Title
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The 3,3,4,4,5,5-hexahydroxystilbene impairs melanoma progression in a metastatic mouse model

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Stilbenes, a group of polyphenolic compounds, have been shown to exert beneficial effects on various malignancies. In an attempt to increase the anti-tumor effects, we generated the stilbene analogue 3,3,4,4,5,5-hexahydroxystilbene termed M8. *In vitro*, M8 dramatically inhibits the proliferation of human melanoma cell including the metastatic M24met melanoma cell line. Cell cycle analyses reveal that M8 promotes G2/M arrest in the M24met melanoma cell line. To further evaluate the underlying mechanisms, we performed proteomic analyses of human melanoma cell lines *in vitro*. We demonstrated that M8 interferes with pathways which are critically involved in the regulation of proliferation, apoptosis and tumor cell migration. In our recently established, spontaneously metastatic human melanoma SCID mouse model, we demonstrate that M8 significantly impairs tumor growth, inhibits cell proliferation and induces apoptosis in a dose dependent manner. Furthermore, mice treated with M8 showed less metastasis to distant lymphnodes compared to littermates receiving solvent. Podoplanin labeling reveals reduced peritumoral lymphatic vessel density in M8 treated mice. In conclusion, M8 exerts pronounced antiproliferative effects on melanoma cells through the induction of apoptosis and cell cycle arrest at the G2/M boundary. Furhermore, the M8 mediated inhibition of melanoma metastasis to distant lymph nodes appears to be achieved through diminution of tumor-associated lymphatic vessels. Our data indicate that M8 is a novel and potent approach for the treatment of melanoma.

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Cobalt and copper are candidates as co-carcinogens in the pathogenesis of cutaneous melanoma

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The complex epidemiology and recent genetic studies of melanoma as well as the limited evidence for ultraviolet light damage in melanoma cells raises the likelihood that the etiologic and pathobiologic factors in melanoma are multi-factorial.Our studies of the role of reactive oxygen species in the pathogenesis of melanoma over the past decade has led us to reconsider the role of ultraviolet light and has led us to explore the role of heavy metals in this process. A systematic experimental exploration of the role of melanin and melanosomes in melanocytes and melanoma cells using electroparamagnetic resonance, electron microscopy and other techniques suggests that the conversion of melanin from an anti-oxidant and reducing agent to a pro-oxidant is an early pathogenic event which is accompanied by melanosomal disruption that serves as an organelle source of free radical generation. The involvement of selected heavy metals is critical to this process and in conjunction with ultraviolet light can lead to phenotypic changes in normal human melanocytes that resemble dysplatic nevi melanocytes in culture. A re-examination of the non-genetic risk factors for melanoma has identified a large(and neglected) epidemiology that implicates heavy metals and includes markedly increased risks for melanoma in printers/lithographers(4 large studies), electrical industry workers(2 large studies), and hip replacements patients(3 large meta-analyses. Clinical observations(imaging studies) and studies that document the importance of metallothionein expression in primary melanoma and prognosis also offers ancillary support for the involvement of metals in the pathogenesis of melanoma. Based on these considerations and studies of heavy metals in a modified JB6 carcinogenesis indicator system, the role of copper in the melanin synthesis pathway and the binding characteristics of metals to melanin we propose that the most likely culprits that accounts for a significant contribution to the pathogenesis of melanoma are Cu+2 and Co+2.

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C-MYC controls senescence in melanoma cells

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Activating mutations of the oncoproteins BRAF (V600E) or NRAS (Q61R) are commonly found in malignant melanomas. Intriguingly, the same genetic changes and often at higher rates are detected in benign nevi composed mostly of senescent melanocytes. We have previously shown that in normal melanocytes ectopic expression of BRAFV600E and NRASQ61R induces different types of stress responses ultimately leading to the activation of cellular senescence. How oncogene-mediated senescence is overcome during melanoma genesis, and whether aggressive melanoma cells retain the ability to undergo senescence is unclear. Here we report that shRNA-mediated inhibition of C-MYC in several BRAFV600E- or NRASQ61R-expressing tumor-derived melanoma cell lines resulted in permanent growth inhibition accompanied by activation of senescence-associated β-galactosidase and formation of senescence-associated heterochromatin foci. Cell morphology of MYC-depleted BRAFV600E- or NRASQ61R-expressing melanoma cells closely resembled that of normal melanocytes undergoing senescence induced by BRAFV600E or NRASQ61R, respectively. Additionally, senescing melanocytes overexpressing either of the above oncoproteins demonstrate gradual reduction in C-MYC mRNA and protein levels. Reciprocally, overexpression of C-MYC in normal melanocytes approximately to the levels observed in melanoma cell lines, delays senescence induced by BRAFV600E and, to lesser extent, by N-RASQ61R, confirming and active role of C-MYC in opposing oncogene-induced senescence. Interestingly, senescence-like phenotype induced by depletion of C-MYC was independent of p16INK4A (usually associated with cellular senescence, but not frequently inactivated in spontaneous melanoma). Instead, MYCshRNA-induced senescence was found to relay, at least in part, on p27KIP1, a cell cycle inhibitor and a bona fide MYC-repressed target. Our data suggest that molecular determinants resulting in BRAFV600E- or NRASQ61R-specific senescence programs are preserved in tumor-derived melanoma cells and are repressed by elevated amounts of C-MYC.

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BMP-4 regulation of MITF expression in cultured human melanocytes

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Bone Morphogenetic Protein-4 (BMP-4), released from keratinocytes and melanocytes, was recently shown to modulate melanogenesis and to decrease protein levels of tyrosinase, PKC-β, TRP-1 and MART-1. To further elucidate the mechanisms by which BMP-4 down-regulates melanogenesis, we examined the effects of BMP-4 on the expression of microphthalmia-associated transcription factor (MITF), the key transcription factor for these melanogenic proteins. When paired cultures of newborn melanocytes were treated with BMP-4 (25 ng/ml) or vehicle alone for 24 hrs, BMP-4 reduced the level of MITF protein ($p<0.03$) by 60-70%. The mRNA level of MITF-M, the isoform known to regulate the transcription of melanogenic proteins, measured in parallel by semi-quantitative RT-PCR, decreased 30~40% in BMP-4 treated cells. Moreover, within 24 hrs, BMP-4 decreased the intracellular level (26 ± 4 vs 14 ± 5 pmol/ug vehicle vs BMP-4) of cAMP, the key second messenger that increases transcription of MITF gene. Because MITF activity is transiently increased by MEKinase-dependent phosphorylation, followed by subsequent proteosome mediated degradation, and BMP-4 acts by initially binding with its cell surface tyrosinase kinase receptor known to activate MEKinase, we asked whether BMP-4 also acutely activates (phsophorylates) MITF. In paired cultures of melanocytes treated with BMP-4 (25 ng/ml) for 15 min, 30 min, 1 hr and 4 hrs, there was a 2-4 fold ($p<0.03$) increase in MITF phosphorylation at 15 and 30 min, but MITF protein level was decreased by 4 hr. Moreover, pretreatment of melanocytes with MEKinase inhibitor PD98056 blocked BMP-4 induced MITF phosphorylation. These results demonstrate that BMP-4 initially activates MITF, but prolonged exposure to BMP-4 decreases both MITF mRNA and protein levels by decreasing intracellular cAMP level. Thus, acute exposure of melanocytes to BMP-4 can transiently promote melanogenesis, while longer exposure down-regulates melanogenesis.

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Identification of novel small molecule inducers of melanin production

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Melanin is a complex biopolymer synthesized in organelles called melanosomes that are a unique feature of melanocytes. Regulation of melanin production occurs at multiple points that primarily involve, but are not exclusive to, the regulation of tyrosinase, the key enzyme in melanin synthesis. Because over 100 genes are implicated in control of pigmentation, other regulatory targets are likely to exist. Agents that increase melanin synthesis are of interest both as tools for the study of melanin production, and because of their potential use as "natural sunscreens" that stimulate the skin's resident melanocytes to produce and transfer more melanin to epidermal keratinocytes, thus decreasing the incidence of skin cancer. Recent efforts by Orlow and coworkers have demonstrated that combinatorial library screening is a viable method for the identification of novel regulators of melanogenesis. To that end, we have synthesized and screened a combinatorial library of 75 organic compounds. Three members of this library with structures unrelated to the triazine derivatives reported by Orlow et al. were found to provide a 2-fold increase in pigmentation of mouse melan-A cells with potency comparable to forskolin. Additionally, cell dendricity was found to increase significantly in the presence of melanin-inducing small molecules. We have subsequently employed a proteomic approach (2-D DIGE) to identify changes in protein expression as a function of small molecule treatment. Expression levels of approximately 80 proteins were altered by the presence of one library member.

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Dermoscopic patterns and melanoma risk: a pilot case-control study in patients with multiple dysplastic nevi

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Dysplastic nevi (DN) are markers of melanoma (MM) risk and potential precursors. The purpose of this case-control pilot study was to identify differences between the dermoscopic patterns of DN in patients with and without a history of MM. Patients were randomly selected from the Memorial Sloan-Kettering image database of patients undergoing full-body photography for pigmented lesion surveillance in 2005. Inclusion criteria were age ≥18 at photography, pathological confirmation for history of invasive MM, and at least 5 back nevi ≥5 mm in diameter with available dermoscopic images. Patients with facial and acral MM were excluded. Dermoscopic images of all back nevi ≥5 mm in diameter were evaluated. The primary outcome was Total Dermoscopy Score (TDS) by the ABCD rule of dermoscopy. Secondary outcome measures were dermoscopic global pattern and specific structures. To date, the study has included 119 nevi from 7 males and 3 females with a history of MM (cases) and 74 nevi from the same number of sex and age matched patients without a history of MM (controls). The average TDS was 4.17 for nevi in cases and 3.75 for nevi in controls. There was a significant difference in frequency of global dermoscopic pattern for reticular-globular pattern (23% for cases vs. 8% for controls) and multi-component pattern (10% vs. 4%, respectively), whereas reticular pattern was more frequent in controls (53%) than cases (21%). Among dermoscopic structures, globules, homogeneous areas, and vascular structures were more frequent in cases (65%, 82%, 65%, respectively) than in controls (31%, 68%, 42%, respectively); dots were more frequent in controls than in cases (51% vs. 22%, respectively). Statistical analysis will be reported following review of additional cases. DN in MM patients appear to have different dermoscopic attributes than in non-MM patients. If further validated, these results may be informative for better defining DN that are markers of increased MM risk.