ABSTRACTS | Pigmentation and Melanoma

507 Melanoma cells can adopt the phenotype of stromal fibroblasts by spontaneous cell fusion
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After the removal of primary cutaneous melanoma some patients develop local recurrences, even after having histologically tumor-free re-excision. We hypothesized that recurrent tumour initiating cells could be present in the peritumoral stroma having fibroblast phenotype as a result of a melanoma cell-fusion with a stromal cell line. To test our hypothesis, we generated a melanoma cell line expressing GFP and infected it with lentiviral vectors to express GFP fusion proteins of the fibroblast specific marker α-smooth muscle actin (α-SMA). Sufficient GFP-expressing cells were transfected with lentiviral vectors to express GFP fusion proteins of the stromal cell-specific marker CD44 and the fibroblast marker vimentin. Using fluorescence-activated cell sorting (FACS), we isolated, expanded and characterized cell lines stably expressing the fusion proteins. The simultaneous expression of melanoma- and stromal-specific markers indicated that the fusion is occurring between melanoma and stromal cells. This cell fusion may play a role in the development of recurrent melanoma by creating a more invasive, aggressive phenotype.

508 Modelling melanoma metastasis using organotypic skin equivalent and zebrafish models
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Currently, mouse melanoma models are the ‘gold standard’ for all studies of melanoma, but are unable to model early stages of human melanoma and are limited by their expensive/time-consuming nature. Development of alternative models is therefore an urgent priority. Zebrafish have been shown to be a useful model for the study of human melanoma, but have so far been used for metastatic studies only. In this study, we used a zebrafish model based on the ZFY transgenic strain, which expresses GFP and RFP under the regulatory elements of the human melanoma specific Melan-A gene. This allowed visualization of melanoma cells in vivo. We haematoxylin and eosin (H&E)-stained transverse sections of whole zebrafish were used to observe melanoma cell growth, migration and invasion in vivo. The efficacy of this approach was confirmed by analyzing the presence of GFP and RFP expressing melanoma cells in the skin of the animal. The results indicated that melanoma cells were able to metastasize in vivo, a result that has not been observed in previous studies. The evidence suggests that zebrafish could be used as a model for the study of melanoma metastasis. Further investigations are required to fully validate the use of zebrafish as a model for melanoma metastasis.

509 Dual role for ADAM-9 in melanoma development and metastasis in vivo
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Several proteases have been implicated in the invasive process of cancer cells and these include also the ADAM family of metalloproteases. We have previously identified ADAM-9 as being induced in melanoma cells at the periphery of the tumor as well as in adjacent peritumoral stromal cells while ADAM-9 is detected at very low levels in normal skin. We used transgenic mice expressing a secretable form of GFP linked to the ADAM-9 promoter to study the role of this newly discovered innate immune pathway in promoting anti-melanoma immunity. We have crossed Adam-9-/- mice with the B16-F10 melanoma cell line and investigated the role of ADAM-9 in the promotion of anti-tumor immunity. In vivo experiments showed that ADAM-9 deficient melanoma cells displayed reduced adhesion to and transmigration through an activated endothelial cell layer. Taken together, these data show that ADAM-9 is an important mediator of the innate immune response to melanoma.

510 Melanoma aggressiveness correlates with low CD271 expression levels in 3-dimensional tumor models
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Melanoma is characterized by a poor prognosis, while few therapeutic options are available for the treatment of advanced stage melanoma. In this study, we identified CD271 expression as a potential marker for melanoma aggressiveness. CD271 is a transmembrane protein that plays a key role in melanoma invasion and metastasis. Our results showed that CD271 expression levels are inversely correlated with melanoma aggressiveness. CD271 expression levels were significantly lower in more aggressive melanomas, suggesting that CD271 expression could be used as a potential prognostic marker for melanoma aggressiveness.

511 STINGing the tumor microenvironment for melanoma immunotherapy
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Melanoma is a highly aggressive but potentially immunogenic cancer. Recent studies using anti-CTLA4 and anti-PD1 antibodies for the treatment of metastatic melanoma have provided encouraging evidence for the ability of the immune system to fight cancer. Much interest has been devoted to the tumor microenvironment as the site for suppression of spontaneous antimelanoma tumor immunity. There is also evidence that this immunosuppressive environment can be antagonized by the induction of strong innate immune responses at the tumor site via activation of pattern recognition receptors. The STimulator of INFGenes (STING) signaling pathway and its ability to sense and respond to dsDNA are of particular interest. Here we report that STING activation in melanoma cells results in the suppression of melanoma growth and metastasis in vivo. Furthermore, we showed that STING activation is able to enhance the efficacy of anti-PD1/PD-L1 immunotherapy in vivo.

512 BRAF inhibitors reactivate tumor suppressor and oncopgenic p31 activities in melanoma
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Melanoma is characterized by a poor prognosis, while few therapeutic options are available for the treatment of advanced stage melanoma. In this study, we investigated the role of BRAF inhibition in the reactivation of tumor suppressor and oncopgenic p31 activities in melanoma. We used melanoma cell lines that were sensitive or resistant to BRAF inhibition and performed a comprehensive analysis of the reactivation of p31 activities. Our results showed that BRAF inhibition induced the reactivation of tumor suppressor p31 activities, such as p53 and PTEN, in BRAF inhibitor-resistant melanoma cells. These results suggest that the reactivation of p31 activities in response to BRAF inhibition can be a potential therapeutic strategy for melanoma treatment.

590 Journal of Investigative Dermatology (2014), Volume 134
Large congenital melanocytic nevus harbors cells clonogenic and tumorigenic properties. C. Charbel, 1 F. Fontaine, 1 N. Kaffub, 1 A. Coulomb, 1 A. Hou-Kit, 1 T. Tost, 1 A. Picard, 1 S. Aractingi 1, 2, 3, 4, 5 1 INSERM U892, CRCNA, Inserm U892, CNRS6299, Laboratoire d'immuno-dermatologie, Institut de Biologie du Cutanéum, Université de Nantes, France, 2 plateforme de Génétique des Cancers, CHU Hotel Dieu, Nantes, France, 3 Platforme de Génétique des Cancers, CHU Hotel Dieu, Nantes, France, 4 Plateforme de Génétique des Cancers, CHU Hotel Dieu, Nantes, France, 5 Department of Pathology, Université de Nantes, France.

These results suggest that the mutation status of tumor cells is not affected by culture conditions. The correlation was of 98% between PCR and immunohistochemistry results for tumor cell-lines in all but one discordant case. The alteration, both in FFPE NRAS (54%) or BRAF (54%) V600E protein was also inves-
tigated in the 63 autologous tumor cell-lines. Mutations were analyzed using either allele-specific PCR or quantitative PCR and sequencing in 63 FFPE tissue samples from stage III and IV melanoma patients and in the 63 autologous tumor cell-lines. Mutations were analyzed using either allele-specific PCR or quantitative PCR and sequencing in 63 FFPE tissue samples from stage III and IV melanoma patients and in the 63 autologous tumor cell-lines. The main objective of this work was to determine the presence of BRAF, NRAS and c-KIT mutation status between tumor tissues and autologous tumor cell-lines of stage IIIB melanoma.

Comparative analysis of BRAF, NRAS and c-KIT mutation status between tumor tissues and autologous tumor cell-lines of stage IIIB melanoma.

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Comparative analysis of TRAIP expression in benign versus malignant melanocytic proliferations: a pilot study

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The TRAF-interacting protein (TRAP) is a RING-type E3 ubiquitin ligation involved in the control of cell proliferation. TRAP was reported to interact with the two tumor suppressor genes CYLD and Syk but its precise role in carcinogenesis remains unknown. Moreover, no data regarding the expression of TRAP in melanocytic cells has been reported. Our aim was to assess the role of TRAP in melanoma pathogenesis. TRAP mRNA relative expression level was analysed in 10 melanoma cell lines and normal melanocytes from two different donors and 33 MLANA positive clinical samples (10 non-dysplastic nevi, 10 melanomas in situ, 13 malignant melanomas) by quantitative RT-PCR, using the delta-delta Ct method. TRAP mRNA synthesis was suppressed in a melanoma cell line using lentiviral infection with two plasmids expressing shRNA targeting TRAP to examine whether TRAP is required for melanoma proliferation. Mean difference between the groups was analysed using unpaired Student’s t-test. TRAP was overexpressed in 5/10 melanoma cell lines by at least two-fold compared with normal melanocytes. The TRAP and CYLD expression was inversely correlated in 6/10 melanoma cell lines. Median expression of TRAP, normalised to an endogenous control gene (RPL11A), was 3 times higher in melanoma in situ (p=0.0263) and 5.9 times higher in malignant melanoma compared with benign nevi (p=0.0121). TRAP positively correlated with MK67 RNA expression in a melanoma cell line with shRNAs giving a knock-down efficiency greater than 75% was leading to MK67 mRNA downregulation by more than 60% indicating that TRAP is important for cell proliferation. We conclude that TRAP is likely involved in proliferation control of the melanocytic cell lineages, with possible implications in melanoma progression and invasiveness.

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Correlation among anti-tumor T cell response and the redox homeostasis in human cutaneous malignant melanoma

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Progression of cutaneous malignant melanoma (CMM) depends on the characteristics of the tumor cells as well as on the host immune response. Molecular markers prescribing the relation between tumor cells and their microenvironment help to identify metastasis risk and to determine new biomarker targets. Our cell lines revealed a high expression of CD68 and GHOX1 staining of tumor cells. The correlation between peritumoral CD68+ cells and the HMX1 expression of the tumor cells was linear (r=0.875, p=0.003), but associated with the presence of HMX1+ cells infiltrating the tumor (p=0.039). These HMX1+ expressing cells exhibited dendritic and spindle cell morphologies. Nevertheless, the presence of peritumoral CD68+ cells was more frequent in primary CMM without haemangioendothelial metastasis (p=0.008). Peritumoral CD68 positivity was associated with the absence of CD66a (p=0.048), CD163+ (p=0.003) cells infiltrating the tumor and with presence of CD1a+ (p<0.005) cells surrounding the tumor. Interestingly, significant correlation was found between CD8b tumoral positivity and tumoral HMX1+ positivity (p=0.014) suggesting that production of reactive oxygen species and activation of the antioxidant pathway in melanoma cells might play a role in the modulation of the anti-tumor host immune response.

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Delta-like protein 3 promotes proliferation and migration of melanoma cells

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Notch pathway is involved in differentiation of many kinds of tissues. It is also important for cancer progression in several kind of tumors. However, little is known about role of notch signaling in melanoma progression. In this study, we attempt to illustrate relevance of Notch signaling, especially of Notch ligands in melanoma progression. We first tested expression of 5 ligands, including DLL1, DLL3, DLL4, JAG1 and JAG2 in melanoma. mRNA of DLL3 was preferentially expressed in melanoma rather than melanocytic nevus. To evaluate role of DLL3 in melanoma, we checked cell proliferation, cell migration, cell cycle and apoptosis using RNA interference and overexpression. Knocking down DLL3 inhibit cell proliferation of melanoma and induced G0/ G1 arrest in vitro. In addition, knocking down of DLL3 reduced both migration ability and colony formation ability of melanoma cells. There are no findings of apoptosis in DLL3-knockdown melanoma cells. Overexpression of DLL3 promoted migration ability of melanoma cells. Lastly, we found down-regulation of phospho-ERK in DLL3-knockdown melanoma cell lines. Down-regulation of phospho-ERK suggested that knocking down of DLL3 was involved in inhibition of MAPK signaling in melanoma. These results were compatible with induction of G1 arrest confirmed by flow cytometry. Consequently, our results suggested that DLL3 may be sufficient for progression and/or survival for melanoma cells.

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Diclofenac sensitizes melanoma cells to TRAIL-induced apoptosis through upregulation of TRAIL-R2/DR5 in the resistant melanoma cells

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Extensive research has led to demonstrate, in both humans and experimental animals, that induction of lytic molecules such as TNF-related Apoptosis Inducing Ligand (TRAIL) (e.g. by IFNα, Imiquimod or interleukins) induces tumor cell death. RTA protein of melanoma cells is overexpressed in the majority of the resistant melanoma cell lines, which may explain the sensitization of melanoma cell lines induced by diclofenac. Interestingly, enough, we also found that stimulation of PBMCs with diclofenac as well as celecoxib results in strong increase of TRAIL induction. According to the above mentioned, we also found that diclofenac treatment of WM983A cell line results in the upregulation of the death receptor TRAIL-R2/DR5 in the resistant melanoma cells (WM983A: 71.70% vs. 27.00%; 1205Lu: 23.98% vs. 2.28% apoptotic cells, respectively). In fact, the enhanced expression of TRAIL by different immune cell subpopulations (monocytes, mDC, pDC) in a magnitude similar to that observed with Imiquimod. These results suggest that inhibition of protein kinase C (PKC) and activation of the antioxidant pathway in melanoma cells might play a role in the modulation of the anti-tumor host immune response.

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Disruption of multiple signaling pathways in senile lentigo

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Senile lentigo (or age spots) are becoming a greater skin concern as the world’s population ages. Senile lentigos are hyperpigmented macules on the skin, having an irregular shape and they normally occur after the age of 40. Effective treatment for reducing the appearance of age spots is a major focus for dermatologists and researchers. However, the mechanism of how the age spots are formed is still not clear. In this study, we utilized a gene array approach to gain a more systematic view of changes in the area of age spots. 12 Caucasian women aged from 55–75 were recruited for the study. Biopsies were taken from lesional and peri-lesional areas of the skin, as well as sun-protected area. We identified differentially expressed genes by comparing gene expression profiles between lesional and peri-lesional area, as well as gene expression profiles between lesional and sun-protected area. Top-differential signaling pathways identified include melanogenesis, cell invasion, cell migration and cell differentiation. This study provides a glimpse into how age spots may be formed and can help identify possible points of intervention.

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Fibroblasts regulate both physiological and pathological pigmentation of skin in vitro and in vivo

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We have previously described that white human skin xenografted onto nude mice could become black or totally white. These modifications of pigmentation pattern were associated with changes in densities of dermal fibroblasts. In vitro, we have compared reconstitutions made on DDD colonized or not with various concentrations of normal fibroblasts and we have reproduced this phenomenon of increase or decrease of pigmentation. Furthermore since, in systemic scleroderma SSc, a disease characterized by an excessive secretion of collagen by fibroblasts, pigmentation changes are often marked, we have constructed epidermis on DDD colonized by fibroblasts of SSc patients and demonstrated that for a same seeding density, SSc fibroblasts can either stimulate or inhibit pigmentation. Consequently, we looked at possible fibroblast-derived molecules and we focused on FGF-2 which is a well-known pigment enhancer. The level of FGF-2 was low in hypopigmented xenografts and high in hyperpigmented xenografts, showing an association between the two phenomena. In patients with SSc, FGF-2 was also differentially expressed. Using our model of reconstitutions on DDD colonized by increasing densities of FGF2 overexpressing fibroblasts we observed that pigmentation changes and epidermal FGF-2 were not in presence of low or high number of FGF-2 transduced fibroblasts. Since low level of FGF-2 was associated with depigmentation we measured it expression in skin biopsies of SSc patients: a significant decrease in FGF-2 in perilesional skin was observed with an increased number of dermal vimentin positive cells. In vitiligo/NV, the pattern of expression of FGF-2 is similar to that of one of its target molecule CCN3. Similar to NSV, CCN3 expression in mouse xenografts and SSc patients followed that of FGF-2. In conclusion, our data associated with clinical observations in pigmentation disorders support the hypothesis that fibroblasts modulate pigmentation by each one factor acting on FGF2-CCN3 axis. CCN3 activity in skin should be investigated as possible target for the treatment of SSc patients with pigmentation troubles.

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Gene Expression Profiling in Korean Women

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To date, there is a paucity of data about the difference in the degree of mRNA transcription of the genes between the melasma lesional skin and its normal adjacent one. We conducted this study to identify novel genes involved in the pathogenesis of melasma using gene expression profiling with microarray. In this study, we performed large-scale gene expression profiling using a microarray analysis and confirmed the results on the quantitative real-time polymerase chain reaction (qRTPCR) in Korean women with melasma. There were 314 genes whose degree of expression showed a significant difference between the melasma lesional skin and its normal adjacent one. Of these, five genes were confirmed on both the microarray analysis and qRTPCR. In the melasma lesional skin as compared with its normal adjacent one, there was down-regulation (>2 fold) of genes involved in the PPAR signaling pathway (adiponectin, C1Q and collagen domain-containing (ADypoQ) and up-regulation of guanine deaminase (GDA) (9 fold), those involved in the functions of stratum corneum barrier (S100 calcium-binding protein A8 (S100A8), small proline-rich protein 2A (SPRR2A), small proline-rich protein 2B (SPRR2B) and kallikrein-related peptidase 6 (KLK6) (10 fold), NAD(P) dehydrogenase, quinine 1 (NQO1) (>2 fold) and those involved in the tyrosine metabolism, the activity of testosterone 17b-dehydrogenase or the arachidonic acid metabolism. In conclusion, our results indicate that the pathogenesis of melasma is associated with the up-regulation and down-regulation of novel genes involved in the PPAR signaling pathway, neuronal component and the functions of stratum corneum barrier in Korean women.

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Genes and microRNAs regulating human skin pigmentation: Bioinformatics model and study on engineered pigmented epidermal epithelia.

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Human skin pigmentation involves the two main cell types of the epidermis: melanocytes and keratinocytes. Melanocytes synthesize melanin with the help of melanocortin receptors, enzymes (tyrosinase...), and transfer it into basal keratoycites, packed into melanosomes. Melanin is an excellent photo-protectant able to absorb harmful solar UV irradiation and determines the photopic appearance of skin. Once in keratoycites, melanosomes are organized as supranuclear caps, playing the highly essential role of protecting cell nuclei from UV radiation. As the keratoycites undergoes terminal differentiation, melanosomes are degraded by controlled autophagy so that no melanosomes are visible in the very upper part of the epidermis. With the goal of studying the regulation of human skin pigmentation, we established a bioinformatics model taking into account several processes: melanocyte signaling pathways involved in the transcriptional regulation of melanoenic enzymes; tyrosinase and related enzymes; melanosomal packaging and transfer; autophagic melanin scavenging in keratoycites; and inflammation. The major microRNAs described as potential controllers of human pigmentation were added to the model. To validate this model, we used 3D reconstructed epidermis containing both keratoycites and melanocytes, and we applied classical compounds (e.g. koic acid) known for their property to reduce skin melanin level. The expression level of specific genes and microRNAs was evaluated by RT-qPCR, following treatment.

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Genomic expression differences between Red hair colour phenotype and Black hair colour individuals

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The aim of the study was to identify differentially expressed genes in skin from Red hair colour (RHC) individuals independently of the UV response. Expression data from cocultured keratoycites and melanocytes from two RHC individuals and two Black hair (BHC) individuals was extracted using Whole Human Genome Micoarray 4x44K (Agilent). Expression data from skin biopsies from 14 RHC individuals and 7 BHC was extracted using Human HT-12 V3 BeadChips (Illumina). Differential gene expression data from co-cultures was evaluated by a protein–protein interaction network analyses to identify key deregulated genes in RHC individuals. The expression levels of those key genes were validated using the expression data from skin biopsies. Based on topological parameters of the networks, 24 genes involved in autophagy, oxidative phosphorylation and mithocondrial ribonucleic acid (mRNA) were selected as key genes. Differentiation of keratoycites (CLN5), ATG10, GBAS, RPA1, BRCAT1, and WIP2 genes were also observed in skin biopsies between RHC and BHC individuals. In conclusion, our results suggest that RHC individuals have a constitutive deregulation of redox homeostasis processes and oxidative stress which can be involved in the increased skin cancer risk observed in these individuals.

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107 cells/mouse). The treatment group were injected with iPS-ML, iPS-ML-IFN-α. Mice with established tumors were randomly divided into control and treatment groups. Mice in the treatment group showed a significant decrease in tumor size and number compared to the control group. In conclusion, our results suggest that iPS-ML-IFN-α may have potential as a therapeutic agent for melanoma treatment.

In inflammatory features of melasma lesions in Asian skin

Mast cell tryptase and chymase in cutaneous melanocytic lesions and lymph node metastases. The lesional dermis contained more CD68+ melanophages, CD117+ mast cells, and LCA+ leukocytes in the inflammatory group than in the non-inflammatory group. Inflammatory features in melasma lesions are associated with an increased number of mast cells and leukocytes, which may contribute to the pathogenesis of melasma.

Inhibition of PKC reduces melanoma invasion

Melanoma is the most deadly skin cancer with high incidence and mortality. Therefore, understanding the molecular mechanisms underlying melanoma invasion is crucial for developing effective therapeutic strategies. In this study, we investigated the role of protein kinase C (PKC) in melanoma invasion using MDA-MB-231 cell line. We found that inhibition of PKCα significantly reduced cell migration and invasion in a wound healing assay and a transwell invasion assay. Furthermore, knockdown of PKCα using shRNA further confirmed the inhibitory effect of PKCα on melanoma invasion. These findings suggest that targeting PKCα may be a promising strategy for the prevention of melanoma invasion.
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Melanoma–macrophage fusion can result in stealth tumor cells
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Macrophage infiltration in melanoma as well as CD68 expression in melanoma cells is associated with reduced survival. A potential mechanism is that melanoma cells fuse with macrophages and gradually lose melanoma phenotype and acquire macrophage features but still have tumorous features. Thus, they can be missed on routine histological examination but might contribute to tumor progression. To investigate whether such tumor–macrophage hybrids can exist and how they can be detected, we analyzed skin biopsies of 39 patients with melanoma. To study tumor–macrophage hybridization, we developed a novel method for detecting melanoma–macrophage hybrids. We showed that tumor–macrophage hybrids can be identified in melanomas by a novel in-silico hybridization approach. This method can be used to detect tumor–macrophage hybrids in patient samples. This study highlights the importance of tumor–macrophage hybrids as potential therapeutic targets.

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miR-330-3p targets Tyrosinase and regulates pigmentation
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miR-330-3p targets Tyrosinase and regulates pigmentation. We applied an in silico sequence-based prediction approach which aimed to identify miRNAs targeting most likely TYR but not MITF. From this approach, miR-330-3p turned out to be the most attractive candidate. miR-330-3p is able to target TYR and act on melanin in 38 patients with human melanoma tissue samples and normal human epidermal melanocytes (NHEM).

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NRAS x NRAS - Signaling of different NRAS mutations and potential clinical implications
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In conclusion, the presence of podoplanin expression in CAFs correlates with aggressive behavior in Merkel cell carcinoma and extramammary Paget’s disease. In addition, podoplanin expression in tumor cells was not correlated with characteristics of tumor progression such as tumor thickness and sentinel lymph node (SLN) metastasis. Podoplanin expression in CAFs (CFA+) was observed in 25 patients (45.5%), including the 11 patients with disease progression (44.0%) on CAFs + SLN metastasis. In contrast, only 4 of 30 patients with negative podoplanin expression in CAFs (13.3%) CFA+ exhibited SLN metastasis. CFA+ was associated with tumor thickness and SLN metastasis. Furthermore, patients with CFA+ had poorer disease-free survival than those with CFA+ (P = 0.0148). On the contrary, podoplanin expression was not observed in tumor cells in Merkel cell carcinoma and extramammary Paget’s disease. In conclusion, podoplanin expression in CAFs was rarely observed in these two carcinomas and not correlated with tumor progression. In conclusion, the presence of podoplanin expression in CAFs correlates with aggressive behavior in melanoma but not in other skin cancers, and may therefore serve as a useful prognostic factor for patients with melanoma.

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Podoplanin expression in cancer-associated fibroblasts predicts poor prognosis in melanoma but not in non-melanoma skin cancers
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Recent studies have demonstrated podoplanin expression in several tumors, which has been associated with lymph node metastasis and poor prognosis. Podoplanin expression in cancer-associated fibroblasts (CAFAs) also correlates with tumor progression in several cancers. However, podoplanin expression and its association with skin cancer remain unclear. To clarify the prognostic significance of podoplanin expression in CAFAs in tumors, podoplanin expression in tumor cells and CAFAs was examined by immunohistochemistry in tissue samples from 55 melanoma, 9 merkel cell carcinoma, and 15 extramammary Paget’s disease. In melanoma, podoplanin expression in tumor cells was identified in 38 patients (69.1%). Podoplanin expression in melanoma tumor cells and fibroblasts was significantly associated with lymph node metastasis and poor prognosis. Podoplanin expression in cancer-associated fibroblasts (CAFAs) also correlates with tumor progression in several cancers. However, podoplanin expression and its association with skin cancer remain unclear. To clarify the prognostic significance of podoplanin expression in CAFAs in tumors, podoplanin expression in tumor cells and CAFAs was examined by immunohistochemistry in tissue samples from 55 melanoma, 9 merkel cell carcinoma, and 15 extramammary Paget’s disease. In melanoma, podoplanin expression in tumor cells was identified in 38 patients (69.1%). Podoplanin expression in melanoma tumor cells and fibroblasts was significantly associated with lymph node metastasis and poor prognosis. Podoplanin expression in cancer-associated fibroblasts (CAFAs) also correlates with tumor progression in several cancers. However, podoplanin expression and its association with skin cancer remain unclear. To clarify the prognostic significance of podoplanin expression in CAFAs in tumors, podoplanin expression in tumor cells and CAFAs was examined by immunohistochemistry in tissue samples from 55 melanoma, 9 merkel cell carcinoma, and 15 extramammary Paget’s disease. In melanoma, podoplanin expression in tumor cells was identified in 38 patients (69.1%). Podoplanin expression in melanoma tumor cells and fibroblasts was significantly associated with lymph node metastasis and poor prognosis. Podoplanin expression in cancer-associated fibroblasts (CAFAs) also correlates with tumor progression in several cancers. However, podoplanin expression and its association with skin cancer remain unclear. To clarify the prognostic significance of podoplanin expression in CAFAs in tumors, podoplanin expression in tumor cells and CAFAs was examined by immunohistochemistry in tissue samples from 55 melanoma, 9 merkel cell carcinoma, and 15 extramammary Paget’s disease. In melanoma, podoplanin expression in tumor cells was identified in 38 patients (69.1%). Podoplanin expression in melanoma tumor cells and fibroblasts was significantly associated with lymph node metastasis and poor prognosis.
Senescence Induction in Metastatic Melanoma during Immunotherapy with Interferon-alpha

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Induction of senescence is recognized as a barrier protecting against cancer development. While it was shown that melanoma immunotherapy can be therapeutically efficient, it remains enigmatic whether the therapeutic success depends on mechanisms inducing cancer cell death, like cytolytic or apoptotic, or whether it also involves growth inhibitory processes, e.g. senescence induction in the tumor cells. We have recently shown that proinflammatory cytokines are capable of stopping cancer growth through induction of senescence in a variety of malignant cells, including primary melanoma cells. To further analyze the effects of interferon (IFN) and tumor necrosis factor (TNF) on melanomas, we treated a panel of cell lines with these two cytokines. FACS analysis showed that the combined action of IFN and TNF can cause apoptosis and a senescence characterizing CDD/G1 arrest. Furthermore, we could detect an upregulation of senescence-associated β-galactosidase and a stable cell cycle arrest in the cytokine-treated melanoma cells that remained stable after withdrawal of IFN and TNF. Moreover, the cytokine-treated melanoma cells showed a senescence-associated secretory phenotype with the production of IL-6, IL-8, IP-10 and CCL-2. In line with this, we could also detect the induction of markers for primary cells from a patient with stage IV melanoma and melanoma in vitro, and, more importantly, during IFN-alpha immunotherapy of the same patient in vivo. The patient had an EOCG performance status of 4 due to a malignant ascites. The tumor biopsy, suggesting that the skin microbiome may fail a specific diagnostic tool for vitiligo and melanocytic nevi. This treatment cleared the ascites completely, and the life quality was dramatically enhanced with an EOCG performance status of 1. In vivo analyses of the ascides-derived melanoma cells confirmed the induction of senescence during the treatment. Thus, interferon can drive human melanomas into senescence in vitro and in vivo, a discovery that is of great therapeutic relevance.

Skin microbiome in melanoma and melanocytic nevi

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High-throughput DNA sequencing has revealed that the skin microbiome varies specifically due to different exogenous and endogenous factors. Interactions between the microbiome and the host immune system seem to play an important role in conducting the changes. Our object was to characterize the microbiome of cutaneous melanoma and melanocytic nevi. Non-invasive microbiome swab specimens were taken of 19 cutaneous melanomas, 15 dysplastic melanocytic nevi and 21 benign melanocytic nevi. Control samples were taken on the contralateral body side from the same patient's healthy skin. Sequencing of the 16S ribosomal RNA gene, a common genetic marker for bacteria, was carried out on the 454 GS-FLX Titanium platform, and the abundances of sequencing reads were analyzed with QIIME. We analyzed the data using different methods to characterize the microbiome of cutaneous melanoma and melanocytic nevi. More data and larger cohorts of patients are needed to confirm these observations. It would be important to determine whether known melanoma risk factors like positive family history of melanoma, multiple melanocytic nevi and former dysplastic melanocytic nevi have an effect on the skin microbiome.

Solar lentigines on the shoulders and upper back in patients with cutaneous malignant melanoma

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Previous studies suggest that solar lentigines of the skin are associated with risk of cutaneous malignant melanoma (CMM). However, these studies have classified the number of solar lentigines into a maximum of 3 categories. The objective of the current observational case-control study was to investigate whether the number of solar lentigines on the shoulders and upper back, counted in an individual, is a risk factor for developing CMM. Forty-eight patients with CMM and 48 healthy controls completed the investigation. Controls matched CMM patients individually not only by age and gender, but also by constitutive skin type and occupation. Solar lentigines > 2 mm in diameter in an area of 95 cm² cut-off was set at the mean – 2SD of the healthy controls. Decreased levels of solar lentigines were found in 28 of the 71 MM patients (39.4%). The sensitivities of decreased serum levels of solar lentigines in stage in situ, III and IV patients were 25.0%, 75.0% and 100%, respectively. Altogether, there was a trend of reverse correlation between tumor thickness and number of lentigines. There was a significant difference in the values between healthy controls and the patients with invasive MM of all subtypes. When the patients were classified into MM subtypes, serum levels of solar lentigines in patients with nodular melanoma and mucosal MM were significantly lower than those with superficial spreading MM. MM were divided into two groups according to the tumor thickness (<2 mm and ≥2 mm), there was a significant difference in the serum levels of solar lentigines between the two groups (P = 0.0016). The production of IL-6, IL-8, IP-10 and CCL-2 was increased significantly in the cytokine-treated melanoma cells that remained stable after withdrawal of IFN and TNF. Moreover, the cytokine-treated melanoma cells showed a senescence-associated secretory phenotype with the production of IL-6, IL-8, IP-10 and CCL-2. In line with this, we could also detect the induction of markers for primary cells from a patient with stage IV melanoma and melanoma in vitro, and, more importantly, during IFN-alpha immunotherapy of the same patient in vivo. The patient had an EOCG performance status of 4 due to a malignant ascites. The tumor biopsy, suggesting that the skin microbiome may fail a specific diagnostic tool for vitiligo and melanocytic nevi. This treatment cleared the ascites completely, and the life quality was dramatically enhanced with an EOCG performance status of 1. In vivo analyses of the ascides-derived melanoma cells confirmed the induction of senescence during the treatment. Thus, interferon can drive human melanomas into senescence in vitro and in vivo, a discovery that is of great therapeutic relevance.

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Wedelolactone inhibits c-kit activation by SCF and reduces melanogenesis

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In previous reports, it was shown that c-kit and SCF, a ligand of c-kit, are overexpressed in the lesion of melasma and that treatment of imatinib, inhibitor of c-kit, to cancer patients gives rise to hypopigmentation in the skin and hair. It means that c-kit inhibitor can be used as a new element to treat melasma and hyperpigmentation. To discover novel c-kit inhibitors, western blot analysis with phosphorylated c-kit antibody was performed using Mo7e cell treated with major compounds present in various natural herbs. Here we report that Wedelolactone, an active ingredient of Eclipta alba and Wedelia calendulacea, exhibits activity that inhibits phosphorylation of c-kit caused by SCF. As a result, Wedelolactone reduced melanogenesis in melanocyte, which was mediated by suppression of melanogenic enzyme expressions. Wedelolactone-induced phosphorylation of ERK and p38, leading to degradation of MITF, may be involved in inhibition of melanogenesis. Taken together, it suggests that Wedelolactone can be used as a new ingredient for cosmetics and drugs to treat the melasma through suppression of the c-kit activation by SCF and of melanogenesis.