased proliferation without altering differentiation or inducing apoptosis. KD 3D cultures had decreased Ki-67 expression. A phospho-array demonstrated decreased phosphorylation of Stat3, confirmed by Western blotting. Live-imaging confocal microscopy demonstrated that PLC-β4 KD cells had absent intracellular calcium-release following specific activation of the PLC-β4 pathway. Moreover, various functional assays, including monolayer scratch, Transwell® and organotypic 3D cultures, revealed significantly decreased cell migration and invasion in SCC cells with KD of PLC-β4 with reduced cell adhesion. PLC-β4 KD cells had decreased β1 integrin expression, alterations in FAK activation and decreased Rho GTPase expression. These findings suggest that PLC-β4 regulates SCC cell proliferation, migration and invasion through the Rho/integrin/FAK/Stat signaling pathway and likely intracellular calcium regulation. The recent finding of mutations in PLCB4 in melanoma suggests that this will be an attractive therapeutic target.

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Yin and Yang: IL-1β promotes, while IL-1α inhibits, lymphoma and melanoma tumor growth in mice

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The roles of Interleukin 1α and 1β have long been controversial in tumor immunity. We showed earlier that transgenic mice overexpressing IL-1α in the epidermis were completely resistant to chemical carcinogenesis; however, the mechanism was elusive. In the present study, we used mice injected with EL4 lymphoma and B16 melanoma tumor cells to further explore the roles of these ancient IL-1 family members. To our surprise, mice deficient only in IL-1β had dramatically reduced growth of both tumors. In contrast, mice deficient in IL-1α, or the IL-1R type I (through which both IL-1α and IL-1β signal) showed normal (poor) tumor resistance. To explore this paradoxical finding in a different fashion, we treated mice with an IL-1β blocking antibody; this treatment inhibited tumor growth dramatically, while antibody depletion of IL-1α did not. This presented a unique puzzle, since both IL-1α and β are thought to signal solely through the same (type I) IL-1 receptor. We next fashioned bone marrow chimeras between IL-1β and wild type mice. Only the mice with wild type bone marrow placed into IL-1β deficient recipients showed resistance to tumor growth—IL-1β deficient bone marrow did not confer this protective effect. Depletion of both CD4 and CD8 T cells demonstrated that the protective effect of IL-1β deficiency could be completely abrogated by T cell depletion, indicating a T cell mediated immune mechanism mediating protection. Taken together, these results indicate that the simultaneous absence of IL-1β and the presence of IL-1α creates a tumor-resistant milieu that is strongly influenced by T cell immunity. This fascinating translational observation requires further study, especially given the recent introduction of IL-1β blocking antibodies as a therapeutic agent for both pediatric and adult inflammatory conditions.

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Topical rapamycin inhibits tumor formation in a murine model of cutaneous T-cell lymphoma

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The mTOR signaling pathway is a central regulator of cellular proliferation, growth, and metabolism which has been demonstrated to be important for tumor formation and growth. Herein, we characterize the antitumor effect of topically applied rapamycin, an mTOR pathway inhibitor, in a murine model of cutaneous T-cell lymphoma (CTCL). In our experimental model, MBL2 murine T lymphoma cells were injected subcutaneously into ears of C57BL/6 mice followed immediately by a single topical application of dinitrofluorobenzene (DNFB), a contact sensitizing agent, to the injection site. From day 6 on, rapamycin 0.1% in petrolatum base (n=8 mice) or petrolatum alone (n=6 mice) was applied daily. At day 14, the rapamycin-treated mice showed markedly decreased tumor size as assessed by ear thickness compared to control animals (0.78 mm vs. 2.26 mm, $p<0.05$). H&E staining of ear tissue revealed a striking decrease in tumor cells in rapamycin-treated ears. This antitumor effect was correlated with decreased activation of mTOR signaling as assessed by an approximately fourfold decrease in phosphorylation of p70S6K, S6, and Akt (Ser473) in Western blots from ear tissues of treated animals. To characterize the effect of rapamycin on MBL-2 cells, in vitro studies were performed. Incubation of MBL2 cells with rapamycin (5 nM) attenuated mTOR pathway activity as determined by phosphorylation of downstream targets such as S6K, S6, and Akt (Ser473) in Western blot assays. Furthermore, rapamycin also decreased the proliferation of MBL2 cells as well as human Hut78 CTCL cells. In summary, we were able to demonstrate for the first time that topical rapamycin is sufficient to inhibit development of tumors in a murine model of CTCL. Moreover, we demonstrate the anti-proliferative effects of rapamycin on murine and human T lymphoma cells in vitro. Future clinical trials will characterize the clinical efficacy and therapeutic potential of topically applied rapamycin in patients with early stage CTCL.

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Topical platelet activating factor receptor (PAF-R) agonist treatment suppresses PMA induced inflammatory effects and cutaneous chemical carcinogenesis: Effects on inflammation requires both the PAF-R and mast cells

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Platelet activating factor (PAF) is a well known mediator of acute edema responses. We have previously reported that PAF-R knockout mice (Pafr-/- mice) exhibit an enhanced chronic inflammatory response to repetitive PMA treatment. Pafr-/- mice also exhibited augmented chemical carcinogenesis that was not associated with DMBA-induced DNA damage responses or PMA-induced epidermal hyperplasia. In this study, we examined the effects of topical CPAF on PMA-induced inflammation. Given the known vasoactive effects of PAF, it was not surprising that topical CPAF (2, 6, and 20 nmole) induced a modest dose-dependent increase in ear thickness that peaked at 2 hrs and resolved within 8 hrs. PMA induced both a rapid acute inflammatory reaction after initial application, an augmented acute response to a second application, and a sustained chronic inflammatory phase with repeated treatments. Cotreatment of mouse ears with both PMA and 6 nmole CPAF resulted in a significant reduction in both acute and chronic PMA-induced ear thickness changes. The effects of topical CPAF were abolished in both Pafr-/- mice and Mast cell deficient mice. In dorsal epidermis treated with both repeated PMA applications, co-administration of topical CPAF suppressed both PMA-induced skin thickness and myeloperoxidase activity. Given the known association between PMA-induced inflammation and tumor promotion, as well as the increased DMBA/PMA-induced tumorigenesis in Pafr-/- mice, it is notable that we also show that topical CPAF suppresses DMBA/PMA-induced tumor formation by 37%. Our data indicate that PAF has potent immunosuppressive effects on PMA-induced skin inflammation. Thus, PAF-R activation may play a more complex immunomodulatory role on cutaneous inflammatory responses than previously appreciated. Moreover, these inflammatory effects appear to be mediated by dermal Mast cells.

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Genetic loss of Srcasm promotes oncogenic signaling and cell growth after UVB exposure

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Cell growth is regulated through a coordination of oncogenic and anti-oncogenic signals. Tyrosine kinases are primary drivers of cell growth and are negatively regulated by Srcasm through lysosomal degradation in transgenic overexpression models. We hypothesized that genetic downregulation of Srcasm by gene deletion or shRNA knockdown would promote oncogenic signaling and cell growth. To test this hypothesis Srcasm null mice and Srcasm lentiviral knockdown in human keratinocytes were generated and studied. Western blotting of epidermal lysates from Srcasm null mice demonstrates that loss of Srcasm promotes activation of EGFR and Src kinases and decreases levels of p53, NICD and p21. Activation of the canonical Erk1/2, STAT-3 and PDK-1 oncogenic pathways also was seen in Srcasm null lysates. The epidermis of Srcasm null mice exhibits a higher Ki-67 index of 8.7 +/- 2.0 compared to 1.5 +/- 1.1 in controls ($p<0.001$). UVB irradiation (500 mJ/cm2 x 15 doses) produced precancerous lesions in Srcasm null mice but not in controls; the precancerous lesions show an increased Ki-67 index and activation of Src kinases, STAT-3 and PDK-1. Acute UVB (1200 mJ/cm2 x 1 dose) of Srcasm null mice induced prominent erythema, epidermal necrosis and a prominent increase in the TUNEL-positive index (14.0 vs 1.3). Srcasm knockdown in human keratinocytes accelerated keratinocyte growth 130%; it also increased levels of EGFR and accelerated the kinetics of EGFR and MAPK activation in response to EGF stimulation. In human keratinocytes, Srcasm knockdown promoted cell growth after UVB exposure. These data show that loss of Srcasm is associated with increased oncogenic signaling, impaired p53 function and keratinocyte proliferation. Loss of Srcasm promotes keratinocyte proliferation post-UVB exposure and promotes the early stages of UVB-induced cutaneous neoplasia.

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Human basal cell carcinoma tumor-initiating cells are resistant to etoposide killing

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The creation of an in vivo orthotopic xenograft model for primary human basal cell carcinoma (BCC) led to the recent identification of CD200+ BCC tumour-initiating cells (TICs) (Colmont et al., 2013). The methodology used to reproducibly propagate BCC in vivo was similar to that for primary human squamous cell carcinoma propagation, but in addition necessitated administration of intraperitoneal etoposide to immunocompromised recipient mice 1 day prior to BCC grafting. We sought to determine if etoposide exerted selection bias by killing BCC cells or otherwise adversely affecting the TIC frequency or expansion. Addition of 60 or 100μM etoposide to freshly-plated or established BCC colonies in culture led to a DNA damage response, resulting in nuclear accumulation of H2AX, stabilisation of p53, cell cycle arrest and DNA repair in viable cells - similar to that which occurred with human osteosarcoma U2OS cells. At etoposide concentrations > 60μm, BCC colonies were reduced in size by 5% but their numbers were unaffected. Because cancer cell resistance to etoposide can result from expression of transmembrane pumps, we assessed BCC tumors and cells for these proteins. BCC tissue samples (n=6) expressed MDR1, MRP1, MRP3 and MRP4, whereas cultured BCC cells (n=5) expressed only MDR1, in the presence and absence of etoposide. Although MDR1 is not expressed by normal keratinocytes, we confirmed its expression by BCC cells by RT-PCR and FACS analysis and verified activity using a rhodamine dye extrusion assay. MDR1 was expressed by 2-3% of all BCC cells and ~50% of CD200+CD45- BCC cells. Both MDR1+ CD200+ CD45- and MDR1- CD200+ CD45- BCC subpopulations initiated tumors in vivo (n=3 BCC samples). In conclusion, BCC TIC's are resistant to etoposide killing, perhaps because they express MDR proteins. This is consistent with previous clinical observations and establishes the appropriateness of etoposide use in this newly developed human skin cancer model.

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NKp46 specific expression on skin-resident CD4+ lymphocytes of mycosis fungoides (MF) and Sézary syndrome (SS)

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In SS, and MF, because the morphological features of malignant T cells are not specific enough for an unequivocal detection of the tumoral cells, specific markers are required. Here, we report the NKp46 expression on the malignant skin resident CD4+ T cells of CTCL. 15 CTCL (5 MF, 5 TMF, 5 SS) were included. Controls were 10 inflammatory skin diseases (5 eczema, 4 psoriasis, 1 drug eruption), 5 primary cutaneous diffuse large B-cell lymphoma, leg type and 5 normal-skin. All patients were treatment-naive before cutaneous biopsies. Immunohistochemistry were performed on formalin-fixed sections using monoclonal antibodies against human CD4, CD8, CD56 or CD30 as primary antibodies. Molecular analyses were performed on frozen skin samples and on CD4+ laser-microdissected cells. Conventional RT-PCR was made on frozen skin samples to value the NKp46 transcript. On the laser-microdissected cells, we used RT-PCR after a preamplification step to control the presence of CD4+ and the absence of CD8+ transcripts. Phenotypic analyses showed that CD3+ CD4+ lymphocytes were prominent in dermal infiltrate of all CTCL. Molecular analyses showed a significant over-expression of NKp46 in the 15 CTCLscompared to the ISD ($p<0.05$), CBCL ($p<0.05$), or normal-skin ($p<0.05$). No significant difference was found between SS, MF and TMF. Using quantitative RT-PCR on CD4+ laser-microdissected cells, NKp46 was detected in the 15 CTCL, without significant difference across MF-TMF-SS. Moreover, NKp46 gene expression levels were significantly higher in CD4+ laser-microdissected cells from CTCL than those from CBCL ($p<0.05$). To note T-plastin mRNA was not expressed in CD4+ T-lymphocytes from SS, MF and TMF patients. NKp46 expression on CD4+ circulating Sézary cells has been recently identified. Here we characterized NKp46 expression on CD4+ resident skin cells and showed that this expression was not restricted to SS, but also present in MF.

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Defining and targeting differentiation pathways in non-melanoma skin cancer

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Human cancer stem cells are proposed to play a critical role in tumor initiation and maintenance by their exclusive ability to regenerate the tumor. Thus cancer stem cells share many of the properties of normal stem cell including self-renewal and ability to give rise to progeny which undergo tissue-specific differentiation. The aim of this project is to determine whether both inward and upward differentiation patterns observed in the normal tissue (hair follicle) are conserved in cancer (basal cell carcinoma). To test this hypothesis we analyzed 6 different human BCC samples with normal hair follicle tissue as controls for 20 different hair follicle specific differentiation keratins using RT-PCR. For the 12 specific keratin genes expressed in the BCC, we analyzed expression by immunofluorescence on 20 different BCC samples, using hair follicle samples as positive controls. Our findings suggest that human BCC demonstrates both inward and upward differentiation patterns similar to the hair follicle, with expression of: outer root sheath (K5,14,16,and k17), companion layer (K75), inner root sheath (K26,27,28,71,72,and k74), and cuticle (K32,35,82,and k85); but not hair shaft (K31) markers. We also observed the mutually exclusive relationship between expression of the early differentiation marker K19 and cell proliferation in the hair follicle and BCC, in keeping with the shared functional characteristics of these cells. Similarly, expression of the outer root sheath keratins coincided with nuclear translocation of both Gli1 and NFIL-6 suggesting that regulation was also similar. To further test the hypothesis that normal tissue factors observed in the hair follicle regulated BCC differentiation we have developed an in vitro BCC assay. Using this tissue culture model we have tested the hypothesis that BCCs are "stuck" in a refractory telogen hair follicle differentiation pattern, by inducing BCC differentiation.

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The expression of IL-1 family cytokines is perturbed in non-melanoma cancers of the skin

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Non-melanoma skin cancers, including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the most common malignancies of the skin. While keratinocyte damage is causal, alteration of immune responses may contribute to their pathophysiology. The IL-1 family comprises 11 ligands and 9 receptors forming several distinct signaling systems; aberrancies in which are seen in several diseases, including malignancies. The objective of this study was to delineate differences in expression of IL-1 family members among SCC, BCC and normal skin (NN). SCC and BCC tissue was obtained from Mohs surgery excisions and NN tissue from healthy patient donors. RNA and protein expression was analyzed using microarray, quantitative real time PCR (QRT-PCR) and immunohistochemistry. Overall, SCCs showed greater changes in expression of IL-1 members than BCCs. QRT-PCR data showed the pro-inflammatory genes IL1A and IL1B were up-regulated in SCCs versus NN 9.6-fold (p=0.001) and 10.5-fold (p=0.006) respectively whereas their receptor, IL1R1, was down-regulated 2.4-fold (p=0.001) and their antagonist, IL1RN, was up-regulated 2.2-fold (p=0.03). In contrast, BCCs had 4.2-fold down-regulation of IL1A (p=0.009) while there were no significant differences in IL1B, IL1R1, or IL1RN expression. Of the members of the novel IL-36 family, IL36A was unchanged, whereas IL36B and its antagonist IL36RN were up-regulated 8.8-fold (p=0.03) and 3.3-fold (p=0.0001) respectively in SCCs. These findings were not mirrored in BCCs as there were no significant changes in the expression of IL-36 ligands or antagonists. Immunohistochemistry confirmed these findings. These data are consistent with prior observations that SCCs are more inflammatory in nature than BCCs, and demonstrate for the first time abnormalities in the IL-36 system in these cutaneous malignancies.

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Oncogene addiction distinguishes malignant from benign skin tumors caused by deregulated Hedgehog signaling

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High-level oncogenic Hedgehog (Hh) signaling underlies the development of basal cell carcinomas (BCCs) whereas low-level signaling leads to the formation of benign basaloid follicular hamartomas (BFHs). The great majority of BCC tumor cells are strictly dependent on Hh pathway activity for growth and survival ("oncogene addicted"), based on studies performed in experimental animals and clinical data from patients treated with Hh pathway antagonists. To determine whether benign tumors caused by low-level Hh signaling are similarly dependent on continued signaling, we used a novel mouse model to reversibly activate expression of the Hh pathway oncogene SmoA1 in skin of adult mice using doxycycline (doxy). SmoA1-expressing mice developed slow-growing BFHs composed of anastomosing cords and strands of bland-appearing epithelial cells, but lesions resembling BCCs, either grossly or histologically, were never observed. Hamartomas expressed follicle outer root sheath markers (K17, Sox9) and HA-tagged SmoA1 in nearly all epithelial cells, but expression of Hh target genes (Gli1, Ptch1, Ptch2, Cyclin D1), proliferative activity (Ki67 immunostaining), and the hair matrix marker CDP were largely limited to cells at the tumor periphery. This pattern differs strikingly from the diffuse expression of markers in BCCs arising in GLI2*-expressing mice with high-level Hh signaling. Withdrawal of doxy led to rapid loss of SmoA1 expression and reduced Ki67 immunostaining, but remarkably, most BFH tumor masses were still present after 7 weeks. Persistent hamartomas expressed the follicle marker K17 and to a lesser extent Sox9, but the peripheral CDP-positive population of tumor cells was lost, suggesting that only those cells with elevated Hh signaling regress upon SmoA1 inactivation. Our findings underscore the molecular and biological differences between malignant BCC and benign BFH, and strongly suggest that tumors with low-level oncogenic Hh signaling activity are not likely to be responsive to treatment with Hh pathway antagonists.

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A miR-34a-SIRT6 axis in the squamous cell differentiation network

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Squamous Cell Carcinomas (SCCs) are highly heterogeneous tumours, resulting from deranged expression of genes involved in squamous cell differentiation. Here we report that microRNA-34a (miR-34a) functions as a novel node is the squamous cell differentiation network, with SIRT6 as critical target. miR-34a expression increases with keratinocyte differentiation, while it is suppressed in skin and oral SCCs, SCC cell lines, and aberrantly differentiating primary human keratinocytes (HKCs). Expression of this miRNA is restored in SCC cells, in parallel with differentiation, by reversion of genomic DNA methylation or wild-type p53 expression. In normal HKCs, the pro-differentiation effects of increased p53 activity or UVB exposure are miR-34a-dependent, and increased miR-34a levels are sufficient to induce differentiation of these cells both in vitro and in vivo. SIRT6, a sirtuin family member not previously connected with miR-34a function, is a direct target of this miRNA in HKCs, and SIRT6 down-modulation is sufficient to reproduce the miR-34a pro-differentiation effects. The findings are of likely biological significance, as SIRT6 is oppositely expressed to miR-34a in normal keratinocytes and keratinocyte-derived tumors.

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Expression of epidermal growth factor receptor and detection of mutant EGFR (EGFRvIII) in human cutaneous squamous cell carcinoma

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Epidermal growth factor receptor (EGFR) is overexpressed in large percentage of cutaneous squamous cell carcinomas (SCC). Therapeutic trials using EGFR blockade to treat high-risk or metastatic SCCs are ongoing. The most common form of mutant EGFR called EGFRvIII has been described in several cancers. It has been shown that EGFRvIII contributes to enhanced growth of SCC and resistance to EGFR inhibitor drugs. Our goal was to assess expression of wild type EGFR and EGFRvIII in SCC and correlate it with tumor biology. SCC specimens from patients were collected consecutively. Tumor specimens were embedded in paraffin and assessed for EGFR expression by immunohistochemistry while EGFRvIII expression was determined by RT-PCR. A representative SCC cell line stably transfected with an EGFRvIII expression construct served as a positive control. Fifteen of the tumors (20%) were collected from solid organ transplant patients. All tumors (100%) expressed EGFR, with variation in intensity and proportion of labeled cells. EGFR expression, however, was not correlated with tumor differentiation, anatomic location or transplant status. Surprisingly, none of the tumors (0%) expressed EGFRvIII mRNA. To verify these results tissue microarray with 275 SCC samples (138 received from solid organ transplant recipients) was stained for the EGFRvIII protein expression. Similarly to the previous results, none of these tumors expressed the mutated form of the receptor. The lack of mutant EGFRvIII expression may have implications on the design of future clinical trials using EGFR inhibitors for treatment of high risk cutaneous SCC.

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Defining the roles of p53, Myc, and Aurora kinase A in skin squamous cell carcinomas from organ transplant recipients

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Organ transplant recipients (OTRs) are highly susceptible to the development of skin Squamous Cell Carcinomas (SCCs) that are likely to recur or metastasize. We have previously shown that skin specific expression of a gain-of-function mutant form of p53 in mice resulted in metastasis-prone SCCs that display high levels of genomic instability as well as preferential gene amplification of *Myc* and altered expression of Aurora Kinase A (Aurora-A) (Oncogene (2012) 31: 2680-90). To define how these oncogenes contribute to the formation of high-risk SCCs in OTRs, we examined the staining pattern of p53, Myc and Aurora-A in 53 SCCs resected from 22 OTR patients. Tumor grade was assessed using the National Institute for Health diagnostic criterion (Grade I=well-differentiated to IV=poorly differentiated). The mitotic index was ascertained in at least 10 high-powered fields from Hematoxylin and Eosin stained sections. A cumulative score for immunoreactivity was determined by multiplying the staining intensity by the percentage of positively stained cells in at least three separate fields. Immunoreactivity for p53, Myc and Aurora-A was detected in all samples. The percentage of p53 positive cells in tumors ranged from 19% to 97% with a mean of 68.6% (95% CI=65.1, 72.0). p53 expression was significantly increased with tumor grade ($p<0.0001$). Mitotic index was positively correlated with tumor grade ($p<0.03$) and with p53 expression ($p<0.05$). High-level expression of p53 is consistent with the expression of mutant forms of p53, not loss of p53. Our results indicate that mutant p53 may contribute to the development of high-risk tumors in OTRs.

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Lgr5+ stem cells give rise to Hedgehog pathway-driven gastric adenocarcinomas

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Deregulated Hedgehog (Hh) signaling drives basal cell carcinoma (BCC) development and has been linked to several internal malignancies, including gastric adenocarcinoma, but studies testing a causal role for Hh signaling in this malignancy have not been reported. In preliminary experiments, mosaic expression of the activated Hh pathway transcription factor GLI2 (GLI2*) led to gastric adenocarcinoma development. To investigate the potential cell of origin of these cancers we targeted GLI2* expression to Lgr5+ stem cells in the gastric antrum of adult mice. Induction of GLI2* led to the development of large antral tumors within 4-6 weeks. At 3 days post-induction, GLI2* was detected in small groups of cells at the base of antral glands near the stem cell compartment, and by day 14 these had expanded to fill the thickened antral mucosa. Tumors were frequently over one cm in size, with occasional submucosal invasion. They consisted of an admixture of disorganized glands comprising GLI2*+ cells which expressed canonical Hh target genes (*Gli1*,*Ptch1*), hyperplastic gastric epithelium, an inflammatory infiltrate, and an increased number of SMA+ myofibroblasts. Expression of proliferation markers was markedly increased, and pSTAT3, which has been strongly implicated in gastric adenocarcinoma development in humans and other mouse models, was highly expressed in epithelial and stromal cell populations. GLI2*-expressing mice also developed BCCs, as previously reported, but epithelial tumors were strikingly absent in intestine, despite GLI2* expression in Lgr5+ intestinal stem cells. Our findings establish that deregulated Hh/Gli signaling can function as an oncogenic driver to produce invasive gastric adenocarcinomas in adult mice; identify Lgr5+ gastric stem cells as the likely cells of origin for these tumors; support the concept that STAT3 activation is a key signaling event in gastric cancer; and underscore the importance of tissue context in defining responsiveness to oncogenic signaling.

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ΔNp63α in squamous cancer pathogenesis

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ΔNp63α, the predominant p63 isoform in epidermis, is expressed in basal keratinocytes and overexpressed in human squamous cell carcinomas. To delineate the biological contribution and identify downstream mediators of ΔNp63α in keratinocyte growth regulation, differentiation, survival, and cancer development, we have used adenoviral and lentiviral vectors to drive transient and sustained overexpression of ΔNp63α.in primary mouse keratinocytes. We previously established that transient overexpression of ΔNp63α inhibits Ca2+-mediated growth arrest and biochemical differentiation of primary murine keratinocytes, and that the block in growth arrest is mediated via the NF-κB subunit c-Rel. c-Rel accumulates in a phosphorylated form in the nucleus of ΔNp63α-overexpressing cells and binds in complex with ΔNp63α to a p63 binding site on the p21waf1 promoter. Consistent with these findings, keratinocytes expressing lenti-ΔNp63α demonstrate enhanced proliferation rates over 15 days in culture relative to lenti-GFP control cultures. These cultures also exhibit a block in senescence associated with decreased levels of p16 and p19. Following grafting to nude mice, keratinocytes expressing lenti-ΔNp63α in combination with oncogenic v-rasHa form undifferentiated carcinomas, in contrast to the well-differentiated papillomas observed with oncogenic v-rasHa alone. Lentivirus-driven ΔNp63α overexpression also results in sustained nuclear accumulation of c-Rel. Lentiviral c-Rel shRNAs have been developed to knock down c-Rel in ΔNp63α-overexpressing keratinocytes and provide a model system for elucidating the contribution of NFkB/c-rel to ΔNp63α /v-rasHa-driven carcinogenesis.