The importance of gene expression profiling for the development of personalized molecular approach for diagnosis and treatment of Cutaneous T-Cell Lymphoma (CTCL)

ABSTRACTS

Dissecting the role of iASPP, a novel regulator of epidermal homeostasis, in keratinocyte skin carcinogenesis

ABC5B inhibition sensitizes Merkel cell carcinoma cells to chemotherapy-induced apoptosis

Complement Factor I promotes progression of cutaneous squamous cell carcinoma

Genome-wide analysis of DNA methylation in Sézary syndrome

The incidence of cutaneous squamous cell carcinoma (cSCC) and its precancerous forms is rising worldwide. Here, we have investigated the role of complement components in the progression of cSCC. Analysis of cSCC cell lines (n=8) and normal human keratinocytes (n=11) with whole transcriptome profiling (SOLID), quantitative RT-PCR, and western blotting showed marked overexpression of complement factor I (CFI) in cSCC cells. Immunohistochemical analysis for CFI showed stronger tumor cell specific staining intensity in invasive sporadic cSCCs (n=83) compared to either chemotherapy alone. Our results establish ABC5B as a novel potential therapeutic target in cSCC.

A potential therapeutic target in cSCC.

Using demethylating agents we found that promoter methylation was associated with gene silencing. Clinically, the differentially methylated sites in SS could be applied as epigenomic biomarkers in the early diagnosis of this disease. The epigenetic instability of SS cells provides a rationale for its poor survival in the clinic. We demonstrated that ABC5B knockdown sensitized tumor cells to carboplatin and etoposide-induced apoptosis and inhibited tumor growth compared to either chemotherapy alone. Our results establish ABC5B as a novel potential therapeutic target in cSCC.
Diverse roles for laminin 332 subunits in squamous cell carcinoma

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The role of Laminin 332 in tumour development is poorly understood. It has been identified as both vital for tumour development and/or as a tumour suppressor. Perturbed expression of laminin 332 subunits, possibly caused by mutations in laminin 332 have a high incidence of squamous cell carcinoma (SCC). To investigate the role of laminin 332 in SCC we generated stable knockdown (KD) of each laminin 332 chain (LnamA, LnamB & LnamC) in cutaneous SCC cell lines. Using these cells we studied their role in cell attachment, motility, in vitro and in vivo invasion, integrin expression, cell-signalling and global gene expression using RNA-seq. The loss of any Lnam332 chain reduced cell attachment with the greatest reduction seen with LnamA and LnamC. The loss of LnamA and LnamC increased cell motility whereas loss of LnamB had no effect on motility. Loss of LnamA and LnamC increased invasion both in vitro (collagen gels) and in vivo (murine xenografts) with distinct patterns of invasion in each cell line. LnamA and LnamC KD increased EMT in vivo with increased Vimentin expression (ROS) was increased in these mice, resulting in a reduction of DMBA-induced oxidative stress and increased Keratin 10 and involucrin. Each cell line demonstrated a unique pattern of integrin expression with increased β1 integrin in LnamA and LnamC KD and increased αv in LnamA and LnamC KD. FAK activation in pFAK was increased in LnamA and LnamC KD with a matched increase in Src. Downstream from FAK changes were seen in Rac, Akt and Wnt pathways. RNA-seq analysis allowed us to identify genes involved in cancer progression differentially regulated due to the loss of each chain of laminin 332. Of particular interest was the increased expression of MMPs and downregulation of MMP inhibitors. These data provide evidence that loss of each chain of laminin 332 leads to distinct phenotypic and genotypic changes highlighting the importance of studying each laminin 332 chain in future studies.

A surprising pro-tumorigenic function of activated Nrf2 in keratinocytes

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Nrf2 is a central regulator of the cellular antioxidant defense system through the regulation of antioxidant enzymes, cytoprotective proteins and transporters. Compounds that activate Nrf2 are therefore in clinical trials as anti-cancer drugs. We tested the consequences of Nrf2 activation on DMBA/TPA-induced skin tumorigenesis using transgenic mice expressing a constitutively active (ca) Nrf2 mutant in keratinocytes. Expression of genes involved in detoxification of DMBA and of reactive oxygen species (ROS) was increased in these mice, resulting in a reduction of DMBA-induced oxidative stress with increased Keratin 10 and involucrin. Each cell line demonstrated a unique pattern of integrin expression with increased β1 integrin in LnamA and LnamC KD and increased αv in LnamA and LnamC KD. FAK activation in pFAK was increased in LnamA and LnamC KD with a matched increase in Src. Downstream from FAK changes were seen in Rac, Akt and Wnt pathways. RNA-seq analysis allowed us to identify genes involved in cancer progression differentially regulated due to the loss of each chain of laminin 332. Of particular interest was the increased expression of MMPs and downregulation of MMP inhibitors. These data provide evidence that loss of each chain of laminin 332 leads to distinct phenotypic and genotypic changes highlighting the importance of studying each laminin 332 chain in future studies.

Analysis of STAT4 expression in cutaneous T-cell lymphoma (CTCL) patients and patient-derived cell lines

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Deregulation of STAT signaling has been implicated in the pathogenesis for a variety of cancers, including CTCL. Recent reports indicate that loss of STAT4 expression is an important prognostic marker for CTCL progression and is associated with the acquisition of T helper 2 cell phenotype by malignant cells. However, little is known about the molecular mechanism behind the down-regulation of STAT4 in this cancer. In the current work we test the expression of STAT4 and STAT6 via RT-PCR in CTCL lesional skin samples and in immortalized-patient-derived cell lines. In these malignant cell lines we correlate the expression of STAT4 and STAT6 with the T-helper (Th) phenotype by malignant cells. Herein, we show for the first time that STAT4 and STAT6 genes are inversely regulated in CTCL. Treatment of HDAC inhibitors upregulates STAT4 expression, while at the same time decreases STAT6 expression in Myc cells. Also, siRNA-mediated knockdown of miR-155 leads to upregulation in STAT4 expression in Myc cells. In summary, our results suggest that loss of STAT4 expression and associared with T-Cell progression may be driven via aberrant histone acetylation and/or upregulation of oncogenic miR-155 microRNA.
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114 Clandin-11 regulates invasion of cutaneous squamous carcinoma cells
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The incidence of keratinocyte derived skin cancer, cutaneous squamous cell carcinoma (cSCC), is increasing globally. Epidermal keratinocytes are connected by tight junctions (TJ), consisting of transmembrane proteins, such as claudins (CL) and occludin, and of zona occludens (ZO) which connect TJs to actin cytoskeleton. Expression of TJ proteins in normal human epidermal keratinocytes (NEHK, n=4) and cSCC cells (n=8) was determined with SOLID whole transcriptome sequencing and Affymetrix analysis. ZO-1 was expressed both in NEHKs and in cSCC cell lines. Clandin-11 (CL-11) was expressed in NEHKs and primary cSCC cells (n=5) but not in metastatic cSCC cells (n=3). Furthermore CL-11 mRNA expression was significantly downregulated in cSCC tumors (n=6) compared to normal skin (n=7, p=0.004). Knock-down of CL-11 in cSCC cells revealed increased cancer cell invasion (p=0.039). Expression of CL-11 was also detected in immortalized non-tumorigenic HaCaT cells and in metastatic HaCaT cells (A5, B4, and RT3) with high basal ERK1/2 activation. Inhibition of ERK1/2 pathway with MEK1 inhibitor PD98059 resulted in restoration of CL-11 expression in HAa-transformed HaCaT cells (A5, B4, and RT3) with basal ERK1/2 activation. These results provide evidence that the loss of CL-11 expression in cSCC cells might serve as a biomarker for an advanced invasive stage of cSCC.

115 Common genetic variations of multiple basal cell carcinomas
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Recent genome-wide association studies (GWAS) have identified several loci involved in susceptibilithy to basal cell carcinoma (BCC). A case-control approach (CGA) methodology was used to assess the association of these loci with multiple BCC (mBCC), i.e., more than one BCC. We combined 19 previously reported mBCC GWAS datasets (n=5,081) and mBCC cases (n=9,985) and performed meta-analysis using the CGA approach. The signal at the most significant SNP was rs78857623, which is an intronic variant in the UBAC2 gene. This is the first GWAS conducted on histopathologically confirmed cases with mBCC. Our results provide a promising candidate region for follow-up studies to confirm our findings.

116 Dasatinib promotes apoptosis of cutaneous squamous carcinoma cells
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Receptor tyrosine kinases are desirable targets for treatment of cancer. Imatinib mesylate is an inhibitor of multiple tyrosine kinases including ABL, KIT, and platelet-derived growth factor receptors. We have provided evidence for the potent effect of dasatinib on viability of cSCC cells suggesting dasatinib as a therapeutic candidate for patients with cSCC.

117 DNA methylation and hydroxymethylation regulate E-cadherin/CDH1 expression in squamous cell carcinoma of the skin
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DNA methylation down-regulates mRNA transcription by 5-methylcytosine, 5-mC, in promoter sequence, and takes significant roles in development, aging, and carcinogenesis. Recently the existence of 5-hydroxymethylation, 5-hmc, in human genome was discovered. Tet family proteins, Tet1, 2, or 3, oxidize 5-mC to 5-hmc, then it is turned into 5-hydroxy-mC. We established new method, non-isotopic cytosine extension assay for 5-hmc, previously, and reported that 5hmcM ratio in skin is firmly conserved in age: 5-mC/5-hmc ratio could be closely regulated to maintain homeostasis in normal condition. Because of the methodological difficulty in analyzing 5-hmc, the 5-hmc data in skin tumor is sparse, and the in situ analysis has not been conducted. With the extracted genomic DNA from the 19 genes previously associated with BCC, six genes (TERT, CTPII, KRT5, PADI6, TGM3, and TPS1) were significantly associated with BCC compared to no BCC in our CGA. In sharp contrast, only one gene (UBAC2) was significantly associated with mBCC. Through our data analysis, E-cadherin (CDH1) was localized on chromosome 2 and 3. The most significant SNP was rs78857623, which is an intrinsic variant in the EHD1 gene (P=1.19x10^-10). The CGAs point out that the genetic composition differs between people who develop only one BCC compared to people who develop mBCC. In addition, the GWAS performed on mBCC identified a new region, 17q11.2, with high density of genetic variants. This is the first GWAS conducted on histopathologically confirmed cases with mBCC. Our results provide a promising candidate region for follow-up studies to confirm our findings.

118 DNA repair specifies regional mutation frequencies in human cancer
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One possible mechanism explaining the prolonged disease development in early stage mycosis fungoides (MF) could be that the patient’s immune system controls tumour progression by an antitumour immune response, in which dendritic cells are thought to actively participate. To focus on studying the 3 lineages of dendritic cells (DCs); Langerhans cells (LCs), dermal dendritic cells (DDCs), and plasmacytoid dendritic cells (pDCs), through determining the expression of their markers CD1a, CD3 and CD123 respectively in early stage MF. 16 patients with early stage MF and 6 controls were included. Skin biopsies underwent immunohistochemical staining for CD1a, Factor XIIIa and CD123 respectively in the early stage MF. The mean number of positive cells of both CD1a+ve LCs and factor XIIIa DDCs were significantly higher in the MF patients in comparison to the controls (P=0.001, P=0.013) respectively. However, no significant difference was documented regarding the mean number of CD123+ve pDCs between MF patients (0.77±0.5) and controls (0.2±0.47) (P=0.13). ROC analysis revealed that the area under the curve of CD1a+ve LCs and factor XIIa DDCs was 0.948 and 0.844, with the best cutoff values 3.167 and 5.83, that achieved sensitivity 100%, specificity 67%, and sensitivity 100%, specificity 67% respectively. A possible role is played by LCs, DDCs and pDCs in the pathogenesis of MF. However, it was shown to tumor cells to antagonize their progression or to be a source for chronic antigenic stimulation resulting in the immune escape of tumor cells and perpetuation of the disease needs more clarification.
HSP72 is a possible target for combination therapy with HDAC inhibitor

Increased frequency of regulatory T cells with impaired suppressive capacity after PUVA in cutaneous T-cell lymphoma

Inhibition of Aurora Kinase-A in skin carcinogenesis

Interaction of CD100 and its receptor Plexin-B1/B2 in the pathogenesis of Cutaneous Squamous Cell Carcinoma

Investigating the Mechanism of Action of Ingenol Mebutate in cutaneous SCC

Infrared Radiation Confers Resistance to UVB-induced Apoptosis in Normal Human Melanocyte

123 Inhibition of Aurora Kinase-A in skin carcinogenesis

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In the present study we investigated the impact of IR on UV-induced apoptosis and DNA repair in normal human melanocytes since malignant transformation of normal melanocytes is a prerequisite for malignant melanoma. UVB (10mJ/cm2) induced apoptosis, caspase-3 cleavage, histone H3 and H4 acetylation, reduction of mitochondrial membrane potential were enhanced in HSP72 knockdown cells. Baseline expression level of bcl-2 was lower in knockdown cells. That of bcl-xl and XIAP was not changed, while HSP72-induced attenuations of these proteins were more evident in knockdown cells. In conclusion, HSP72 is a possible target for combination therapy with HDAC inhibitor.

Cutaneous T-cell lymphoma (CTCL) is a disease of proliferative CD4 T cells housing in the skin and caused by the human T-cell lymphoma virus type 1 (HTLV-1). While CTCL is a chronic and life-long disease, there is a high risk of transformation into more aggressive and fatal condition. Photochemotherapy induces apoptosis of malignant cells and is highly effective in the initial stages of the most common CTCL malignancy mycosis fungoides (MF). However, PUVA is also known for inducing the formation of high-risk SCCs in mice, especially in the underlying therapeutic mechanism in MF is not yet well understood. We addressed this issue by examining skin biopsies and blood of MF patients before and during PUVA treatment. At baseline we found a low number of Fosq1+ cells in the lymphoid infiltrate in the skin, however, the relative frequency of Treg cells was enhanced. The frequency of Treg cells increased further after treatment with PUVA, conversely, their suppressive capacity decreased. Moreover, we observed a decrease in TH17 (RORγT+) cell frequency and the moni-
β4) belongs to the phospholipase C family of enzymes, regulating

Phospholipase C beta 4 (PLC-beta4) regulates invasion and proliferation in cutaneous SCC

and identify miR-31 as a potential target for cSCC treatment.

phenotypes. These results indicate that miR-31 regulates cancer-associated phenotypes of cSCC

migration, invasion and colony forming ability, whereas overexpression of miR-31 induced these

situ hybridization revealed that miR-31 was specifically up-regulated in tumor cells. Mechanistic

responsible for approximately 20% of skin cancer-related death yearly. We have previously com-

MicroRNA-31 is overexpressed in cutaneous squamous cell carcinoma and regulates cell

motility and colony formation ability of tumor cells

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Withdrawn

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Possible association between actinic keratosis and polymorphism rs7208422 (c.917A→T, p.N306I) of EVER2 gene in non-epidermodysplasia verruciformis patients.

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In recent years, two novel genes, EVER1 and EVER2, have been detected, mutations in which play a role in development of epidermodysplasia verruciformis (EV), a genodermatosis associated with precancerous lesions/skin cancers. Recently it has been found that, polymorphism rs7208422 (c.917A→T, p.N306I) in EVER2 is related to an increased risk of squamous skin cancers (SCC) in non-EV patients. We hypothesized, that this polymorphism might be also associated with actinic keratosis (AK). The aim of the study was to determine whether polymorphism rs7208422 of the EVER2 gene is associated with AK in non-EV patients. We genotyped rs7208422 in 65 patients with AK and 280 control group using RT-PCR. We detected a possible association between AK and rs7208422 TT (frequency of the genotype TT in AK was 38.5% and 26.3% in the controls; OR=1.75, p=0.056 for recessive model of inheritance). We also found an association between rs7208422 TT and the age in which AK appeared and the extent of AK. This variant was more frequent in AK comprising of more than 3 areas (OR=3.14, p=0.031 for recessive model; OR=2.34, p=0.01 for allelic comparison). These associations remained significant in a multivariate regression analysis, which evaluated the compound point of these parameters showing, that both of them were independently associated with genotype TT (p=0.031). Summarizing this study could point to the potential role of polymorphism rs7208422 (c.917A→T, p.N306I) of EVER2 gene in AK.

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

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Phospholipase C beta 4 (PLC-beta4) belongs to the phospholipase C family of enzymes, regulating cell growth, differentiation and migration through activation of protein kinase C (PKC) and intracellular calcium release. We found using gene expression arrays that PLC-beta4 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. Using RNAi technology, we achieved transient and lentiviral stable knock-down (KD) of PLC-beta4 in SCC cell lines. SCC cells with KD of PLC-beta4 had decreased proliferation without altering differentiation or inducing apoptosis. Live-imaging confocal microscopy demonstrated that PLC-beta4 KD cells had absent intracellular calcium-release following specific activation of the PLC-beta4 pathway. Moreover, various functions role for human papillomavirus (HPV) with ultraviolet radiation (UVR) in the aetiology of cutaneous SCC (cSCC) is supported by epidemiological data confirming a significant association with beta genus HPV. Possible pathogenic mechanisms include interactions between host cellular proteins and HPV oncogenes. For example, UV-induced pro-apoptotic activity of the mitochondrial effector BAK is abrogated by HPV E6-mediated degradation. Beta genus HPV E6 oncogenes have also been shown to repress NOTCH signalling in vitro through their interaction with the transcriptional co-activator Mastermind-like 1 (MAML1). NOTCH mutation has recently been identified as a common early event in cSCC development. We hypothesised that HPV E6-dependent inhibition of MAML1 transactivation may represent an alternative mechanism for abrogating NOTCH signalling in the absence of NOTCH mutation. The mutational status of NOTCH1 and NOTCH2 and the presence of HPV DNA were investigated in a series of 79 cSCC. A majority (66%, 52/79) of the samples were positive for beta genus HPV while 86% (68/79) had NOTCH1 and/or NOTCH2 mutations. HPV DNA was detected in a higher proportion of NOTCH wild-type (82%, 9/11) than NOTCH mutation (63%, 43/68) tumours, although the difference was not statistically significant (p=0.3), likely due to the small number of NOTCH wild-type samples. Our data suggest that NOTCH mutation is the major mechanism by which the NOTCH signalling pathway is repressed in cSCC. Further investigation of a larger sample series should determine whether beta HPV prevalence is indeed higher in NOTCH wild-type tumours, which may further support role for HPV in the abrogation of NOTCH signalling and the pathogenesis of cSCC.

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Investigation of a possible role for human papillomavirus as a repressor of notch signalling in skin carcinogenesis

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132 Premature aging features rescue by inhibition of NADPH oxide activity in XPC deficient mice
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Progeroid syndromes are a group of diseases characterized by signs of premature aging. Most of these syndromes include defects in different DNA repair systems such as nucleotide excision repair (NER). Among NER defects, xeroderma pigmentosum type C (XPC) is a rare hereditary disease characterized mostly by a predisposition to skin cancers and accelerated photocaging, but little is known about premature aging in this disease. Comparing young and old mice, we found that the level of premature aging was increased in the young Xpc−/− mice. The expression level of mitochondrial complexes and mitochondrial functions in the skin of young Xpc−/− animals was lower as in control aged Xpc+/+ animals. Furthermore, metabolic profile in young Xpc−/− resembled to the profile found in the aged Xpc+/+ mice. Our study further supported the role of premature aging features in young Xpc−/− mice were mostly rescued by inhibition of NADPH oxide 1 (NOX1) activity using a novel NOX1 peptide inhibitor. Our results suggest that the continuous oxidative stress due to overactive NADPH extends the functional impairment in the epidermis of XPC-deficient mice.

133 PTCH1 mutations impair specifically base excision repair in Gorlin's fibroblasts
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Gorlin syndrome is an autosomal dominant disorder whose incidence is estimated at about 1 per 60,000 individuals. It is characterized by radio-sensitivity and an increased predisposition to basal cell carcinomas (BCCs). Mutations in the tumour suppressor gene PTCH1 are responsible for these clinical manifestations. As several genetic mutations in the DNA repair genes are responsible for photo or radiosensitivity and high predisposition to cancers, we hypothesized that these effects in Gorlin syndrome might be due to a defect in the DNA damage response (DDR). Therefore, the objective of this work is to study the effect of PTCH1 mutations on the DNA damage response in order to better understand the cellular and molecular mechanisms leading to hyper-radio-sensitivity and high susceptibility to various cancers in Gorlin’s patients. To that aim, we used fibroblasts from Gorlin patients or healthy individuals and compared their radiosensitivity, their resistance to several types of drugs and the DNA repair capabilities in base excision repair and mismatch repair pathways. Our results suggest that PTCH1 mutations in Gorlin’s fibroblasts do not only impair the BER system under basal conditions but also prevents the cell from adapting to an oxidative environment. The synergistic effects of increased ROS, accumulated DNA damage and impaired DNA repair could participate and partly explain the increased radiosensitivity of Gorlin’s patients. Perturbations in the PTCH1 signaling pathway might provide a potential therapeutic strategy to treat patients affected by Gorlin syndrome.

134 Quantification of CD8+ T cells and FOXP3+ regulatory T cells in actinic keratoses and cutaneous squamous cell carcinomas
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Skin cancer is the most common form of cancer in Germany with an incidence of 120 to 130 cases per 100,000 inhabitants. Previously, we established that the CC chemokine CCL27 is exclusively expressed in human skin and observed its progressive loss during cutaneous carcinogenesis (actinic keratosis, basal cell carcinoma, squamous cell carcinoma). Furthermore, the activation of the EGFR/Ras signaling pathway resulted in the marked downregulation of CCL27 in keratinocytes in vitro and in the epidermis in vivo. Our recent findings suggest that CCL27 plays a crucial role in skin homoeostasis and that its progressive loss enhances cutaneous carcinogenesis. To further investigate the role of CCL27 in cutaneous carcinogenesis, we took advantage of a CCl27 null mouse strain and analyzed the role of CCL27/CCL11 in a two-stage chemo-carcinogenesis model used by Damia and colleagues. Twelve-week-old Ccl27−/− female BALB/c mice and wild-type (WT) controls received a single application of 25 mg/200 ml of the initiating agent DMBA followed by multiple applications of 5 mg/200 ml of the promoting agent TPA for 40 weeks. Repetitive TPA applications induced cutaneous tumors and altered skin barrier function with treatment of TPA progressing to normal Ccl27−/− controls. During the experiment we measured the number of the occurring tumors per mouse and in addition, the tumor size at the end of the experiment. Surprisingly, Ccl27−/− mice developed significantly less and smaller tumors when compared to WT controls, which was accompanied by a significantly increased survival of Ccl27−/− mice (***p=0.009). Since the DMBA/TPA model of cutaneous carcinogenesis critically depends on skin inflammation, findings of the present study suggest that the loss of CCL27 signaling within the skin impairs cutaneous inflammation and subsequent tumor progression in a lower percentage of tumor development. Consequently, the use of a non-inflammatory-induced UV-induced mouse model of cutaneous carcinogenesis will further elucidate the role of CCL27/CCR10-interactions.

135 Rosuvastatin: is it a promising agent for melanoma chemoprevention?
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Evidence from preclinical and clinical studies indicates that statin use may be associated with lower incidence of melanoma. Inhibition of HMG-Coa reductase decreases the synthesis not only of cholesterol but also other lipid-soluble compounds involved in protein pre-nylation. Considering the role of prenylated proteins in regulation of cellular processes connected with melanomagenesis, we studied antioxidant activity of rosvastatin against human melanoma cells in vitro. In our study cell-surface cell lines (A549 and WM1552C) established from a primary superficial spreading melanoma were used. Cells were treated with rosvastatin at concentrations ranged from 10-5000μg/ml for 24-72h. To assay in vitro cytotoxicity of rosvastatin, MTT assay and real time cell growth analysis (clonogenic assay) were performed. We also analyzed induction of apoptosis and influence of rosvastatin on cancer cell cycle progression. Our analysis revealed that rosvastatin reduce viability of A549 melanoma cells. Cytotoxic effect was observed after 24h incubation at concentration of 1000μg/ml. Under the same conditions rosvastatin induced relatively lower activity against WM1552C cells. We also did not observe induction of apoptosis in any of melanoma cell lines analyzed. The results of our study indicates that sensitivity to rosuvastatin is highly dependent on the tumor cell line assessed. However, concentrations required for decreasing cancer cells viability in vitro exceeds 10 to 50-fold plasma concentrations reached in patients treated with rosvastatin, what probably preclude its use in melanoma chemoprevention.

136 Skin carcinogenesis is reduced in immune-deprived Ccr10-deficient mice
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Skin cancer is the most common form of cancer in Germany with an incidence of 120 to 130 cases per 100,000 inhabitants. Previously, we established that the CC chemokine CCL27 is exclusively expressed in human skin and observed its progressive loss during cutaneous carcinogenesis (actinic keratosis, basal cell carcinoma, squamous cell carcinoma). Furthermore, the activation of the EGFR/Ras signaling pathway resulted in the marked downregulation of CCL27 in keratinocytes in vitro and in the epidermis in vivo. Our recent findings suggest that CCL27 plays a crucial role in skin homoeostasis and that its progressive loss enhances cutaneous carcinogenesis. To further investigate the role of CCR10 signaling during cutaneous carcinogenesis, we took advantage of a Ccr10-deficient mouse strain and analyzed the role of CCL27/CCR10 in a two-stage chemo-carcinogenesis model used by Damia and colleagues. Twelve-week-old Ccr10−/− female BALB/c mice and wild-type (WT) controls received a single application of 25 mg/200 ml of the initiating agent DMBA followed by multiple applications of 5 mg/200 ml of the promoting agent TPA for 40 weeks. Repetitive TPA applications induced cutaneous tumors and altered skin barrier function with treatment of TPA progressing to normal Ccr10−/− controls. During the experiment we measured the number of the occurring tumors per mouse and in addition, the tumor size at the end of the experiment. Surprisingly, Ccr10−/− mice developed significantly less and smaller tumors when compared to WT controls, which was accompanied by a significantly increased survival of Ccr10−/− mice (***p=0.009). Since the DMBA/TPA model of cutaneous carcinogenesis critically depends on skin inflammation, findings of the present study suggest that the loss of CCL27 signaling within the skin impairs cutaneous inflammation and subsequent tumor progression in a lower percentage of tumor development. Consequently, the use of a non-inflammatory-induced UV-induced mouse model of cutaneous carcinogenesis will further elucidate the role of CCL27/CCR10-interactions.

137 Skin response to a carcinogen involves the pregnane X receptor
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The pregnane X receptor (PXR) is a transcription factor expressed in liver and intestine that is activated by xenobiotic chemicals including drugs and environmental toxicants. Topical application of the tumour initiator 7,12-dimethylbenz[a]anthracene (DMBA) enhances PXR, CY1A1, CYPIB1, and CYPA1, but not AHR expression. Surprisingly, PXR is expressed in Langerhans cells (LC), and DMBA-induced PXR up-regulation is largely impaired in Langerin+ cells-depleted skin. While PXR and AHR deficiency protects against DNA damage in epidermal cells, only PXR deficiency increases LC migration after skin exposure to low-dose of DMBA, potentially explaining the delay in skin carcinogenesis in PXR-deficient mice. Control of LC migration by PXR occurs via COX-2 and autocrine TGF-beta1 signalling. Human cells and PXR-humanized mice established the human relevance of these findings. This is the first report suggesting that PXR might be involved in cutaneous carcinogenesis and thus a potential target for anti-cancer therapy.
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The mammalian target of rapamycin (mTOR) signaling pathway is active in invasive neoplastic skin proliferations

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The mammalian target of rapamycin (mTOR) is a gatekeeper for various cellular signals which control cell growth, proliferation, and metabolism. mTOR is dysregulated in various human diseases such as cancers and cardiovascular disorders. There is evidence that PI3K-mTOR cell signaling is active in squamous cell carcinomas (SCC) of the skin. However, there is only limited data on the activation of components of this pathway in invasive neoplastic proliferations as compared to healthy control skin (HC). In our study, we evaluated immunohistochemical staining of skin biopsies with antibodies specific for phosphorylated forms of Akt as well as ribosomal S6 protein, a downstream substrate of mTOR. We found a significant increase of these pathway activation markers not only in SCC (n=8) and SCC in situ (Bowen’s disease, BD, n=8), but also in actinic keratoses (AK; n=13) when compared to normal, healthy appearing skin (n=22). These results indicate that the up-regulation of this pathway is associated with even early stages of enhanced keratinocyte proliferation and progression of these pre-invasive conditions to an overt carcinoma. Remarkably, the staining data for phos-(S473)Akt support the notion that an increased PI3K-mTOR pathway activity may already be seen in sun-damaged skin lesions (n=22), although other malignancies in the skin, such as basal cell carcinoma, have not been evaluated in this respect. From these data we conclude that targeting the mTOR signaling pathway might be a treatment option of pre-invasive neoplastic skin proliferations such as AK and BD.

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The TRAF-interacting protein (TRAP) is a regulator of the spindle assembly checkpoint

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The TRAF-interacting protein (TRAP) is a ubiquitously expressed nuclear E3 ubiquitin ligase that interacts with CYLD, SYK and DNA Polymerase h. Mutations in the CYLD gene cause the Brooke-Spiegler syndrome characterized by the presence of skin appendage tumors such as cylindromas and trichoequileithelomas. Accurate chromosome segregation during mitosis is temporally and spatially coordinated by fidelity-monitoring checkpoint systems. Deficiencies in these checkpoint systems can lead to chromosome segregation errors and aneuploidy, potentially promoting tumorigenesis. TRAP knock-down in keratinocytes leads to cell cycle arrest in the G1/S phase. We report here that TRAP was preferentially localized around mitotic chromosomes in early mitosis and incorporated into nuclear-derived foci and premature re-entry upon mitosis exit and early G1 phase. Its functional inactivation in HeLa cells by siRNAs accelerated the early mitosis progression from nuclear envelope breakdown to anaphase onset and increased the percentages of chromosome alignment defects in metaphase and lagging chromosomes in anaphase compared to control cells. TRAP depletion weakened the spindle checkpoint response in the presence of nocodazole and significantly reduced kinetochore levels of MAD2 but not of other spindle checkpoint proteins. These results imply that TRAP is involved in the spindle assembly checkpoint by regulating MAD2 abundance at kinetochores and the accurate distribution of chromosomes between daughter cells. Therefore, TRAP is implicated in the control of mitosis and tumor development.

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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 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 in parallel using a specific blocking peptide against TLR4 (viper vs control peptide cpi). Keratinocytes reacted with an increased proliferation in response to MDII peptide but not in response to viper. In addition, we detected that the blocking HTA125 antibody induces the phosphorylation of SAPK/JNK and ERK1/2 in primary keratinocytes. We also observed that with the subsequent growth of normal primary keratinocytes up to full confluence and differentiation, the surface expression of TLR4 increased significantly. This correlates with the differential TLR4 expression within the layers in normal skin and skin from patients with Squamous cell carcinoma. Furthermore, we found that the tumor SCC13 cell line, stably expressing TLR4 showed lower proliferation capacity and higher motility. SCC13 TLR4 positive tumors showed a decrease in their proliferation and may be operative during the progression of these pre-invasive conditions to an overt carcinoma. Interestingly, HTA125 antibody revealed increased RNA levels of the proto-oncogene Ki67 which may well explain the changes in cellular proliferation rates and might point out TLR4’s important role in cutaneous SCC.

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Ubiquitin-Specific Protease 2 promotes chemophototherapy resistance in cutaneous T-cell lymphoma

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In this study, we aim to investigate the roles of Ubiquitin-Specific Protease 2 (USP2) in the pathogenesis and treatment of cutaneous T-cell lymphoma (CTCL). CTCL is the most common extra-nodal lymphoma arising from skin-homing T-lymphocytes. Mycosis fungoides (MF) and Sézary syndrome (SS) are the most common CTCL. To date, we still lack effective tools for diagnosis and treatment of CTCL. USP2s are de-ubiquitinating enzymes which remove ubiquitin from protein and allow them salvage from proteasome degradation. Due to their protease activity and involvement in several diseases, USPs become potential targets for pharmacological interference. USP2 plays an important role in the protection of prostate cancer from apoptosis. Until now, the role of USP2 in CTCL has not been very clear. First, we set out to investigate the mRNA expression of USP2 in MF plaque, CTCL, normal, and psoriasis lesion. Interestingly, USP2 showed decreased expression in MF plaque and tumor compared to normal and psoriasis biopsies. USP2 showed the highest expression in MyLa2000 cells among three CTCL cell lines. Subsequently, we investigated the treatment of USP2 in MF plaque. USP2 mRNA expression was significantly increased in MyLa2000 cells, not Hut-78 and SeAx. Combined with chemophototherapy, USP2 knockdown resulted in decreased MD2 expression and upregulation of p33 target gene, p21, indicating the anti-apoptotic property of USP2 is p53-dependent. Taken together, USP2 promotes chemophototherapy resistance in CTCL. In the future, we may retrieve better therapeutic outcome in CTCL by manipulating USP2.