



RACK1 labelling distinguishes melanocytoma from melanoma in dogs and horses

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antigen-specific response in C57BL/6 *wildtype* mice bearing established s.c. B16.F10 melanoma. This treatment mediated the systemic induction of CD8⁺ melanocyte antigen-specific T cells of enhanced avidity, a B16-specific serum IgG response and a sustained NK cell expansion, leading to the inhibition of melanoma outgrowth in 6 of 7 mice, and 4 of 7 mice surviving tumor-free for 200 days. Furthermore, effective CD8⁺ T cell mediated immunological memory was formed. Our data show that by using monobenzone to induce targeted autoimmunity to the melanocytes the tumor originated from, an effective and lasting anti-melanoma immune response orchestrating different arms of the immune system was induced.

OP06 Pregna-5,7-diene-3 β ,21-diol and its vitamin D-like derivatives as potential anti-melanoma factors

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Pregna-5,7-dienes and their hydroxylated derivatives with minimal (low/none) calcemic effects can show comparable anticancer activities to the active form of vitamin D3 (calcitriol). We have synthesized for the first time prena-5,7-diene-3 β ,21-diol (21-OH-7DHP) and, using ultraviolet B (UVB) radiation, induced photoconversion of 21-OH-7DHP to 21-OH-pregnacalciferol, 21-OH-pregnalumisterol₃ and 21-OH-pregnatachysterol derivatives (vitamin D-like compounds). The number and character of the products and the dynamics of the process were dependent on the UVB dose, and derivatives could be distinguished by characteristic UV absorption. At high UVB doses, the formation of multiple oxidized derivatives of the primary products, was observed. Subsequently, the products of irradiation were separated and characterized by means of RP-HPLC chromatography, MS and NMR spectroscopy. Newly synthesized compounds inhibited human pigmented and non-pigmented melanoma SK-MEL 188 growth in a dose dependent manner, with comparable potency to calcitriol. Interestingly, those compounds had none or slight stimulatory effects on HaCaT keratinocytes growth. In summary, we have characterized for the first time the products of UV induced conversion of 21-OH-7DHP and documented that the precursor and its photoderivatives have selective, inhibitory effects on melanoma cell proliferation.

SESSION I: DEVELOPMENTAL BIOLOGY OF PIGMENT CELLS

OP07 Implication of notchless and strawberry notch homolog 2 genes in melanocyte stem cells homeostasis

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Seven genes only have been reported to control melanocyte stem cells (MSCs) homeostasis. Out of these, three encode

components of the Notch pathway, namely *Notch1*, *Notch2* and *RBP-Jk*. *Notchless homolog 1* (*Nle1*) and *Strawberry Notch homolog 2* (*Sbno2*) genes are mouse orthologs of regulators of Notch pathway in *Drosophila*. If the roles of *Nle1* and *Sbno2* are conserved, mutations in *Nle1* and *Sbno2* should alter MSC homeostasis in the mouse. To test this hypothesis, *Nle1*^{fl/fl} mice have been crossed with *Tyr:Cre* mice, which express Cre recombinase in the melanocyte lineage, to produce conditional *Nle1* knock-out (*cNle1* KO) mice. In parallel, *Dct:Sbno2* transgenic mice overexpressing *Sbno2* in the melanocyte lineage were produced. We found that during embryogenesis, elimination of *Nle1* drastically affected melanoblasts survival, whereas *Sbno2* overexpression had a slight effect on melanoblasts migration. Hence, *cNle1* KO pups had an essentially white coat with occasional wild-type spots, while *Dct:Sbno2* pups had a wild-type coat with white tail tip and feet. As the mice got older, the wild-type areas of both mutants whitened rapidly. This accelerated whitening was associated with a reduced number of MSCs in the hair follicle bulges. Moreover, from the second hair cycle onwards pigmented melanocytes were seen in the bulge area of hair follicles from both *cNle1* KO and *Dct:Sbno2* mice, indicating anticipated differentiation of MSCs or melanocyte progenitors. Altogether, these observations indicate that *Nle1* and *Sbno2* genes are involved in MSCs maintenance.

SESSION II: GENETICS OF PIGMENTATION

OP08 MART-1 gene expression and function

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MART-1 is an important melanoma-associated antigen, which has been widely studied in the context of immunotherapy to develop a vaccine for malignant melanoma. We are studying different aspects of the biology of this pigment cell specific gene. We analyzed its promoter and potential regulatory elements. We observed that the promoter alone was sufficient to drive a strong expression in RPE, however it was not able to drive a strong melanocyte-specific expression, which indicates that further regulatory elements might be implicated in MART-1 expression. To address this issue, we used a BAC transgenics approach. We inserted a LacZ gene into a MART-1 BAC clone and produced a transgenic reporter mouse line. We observed the specific expression of the reporter gene both in melanocytes and RPE. We also tested some of the conserved noncoding sequences located upstream of the MART-1 genomic region; however none of these regions showed a regulatory activity. As well as regulation of MART-1 expression, we are interested in understanding the function of MART-1. The exact function of MART-1 is not known, however it has been shown to interact with the melanosomal protein Pmel17, and this interaction suggests a role in maturation of Pmel17. We believe that the results of our study would provide important information concerning the effect of MART-1 on processing of Pmel17 and its involvement in melanosome biogenesis.

OP09 Mitf regulates expression of the interferon regulatory factor 4 (IRF4)

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Department of Biochemistry and Mol. Biology, University of Iceland, Iceland (*contributed equally)

A recent gene-expression analysis identified target genes of the microphthalmia-associated transcription factor (MITF). Among

PP54 MicroRNA miR-196a is a central regulator of HOX-B7 and BMP4 expression in malignant melanoma

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Since bone morphogenetic proteins (BMPs) play an important role in melanoma progression, we aimed to determine the signaling cascade leading to over-expression of BMP4 in melanoma cell lines compared to melanocytes. Recent analyses demonstrated that Ets-1 is a positive regulator of BMP transcription and that activity of Ets transcription factors in turn is regulated via fibroblast growth factors. Indeed, in this study, over-expression of bFGF in melanocytes and melanoma cells led to increased Ets-1 activity and thus activated the *BMP4* promoter resulting in induction of BMP4 expression. Moreover, we demonstrated that bFGF mediated induction of migration is achieved via activation of BMP4, thus determining BMP4 as major modulator of migration in melanoma.

HOX-B7 was reported to constitutively activate bFGF and is over-expressed in melanoma cells. Knockdown of HOX-B7 led to down-regulation of bFGF and BMP4. Computer algorithms suggested miR-196a a potential regulator of HOX-B7 expression. In accordance, expression of miR-196a was significantly diminished in melanoma cells compared to melanocytes. Melanoma cell clones re-expressing miR-196a showed decreased expression of HOX-B7, bFGF and BMP4. Finally, we were able to demonstrate direct binding of miR-196a to the HOX-B7 3'UTR. In summary, we were able to show that BMP4 expression is regulated by Ets-1 and bFGF, and that expression of bFGF in turn is controlled by miR-196a via HOX-B7. The analysis of this signaling cascade provides insights into early steps of melanoma progression and might thus harbor therapeutic relevance.

PP55 Proteomics analysis of melanoma versus melanocyte cell lines

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Objective: Identification of melanoma biomarkers that would be useful to improve clinical diagnosis and treatment options for melanoma patients.

Methods: To understand the molecular changes involved in the pathogenesis of melanoma, in the present study we have compared proteomic profiles of four different melanocyte cell lines and six human melanoma cell lines (three primary and three metastatic melanomas), using two-dimensional polyacrylamide gel electrophoresis (2-D PAGE).

Results: In the image analysis, we found 44 gel spots differentially expressed ($P < 0.05$) and with a change of volume bigger than 40%. Among them, 31 gel spots were of greater intensity and 13 gel spots of lesser intensity in the melanoma lines relative to the melanocyte ones. These spots were analysed by liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) and 134 proteins were identified, which were grouped based on

their functional distribution into the following categories: metabolism (22%), gene transcription (16%), cell cycle and proliferation (12%), transport (11.5%), protein folding and proteolysis (11.5%), response to stress (9%), apoptosis (6%), signal transduction (6%) and cell motility (5%). From proteomic analysis, we have chosen 18 differentially expressed proteins to verify their expression by Western Blot. Our preliminary results shown that melanoma cells might have disturbed the cellular response to free radicals and apoptosis.

PP56 The transcription factor c-Jun is regulated by loss of active cell-cell contacts during development and progression of malignant melanoma

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The transcription factor c-Jun forms homodimers or heterodimers with other members of the transcription factor super-family AP-1, influencing the expression of a multitude of regulators involved in tumor development and metastasis. We could show that c-Jun protein is upregulated in melanoma cells, whereas in melanocytes c-Jun protein is not expressed. Moreover, reporter gene assays revealed a strong AP-1 activity in melanoma cells. Gel shift assays confirmed a significant binding to the AP-1 consensus sequence in melanoma cells. Inhibition of c-Jun by a dominant negative form of c-Jun (Tam67) leads to loss of the transcriptional activity of AP-1 in melanoma cells. This indicates an essential role of c-Jun for AP-1 activity in melanoma. The cell-cell-adhesion molecule E-Cadherin plays a key role during development and progression of malignant melanoma. Interestingly, there is a coincidence in the loss of E-Cadherin expression and up-regulation in c-Jun protein in malignant melanoma. Our data could show that loss of E-Cadherin expression during melanoma development induces c-Jun activity, whereas in melanocytes active cell-cell-contacts via E-cadherin have a negative impact on c-Jun, suggesting a direct link between E-Cadherin and c-Jun. Further experiments show that the cytoskeleton is involved in the cell-cell contact dependent regulation of c-Jun. Treatment of melanoma cells, which reexpress E-Cadherin with Nocodazole reveals a strong induction of c-Jun protein in the nucleus. Reporter gene assays confirmed an up-regulation in AP-1 activity and gel shift assays showed an increased binding to the AP-1 consensus binding sequence. Melanoma cells treated with Taxol, a stabilizing agent, show the reverse effects. These experiments point to an involvement of the cytoskeleton in the E-Cadherin dependent regulation of c-Jun.

PP57 RACK1 labelling distinguishes melanocytoma from melanoma in dogs and horses

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Melanoma affects dogs and horses. Histological features of the tumors are not always sufficient to differentiate benign and malignant lesions. RACK1 protein expression has been shown to mark malignancy in human (Egidy et al., 2008). In veterinary pathology, benign melanocytic lesions are named "melanocytomas". We performed RACK1, MITF and cytokeratin 5 immuno-

labellings in melanocytic proliferations from dogs and horses. Healthy skin melanocytes from horse and dog exhibited no detectable RACK1 signal. By contrast, in melanoma lesions from dogs and horses, high homogeneous and diffuse RACK1 labelling was found in all neoplastic cells. In melanocytoma, RACK1 signal was also abundant but its cellular distribution was different from melanoma. The signal was focally detected in the cytoplasm. In conclusion, RACK1 labelling of melanocytoma lesions was not as low and heterogeneous in canine and equine benign tumors as seen in human nevi, thus suggesting these entities are not similar. Furthermore, the fact that RACK1 is over-expressed in cutaneous and mucosal melanomas from several mammalian species suggests a role for RACK1 in melanocytic proliferation and transformation.

Egidy G, Julé S, Bossé P, Bernex F, Geffrotin C, Vincent-Naulleau S, Horak V, Sastre-Garau X, Panthier JJ. Transcription analysis in the MeLiM swine model identifies RACK1 as a potential marker of malignancy for human melanocytic proliferation. *Mol Cancer*. 2008 Apr 28;7: 34.

OTHERS

PP58 Extracellular matrix proteins during progression and spontaneous regression of the MeLiM swine melanoma

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The MeLiM (Melanoma-bearing Libechov Minipigs) strain with hereditary melanoma is a suitable animal model for study this cancer disease. Histological analyses showed depigmentation of melanoma cells and rearrangement of ECM. Immune cells and melanoma cell destruction appeared firstly at the 10 weeks old piglets. For this reason, we appointed melanomas from piglets younger than 10 weeks as progressing ones. We studied expression of extracellular matrix proteins (ECM) in skin melanomas from 27 MeLiM piglets of different age (3 weeks – 8 months) in relation to progression / spontaneous regression processes (SR is also described in human melanoma). Tenascin C, collagen IV, laminin and fibronectin were generally localized in blood vessel walls by indirect immunofluorescence. Gradual remodelling of fibronectin and tenascin C structures from short to wide fibres and changes of its expression in SR melanomas was observed. Preliminary results of Western blotting showed low expression of laminin and high expression of tenascin C, collagen IV and fibronectin in progressing melanomas. During SR, expression of these proteins decreased and from the 3rd month of age again increased. These findings acknowledge that progression followed with SR in the MeLiM melanoma is a dynamic process characterized by active remodelling of ECM in relation to tumour rebuilding into connective tissue.

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PP59 Caveolin-1 functions both as a tumor suppressor and promotes metastasis in an *in vivo* melanoma model

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Caveolin-1 (cav-1) has frequently been attributed a role as tumor suppressor. However, data is also available associating presence of this protein in late-stage tumors with enhanced metastasis. Thus, the role of this protein in cancer remains highly controversial. The aim of this study was to establish an *in vivo* model showing that indeed caveolin-1 displays both characteristics. B16-F0 cells, a melanoma line isolated from C57BL/6 mice that express readily detectable basal levels of caveolin-1, were repetitively (10 cycles) injected intravenously and recovered from lung melanoma metastases, to yield B16-F10 cells that expressed almost undetectable basal caveolin-1 levels. When subcutaneously injected, B16-F10 cells formed solid tumors while intravenous injection resulted in lung metastasis. Derivatives of B16-F10 cells were generated by stable transfection with either empty vector (pLacIOP) or vector encoding caveolin-1 (pLacIOPcav-1) and tested in isogenic C57BL/6 mice. Primary tumor volume was recorded in animals injected subcutaneously with 300,000 cells. For metastasis assays, animals were injected intravenously in the tail vein with 200,000 cells.

Lungs were removed and fixed on day 21 post-injection. Black tissue mass, corresponding to lung metastases was determined. For B16-F0 cells, tumor formation in C57BL/6 mice was substantially lower than for B16-F10(mock) controls and B16-F10(cav-1) cells behaved like B16-F0 cells in this respect. However, in *Metastasis Assays* B16-F10(cav-1) cells generated at least twice as much metastatic lung tissue as did B16-F10 cells ($P < 0.001$). These results provide for the first time direct experimental evidence that caveolin-1 can function *in vivo* both as a tumor suppressor and promoter of metastasis.

PP60 p53 Expression and Melanocyte Colonization in Basal Cell Carcinomas

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Basal cell carcinoma (BCC) is the most common skin cancer. Even though they are keratinocyte-derived neoplasms a fraction of BCC tumors is pigmented due to melanocytic colonization. BCCs are also commonly associated with p53 mutations. To assess a possible interplay between functionality of p53 and melanocytic colonisation we examined in 40 BCC specimens the expression of p53, melanin and melan-A by means of immunohistochemistry. No correlation between the frequency of melanocytes in the tumors and the degree of pigmentation was observed. The level of p53 expression in the tumor cells inversely correlated with the percentage of melan-A positive cells, supporting a recently suggested model that functional p53 signalling in basalioma cells mediates chemoattraction of melanocytes.