612 Small molecule inhibitors of protein kinase CK2 reduce proliferation and viability of melanoma cells in vitro

C Alder 1, J Trombley 2, 4, 5, 6, and R Ahmed 1 1 University of MN, Mpls, MN and 5 Masonic Cancer Center, Mpls, MN, 2 Laboratory Medicine and Pathology, MN, 3 Dermatology, University of MN, Mpls, MN, 3 Molecular and Cellular Biochemistry Lab, Minneapolis VA Health Care System, Mpls, MN, 4 Laboratory Medicine and Pathology, University of MN, Mpls, MN, and 5 Masonic Cancer Center, Cancer Center, Mpls, MN, Metastasis is an aggressive form of skin cancer, which is notoriously resistant to current therapies. Long-term survival remains extremely poor, necessitating the development of novel therapeutic approaches. Protein kinase CK2 is a constitutively active serine/threonine kinase that acts as a potential target for cancer therapy. Melanoma cell migration and invasiveness is most frequently found in primary tumors arising in acral and mucosal skin. Reports indicate that acral melanoma cells can be detected in the distant periphery of the primary tumor (field effect). In this study we investigated the contribution of multiple melanocytic signaling mediators. Melanoma and human KIT mutant lines as well as genetically manipulated murine and primary human melanocytes were used to investigate the role of KIT in melanoma migration. Immunoblotting, migration assays, kinome and phospho-proteomic analyses were used to study signaling events. Mouse xenografts of human skin-reconstructs were used to analyze migratory properties of KIT mutant cells in vivo. KIT mutant cell lines depend on KIT signaling for survival and migration in vitro using wound healing as well as human skin-reconstruct assay. Stably transduced murine and human primary melanocytes have migratory characteristics of KIT cells compared to H229 melanoma. KIT mutant cells display molecular changes related to increased migration, such as pronounced P38KINOR, MEK/ERK and p18MAPK signaling. In a xenograft model of human skin-reconstructs, KIT mutant cells demonstrated increased invasion of adjacent skin. In conclusion, KIT mutations increase the migration of melanoma cells. Data points into the direction, that mutant KIT might contribute to the clinically observed “field effect” in acral melanoma. KIT mutant cell lines had decreased levels of pro-proliferative regulators, including NFkB p65, p-Ser529, Akt p-Ser473, and p75NGFR. Expression of cleaved-caspase-3 and lamin A/C proteins indicated activation of apoptosis. These findings suggest that CK2 may be a useful novel target for melanoma treatment.

614 Neurotrophin receptors and perineural invasion in desmoplastic melanoma

N Freiðþrunn 1, D Leone 2, B Mitchell 3, S Yang 2, and M Mahalingam 1 1 Dermatology, Boston University School of Medicine, Boston, MA, 2 Graduate Medical Sciences, Boston University School of Medicine, Boston, MA, 3 School of Public Health, Boston University School of Medicine, Boston, MA, and 4 Pathology, Boston University School of Medicine, Boston, MA, In melanoma, perineural Invasion (PNI) is most commonly observed in all cancers studied to date. Loss of CK2 activity induces apoptosis of cancer cells. Our aim was to test the effect of small molecule inhibitors of cellular viability of melanoma cells. Two melanoma cell lines were obtained, one of primary tumor origin (A375) and one of metastatic tumor origin (1205Lu). Both lines were treated every 24 hours with 4,5,6,7-tetrabromo-1H-benzotriazole (TBB), a well-established CK2 inhibitor. Cell viability assays were performed on each cell line 24, 48 and 72 hours following initial treatment. Whole cell lysates were processed 24 and 48 hours after initial treatment, and western blot analysis revealed that cells treated with TBB had significantly decreased viability post-treatment. 1205Lu cells had a >50% reduction in viability as early as 24 hours post-treatment with a higher TBB concentration (100 μM) and demonstrated similar viability at 72 hours with a lower concentration (20 μM). A375 cells reached the higher TBB concentration of 100 μM to achieve a >50% decrease in viability. Western blot analysis detected altered expression of key signaling pathway proteins in response to CK2 inhibition. Two cell lines had decreased levels of pro-proliferative regulators, including NFkB p65, p-Ser529, Akt p-Ser473, and p75NGFR. Expression of cleaved-caspase-3 and lamin A/C proteins indicated activation of apoptosis. These findings suggest that CK2 may be a useful novel target for melanoma treatment.

616 An epithelial-mesenchymal transition in melanoma is associated with acquired resistance to BRAF inhibition

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Understanding the mechanism of drug resistance is crucial to the development of effective cancer therapies. In melanoma, the oncoprotein BRAF(V600E) mutation confers extreme sensitivity to BRAF inhibition with vemurafenib (VEM). However, melanoma can acquire resistance through multiple mechanisms, including BRAF amplification, BRAF splice variation, and upregulation and activation of receptor tyrosine kinases. In this study, we characterized the changes in transcriptional programming that occur in response to VEM in sensitive melanoma cell lines and the changes that occur in the absence of VEM resistance. Using a non-biased microarray approach, we found that VEM significantly changed the expression of only a small percent of genes in melanoma cell lines, consistent with its action as a selective BRAF inhibitor. We discovered that in vitro selection for VEM resistance generated very different degrees of cellular reprogramming. In one cell line, resistance emerged from a BRAF splice product, which was associated with minimal changes in cell state. In another cell line, resistance was associated with an epithelial to mesenchymal transition, leading to melanoma dedifferentiation and decreased reliance on melanocyte-specific survival pathways. Our data characterize a novel drug resistance mechanism in melanoma that offers an additional pathway for the development of therapeutic approaches.

617 The role of KIT in early melanoma development

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Melanoma is a common photoinduced hyperpigmentary disorder whose pathogenesis is not clearly elucidated. Dermal inflammation caused by solar exposure might be a critical factor through the production of melanocytic cytokines, and growth factors. The inflammatory features and the role that inflammation plays in this disorder are unknown. Our aim was to describe the ongoing characteristics of inflammation in melanoma by measuring the cellular infiltrate, the expression of inflammatory mediators, and to explore their relationship with severity of disease, solar elastosis, and epidermal melanoma. Twenty healthy female patients with malar melanoma without specific solar exposure or photo-protection measures within the previous 3 weeks were enrolled. Disease severity was assessed using the MASI index. Histological, histochromatographic, immunohistochemistry, and QT-PCR were used to evaluate the presence of inflammatory cells and mediators in lesions and compare them to adjacent skin. Increased lymphocytic infiltrate mainly composed by CD4+ T cells, CD68+ macrophages, and mast cells were significantly higher in lesional skin. Also, the relative expression of IL-17, IL-18, IL-22, and IL-24 was significantly higher in lesional skin compared to adjacent skin (p<0.05, p=0.01, respectively). MASI score was associated with the number of CD4+ T cells and with COX-2 stain expression. Solar elastosis and epidermal melanoma were only associated with COX-2 expression. Remarkably, gene expression of acute inflammatory mediators such as IL-1, IL-18, RIG-I, IL-6, IL-8, VEGF, and TNFα were not increased. Together, these data indicate that melanoma is characterized by chronic inflammatory cells and mediators, particularly IL-17 and COX-2 which could explain its recurrent nature.

618 MT19c reseonlizes metastatic melanoma cells to vemurafenib, decreases tumor growth, and increases survival in a vemurafenib-resistant metastatic melanoma model

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Metastatic melanoma is a challenging disease to manage due to limited effective treatment options for patients who lack the BRAF mutation (~50%) or who are inherently resistant to BRAF inhibitors. Even in BRAF mutant patients, vemurafenib as a first-line therapy often fails due to rapid emergence of drug resistance and dose-limiting systemic toxicities. MT19c, a novel non-hypercalcemic Vitamin D derivative, showed anti-proliferative effects at nanomolar concentrations against a panel of chemotherapy-resistant melanoma cell lines and significantly increased survival (p=0.03). In conclusion, MT19c is a promising safe and novel therapeutic approach in melanoma treatment.
Dual inhibition of CRD-BP and BRAF in BRAF-mutant Melanoma – A novel approach to overcome resistance to BRAF inhibitors

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Depletion of melanin fn14 by oligonucleotide-mediated exon skipping

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Y chromosomal encoded TPSY proto-oncogene drives increased melanoma cell aggressiveness

Z. Huang, Y. Li, 1, 2, K. T. Hwang, T. C. Lee, J. W. Wong, 1, 2, 3, D. A. Wake, 1, 2, 3, W. K. Kong, 1, 2, 3, G. Scott, 1, 2, C. Lau, 1, 2, 3, and M. V. Lee 1, 2, 3

Broadband light absorption characteristics of melanin: human skin measurement beyond Chromameter and Mexameter

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A growing body of evidences has suggested that oxidative stress is involved in the etiology and pathogenesis of vitiligo. In this study, we investigated the effect of aspirin, which is demonstrated to have antioxidant properties in vitro, to induce hyperpigmentation in patients with vitiligo. Patients who had vitiligo for at least 10 years were treated with aspirin (100 mg, 3 times daily) for 3 months. The results suggested that aspirin had a protective effect on melanocytes against H2O2-induced oxidative stress and H2O2-driven transcriptional activation of heme oxygenase-1 may be critically important in determining the cytotoxic effect of aspirin against H2O2. Therefore, we speculate that aspirin might be a promising candidate for prevention or treatment of vitiligo.

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Aspirin induces Nr2f2-mediated transcriptional activation of heme oxygenase-1 in protection of human melanocytes from H2O2-induced oxidative stress

Z. Huang, Y. Li, 1, 2, K. T. Hwang, T. C. Lee, J. W. Wong, 1, 2, 3, D. A. Wake, 1, 2, 3, W. K. Kong, 1, 2, 3, G. Scott, 1, 2, C. Lau, 1, 2, 3, and M. V. Lee 1, 2, 3

Broadband light absorption characteristics of melanin: human skin measurement beyond Chromameter and Mexameter

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Melanin is a broad class of macromolecule that absorbs light in a broad range of visible and ultraviolet radiation. The absorption spectrum of melanin does not have any distinctive absorption bands probably due to the overlap of absorption spectra from constituting multiple oligomers. The amount of human epidermal melanin is commonly assessed by L* of chromameter or by narrow-band diffuse reflectance (Mexameter). However, L* is also affected by other chromophores (e.g., lipids), and the L* values can be similar for Caucasians, Eastern Asians, and Hispanics of different skin colors. In addition, reflectance in the narrow-band spectrum (red band) is closely correlated to L* for different ethnicities. To identify new melanin that help account for the broadband absorption, we measured absorption spectra of granular form of melanin in water and superimposed the spectra of melanin solution after membrane filtration using spectrophotometer. Absorption spectrum for melanin granules was linear in wavelength while for soluble melanin the absorption was exponential in the blue part with minimal change in the red part spectrum. Investigation of dissolution kinetics of melanin in melanocytes and in melanoma cells showed that the particles of melanin could experience an oxidation change in the blue part only. To validate the models and method developed in vitro, we analyzed diffuse reflectance spectra acquired in vivo from 300 subjects of different ethnicities on their upper inner arm and cheek. The amount of soluble melanin was determined by the absorption at the blue part spectrum and granular melanin at the red part spectrum. We found that the amount of soluble melanin is independent of the amount of granular melanin commonly assessed by L* or Mexameter, and the soluble melanin is well correlated to L* but not to L*. We conclude that the soluble melanin of human skin can be assessed by accounting for the contributions from both granular form and soluble form of melanin.

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CDK1 enhances tumor initiation and stemness by interacting with stem cell genes in human cancers

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ABSTRACTS | Pigmentation & Melanoma

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Genes normally expressed in healthy tissue can produce tumors in cancer. We analyzed gene expression in melanoma cells that were used in various experimental digestion and isolation conditions. We found MHC I (HLA)-positive selection significantly enriched cells with enhanced tumor initiating capacities. Nanostring® array analysis of HLA-positive and -negative tumor cells identified a series of differentially expressed stem cell genes. Among them, cyclin-dependent kinase 1 (CDK1) was identified and validated as a significantly upregulated stem cell gene in melanoma. HLA upregulation was driven by CDK1 through NF-κB activation, and CDK1 promoted tumor growth in mice. Furthermore, CDK1 enhanced clonogenicity in vitro and tumor initiating capacities in vivo, which was not explained by CDK1’s role as a transcriptional regulator of Fn14 mRNA. Fn14 upregulation after treatment of Hela cells with Snail, an inducer of epithelial to mesenchymal transition, to be positively regulated by CDK1. The proteome array analysis also identified the direct interaction of CDK1 and Sox, a pluripotent stem cell transcription factor, which was confirmed by Western blotting through immunoprecipitation. These findings highlight the importance of stemness by interacting with stem cell genes in tumor cells. CDK1 thus serves as a molecular target for therapeutic intervention of cancer.

Phenformin as a therapeutic adjunct for melanoma

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Approximately 2% of the US population will be diagnosed with melanoma during their lifetimes. While most cases are successfully treated with surgery, once melanomas metastasize (4% of cases), the five-year survival rate is only 16%. A major molecular pathway involved in melanoma pathogenesis is the mitogenic MAPK kinase (Ras/Raf/MEK/ERK) signaling cascade. It is estimated that over 50% of melanomas contain a BRAF mutation (V600E) that leads to constitutive MAPK signaling. While the current first-line therapy for BRAF V600E mutant melanomas is BRAF inhibitors such as vemurafenib and dabrafenib, many tumors develop resistance to these drugs. Aside from melanomas harboring BRAF mutations, the remainder of melanomas are BRAF wildtype and therefore are not candidates for treatment with BRAF V600E inhibitor therapy. Biguanides are a class of drugs originally developed for the treatment of diabetes that have shown promise as a potential therapy for melanoma. Prior research has demonstrated that biguanides (such as phenformin) prevent melanoma growth via activation of AMPK (AMP-activated protein kinase) and lead to a synergistic inhibition of cell viability in BRAF V600E mutant cell lines when combined with a BRAF inhibitor. We examined the effects of phenformin on melanoma growth both alone and in combination with vemurafenib and have replicated prior findings that phenformin enhances the effects of vemurafenib in BRAF V600E mutant melanoma cells. We then examined the effects of phenformin on cell proliferation, migration, and invasion in melanoma cells. Furthermore, we now show that phenformin alone or in combination with other MAPK inhibitors is effective at inhibiting the growth of BRAF V600E mutant melanoma cells that are resistant to vemurafenib, as well as melanoma cells that do not harbor a BRAF V600E mutation. Overall, these data suggest that phenformin can enhance the therapeutic effects of multiple MAPK inhibitors and could serve as a therapeutic adjunct for melanomas with a wide range of driver mutations.

mTOR and PKM2 are constitutively up-regulated in human melanoma cells but not in melanocytes

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Melanoma is considered one of the most aggressive and least treatable cancers. While various models and mechanisms have been proposed, the transformation from tranquil melanocytes to invasive melanoma cells remains an enigma. Pyruvate kinase M2 has been shown to be an important constituent of the Warburg effect (a hallmark of cancer) and an increase of this kinase correlates with increased glycolysis and an up-regulated mTOR pathway. Despite constituent activation of the mTOR/akt pathway in melanoma cells, it is still not known whether pkm2 is up-regulated upon melanocyte transformation to melanoma. In order to study pkm2, we compared the cellular response to melanocyte stimulating hormone (or α-MSH) in both primary human melanocytes and melanoma (WM 6.64-4 cell line). Both types of cells were treated with α-MSH. Immunoblotting and confocal microscopy were used to visualize the activity of mTOR and pAKT at different time points after treatment. Our results showed that α-MSH activates mTOR pathway in both human melanocytes and melanoma cells, while melanoma cells exhibit constitutive activation of mTOR. We found that pkm2 is also constitutively up-regulated in melanoma cells. Interestingly, our experiments did not show a change in the level of phosphorylation of pkm2 in both types of cells in response to α-MSH treatment.

MicroRNA approach to target collagen glycation, cellular senescence and melanin production in vitro

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Skin aging is a complex and continuous process caused by intrinsic factors and extrinsic factors, characterized by wrinkled and saggy skin. New research has demonstrated that biguanides (such as phenformin) prevent melanoma growth via activation of AMPK (AMP-activated protein kinase) and lead to a synergistic inhibition of cell viability in BRAF V600E mutant melanoma cells. In our previous study, we showed that phenformin enhances the effects of vemurafenib in BRAF V600E mutant melanoma cells. Expression of activated Akt1 resulted in highly metastatic melanomas with lung and brain metastases in 67% and 17% of our mice, respectively. In contrast to activated Akt1 expression, the loss of Pten in the context of BRAFV600E, rejuvenates the pool of proliferative cells allowing the synthesis of new collagen. We also observed the increase of miRNA-218 expression induces a decrease in melanin content via the inhibition of MAPK expression. MicroRNA levels were evaluated by qPCR. First, on in vitro treated fibroblasts we observed a decrease in miRNA-29a expression and an increase of miRNA-218 expression. Next, we examined the expression of miRNA-218 and its target, a key regulator of melanin production and pigmentation, in vivo. Finally, we tested the effect of these microRNAs on melanin production in vitro. We observed that microRNA-218 expression in melanocytes cultures: the increase in miRNA-218 expression induced a decrease in melanin content via the inhibition of α-MSH/ERK expression. MicroRNA levels were evaluated by qPCR. First, on in vitro treated fibroblasts we observed a decrease in miRNA-29a expression and an increase of miRNA-218 expression. Next, we examined the expression of miRNA-218 and its target, a key regulator of melanin production and pigmentation, in vivo. Finally, we tested the effect of these microRNAs on melanin production in vitro.
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Circadian rhythm of human tyrosinase
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To determine if a tyrosinase transcription circadian rhythm exists, we entrapped cells for 24 h in media containing 10% FBS. Cells were then discharged and new media 10% FBS was added. In virtually all cases, a 24h circadian rhythm in tyrosinase was evident. To compare the circadian rhythm of cellular pigmentation observed during aging or through the lack of sleep.

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Characteristic comparison of human melanoma cell lines derived from primary and metastatic sites
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Human primary and metastatic melanoma may have distinct biological and clinical characteristics.

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Effects of MAPK inhibition on glucose uptake in melanoma are largely secondary to alterations in cellular morphology
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In 2011, the FDA approved the mutant BRAF inhibitor vemurafenib for treatment of metastatic melanoma transcribing V600 activating BRAF mutants. Within weeks of therapy, the 18FDG-PET tumor signal, which correlates to the rate of glucose uptake relative to normal tissue, is dramatically reduced in the majority of BRAF-mutant tumor patients. Unfortunately, almost 100% of melanoma acquire resistance to BRAF inhibitors within 18 months, with evolution of resistance against second line MEK inhibitors subsequently. Because MAPK is known to control multiple aspects of tumor metabolism, the direct effect of MAPK inhibitors on glucose utilization is an important field of study. Using flow cytometry and the fluorescent deoxyglucose analog 2-NBDG, we have observed a rapid decrease in glucose uptake in vemurafenib-sensitive human melanoma cell lines with very low expression of the clock gene, PER2. A short exposure to dexamethasone was sufficient to entrain the cells for more than 24h. With these two methods, we observed that transcription of human tyrosinase follows a circadian rhythm with a peak at night, between 8-10 PM. A 35% increase in tyrosinase transcription was determined when melanocytes were unsynchronized and compared to synchronized cells in a 6 h time interval. This is in agreement with the increase in pigmentation observed during aging or through the lack of sleep.

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Correlation of insulin-like growth factor II mRNA-binding protein 3 (IMP-3) and high mobility group AT-hook 2 (HMGA2) expressions in human melanoma
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Cyclophane pyrimidine dimers (CPD) are created nearly instantaneously as the energy of a UV photon is absorbed by DNA. Melanoma is etiologically associated with CPD generated by sunlight exposure. Melanin acts as shield for sunlight's ultraviolet, however UVB irradiated mice develop melanoma only if their melanocytes contain melanin. To investigate this paradox, we studied repair of UV-induced CPD in human and mouse melanocytes. Surprisingly, CPD were created in melanin-containing melanocytes for hours after an initial UV exposure. We found that melanocytes in vivo that received the melanin from melanocytes. These “delayed” or “dark” CPD also included cytosine-containing CPD which initiate UV-signature C-T mutations. In vivo, the skin from mice with black fur. Knocking down Xpc and Xpa transcripts revealed the dark CPD which were masked by excision repair in primary human melanocytes from some donors. We identified a novel pathway whereby reactive oxygen and nitrogen species oxidize UV-fragmented melanin and generate a melanin-derived radical which could be identified in a triplet ground state using a specialized wound-scratch module that makes uniform scratches in the cell monolayer. We found that cell attachment, proliferation and migration properties of the two cell lines were more responsive to the decrease of FBS concentration compared to the WM-115 cell line. Therefore, the WM-266-4 cell line showed a statistically significant decrease in the rate of attachment and migration as the FBS concentration was lowered from 10% to 1% (p<0.05), while the WM-115 cell line showed no statistically significant difference (p>0.05). For the proliferation assay, the lowering of FBS concentration from 10% to 1% or 0.1% greatly decreased the proliferation rate at 48 h in the WM-266-4 cell line compared to the WM-115 cell line (p<0.05). Our results suggest that critical biological differences between the two unique melanoma cell lines exist, and add information to the understanding of human solid tumors in primary and metastatic sites.

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Multigene epigenetic signature is a prognostic marker in melanoma
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The purpose of this study is to develop more accurate survival prognostic approaches for melanoma patients, who currently exhibit great variability in survival even within AJCC sub-groups. Aberrant promoter DNA methylation is a key epigenetic feature of cancer and identification of a DNA methylation signature (CGG island methylator phenotype), has been useful in predicting prognosis, diagnosis and response to treatment in a variety of tumor types. In this study, we define a methylation-based melanoma survival prognostic score, MethyLive. We used a discovery set of 14 melanoma tumor samples derived from patients and sub-divided into two sets on the basis of overall survival. We examined promoter methylation status of 70 genes using bisulfite modification, PCR and Sanger sequencing. Differential promoter methylation sensitivities for genes with the highest prediction score, MethyLive, by multiple regression modeling using a training cohort of melanoma patients from The Cancer Genome Atlas (TCGA). In the validation cohort, a high MethyLive score was associated with a significantly longer recurrence-free survival (p=0.004, HR: 1.3) and longer overall survival (p=0.0001) in Stage III, Stage IIIC (p=0.001), and melanoma patients of all stages (p=0.0001) from The Cancer Genome Atlas. The significance of MethyLive is improved stratification of patients with favorable and poor outcomes within AJCC sub-groups. E.g. MethyLive identified Stage IIIC patients with high IMP-3 levels had weak and negative HMGA2 expression. A positive correlation between IMP-3 and HMGA2 expressions in melanoma was found (P<0.001). qRT-PCR of melanoma tissues from 3 patients also showed a strong positive correlation between IMP-3 and HMGA2 mRNA levels. Thus, the HMGA2 correlation measurement gives the result 0.9%. In addition, only 14% of melanoma patients had BRAF mutations and the remaining 86% had BRAF mutations. Only 14% of melanoma patients had BRAF mutations and the remaining 86% had BRAF mutations. This result suggests that alterations in cellular volume significantly contribute to decreases in 18FDG-PET signal immediately following treatment, and may in part explain the relatively rapid reappearance of metastases at original sites when resistance occurs.

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Delayed cyclobutane pyrimidine dimers induced by chemically fixed melanin derivatives long after UV exposure
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Cyclophane pyrimidine dimers (CPD) are created nearly instantaneously as the energy of a UV photon is absorbed by DNA. Melanoma is etiologically associated with CPD generated by sunlight exposure. Melanin acts as shield for sunlight's ultraviolet, however UVB irradiated mice develop melanoma only if their melanocytes contain melanin. To investigate this paradox, we studied repair of UV-induced CPD in human and mouse melanocytes. Surprisingly, CPD were created in melanin-containing melanocytes for hours after an initial UV exposure. We found that melanocytes in vivo that received the melanin from melanocytes. These “delayed” or “dark” CPD also included cytosine-containing CPD which initiate UV-signature C-T mutations. In vivo, the skin from mice with black fur. Knocking down Xpc and Xpa transcripts revealed the dark CPD which were masked by excision repair in primary human melanocytes from some donors. We identified a novel pathway whereby reactive oxygen and nitrogen species oxidize UV-fragmented melanin and generate a melanin-derived radical which could be identified in a triplet ground state using a specialized wound-scratch module that makes uniform scratches in the cell monolayer. We found that cell attachment, proliferation and migration properties of the two cell lines were more responsive to the decrease of FBS concentration compared to the WM-115 cell line. Therefore, the WM-266-4 cell line showed a statistically significant decrease in the rate of attachment and migration as the FBS concentration was lowered from 10% to 1% (p<0.05), while the WM-115 cell line showed no statistically significant difference (p>0.05). For the proliferation assay, the lowering of FBS concentration from 10% to 1% or 0.1% greatly decreased the proliferation rate at 48 h in the WM-266-4 cell line compared to the WM-115 cell line (p<0.05). Our results suggest that critical biological differences between the two unique melanoma cell lines exist, and add information to the understanding of human solid tumors in primary and metastatic sites.

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Oncogene starvation via selective CDK7 inhibition: A novel approach for targeting traditionally undruggable oncogenic molecules in melanoma

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Cyclopentyl kinase 7 (CDK7), a unique member of the CDK family, has garnered attention as an anti-cancer target given its regulatory roles in the cell cycle and RNA polymerase (RNAP) II transcriptional machinery. Interestingly, it has been shown that selectively targeting CDK7 with THZ1 (3.2 nM IC50), a covalent CDK7 inhibitor, causes preferential downregulation of critical oncogenes, especially those associated with super-enhancers. Here, we present novel, preclinical results of THZ1 in melanoma. A panel of genetically diverse human melanoma cell lines treated with THZ1 demonstrated exquisite sensitivity with 0.2 nM CDK7 inhibitor, a 100-fold lower concentration than that of the CDK2, CDK4 and CDK6 inhibitors, respectively. We also examined the effect of THZ1 on differential expression of several melanoma lines, including one with a documented super-enhancer complex at the MITF promoter. We have shown that THZ1 (3.2 nM IC50), a covalent CDK7 inhibitor, causes preferential downregulation of critical oncogenes, including MITF transcriptional machinery. Interestingly, it has been shown that selectively targeting CDK7 with THZ1 (3.2 nM IC50), a covalent CDK7 inhibitor, causes preferential downregulation of critical oncogenes, especially those associated with super-enhancers. Here, we present novel, preclinical results of THZ1 in melanoma. A panel of genetically diverse human melanoma cell lines treated with THZ1 demonstrated exquisite sensitivity with 0.2 nM CDK7 inhibitor, a 100-fold lower concentration than that of the CDK2, CDK4 and CDK6 inhibitors, respectively. We also examined the effect of THZ1 on differential expression of several melanoma lines, including one with a documented super-enhancer complex at the MITF promoter. We have shown that THZ1 (3.2 nM IC50), a covalent CDK7 inhibitor, causes preferential downregulation of critical oncogenes, especially those associated with super-enhancers. Here, we present novel, preclinical results of THZ1 in melanoma. A panel of genetically diverse human melanoma cell lines treated with THZ1 demonstrated exquisite sensitivity with 0.2 nM CDK7 inhibitor, a 100-fold lower concentration than that of the CDK2, CDK4 and CDK6 inhibitors, respectively. We also examined the effect of THZ1 on differential expression of several melanoma lines, including one with a documented super-enhancer complex at the MITF promoter. We have shown that THZ1 (3.2 nM IC50), a covalent CDK7 inhibitor, causes preferential downregulation of critical oncogenes, especially those associated with super-enhancers. Here, we present novel, preclinical results of THZ1 in melanoma. A panel of genetically diverse human melanoma cell lines treated with THZ1 demonstrated exquisite sensitivity with 0.2 nM CDK7 inhibitor, a 100-fold lower concentration than that of the CDK2, CDK4 and CDK6 inhibitors, respectively. We also examined the effect of THZ1 on differential expression of several melanoma lines, including one with a documented super-enhancer complex at the MITF promoter. We have shown that THZ1 (3.2 nM IC50), a covalent CDK7 inhibitor, causes preferential downregulation of critical oncogenes, especially those associated with super-enhancers. Here, we present novel, preclinical results of THZ1 in melanoma. A panel of genetically diverse human melanoma cell lines treated with THZ1 demonstrated exquisite sensitivity with 0.2 nM CDK7 inhibitor, a 100-fold lower concentration than that of the CDK2, CDK4 and CDK6 inhibitors, respectively. We also examined the effect of THZ1 on differential expression of several melanoma lines, including one with a documented super-enhancer complex at the MITF promoter. We have shown that THZ1 (3.2 nM IC50), a covalent CDK7 inhibitor, causes preferential downregulation of critical oncogenes, especially those associated with super-enhasers. Here, we present novel, preclinical results of THZ1 in melanoma. A panel of genetically diverse human melanoma cell lines treated with THZ1 demonstrated exquisite sensitivity with 0.2 nM CDK7 inhibitor, a 100-fold lower concentration than that of the CDK2, CDK4 and CDK6 inhibitors, respectively. We also examined the effect of THZ1 on differential expression of several melanoma lines, including one with a documented super-enhancers complex at the MITF promoter. We have shown that THZ1 (3.2 nM IC50), a covalent CDK7 inhibitor, causes preferential downregulation of critical oncogenes, especially those associated with super-enhancers. Here, we present novel, preclinical results of THZ1 in melanoma. A panel of genetically diverse human melanoma cell lines treated with THZ1 demonstrated exquisite sensitivity with 0.2 nM CDK7 inhibitor, a 100-fold lower concentration than that of the CDK2, CDK4 and CDK6 inhibitors, respectively. We also examined the effect of THZ1 on differential expression of s...
Malignant melanoma is the most aggressive form of skin cancer. It typically originates in melanocytes located in the epidermis' stratum basale. Melanoma arises in part due to abnormal melanin biosynthesis and in its more aggressive state is often resistant to standard forms of chemotherapy. Melanin has been hypothesized to play the role of a protective pigment by serving as a barrier for UV-induced DNA damage. The present study aims to identify the role of epidermal melanocytes in the occurrence of melanoma.

Inhibition of histone deacetylase 3 overcomes BRAF-inhibitor resistance

In this study, we investigated the role of histone deacetylase 3 (HDAC3) in melanoma cells and its potential as a therapeutic target. We used a melanoma cell line, the A375 cell line, to test the effect of HDAC3 inhibition on cell proliferation, cell cycle, and apoptosis. We found that HDAC3 inhibition significantly reduced cell proliferation and cell cycle arrest at G1 phase. Moreover, HDAC3 inhibition increased the apoptosis rate in A375 cells. These findings suggest that HDAC3 inhibition may be a potential therapeutic strategy for melanoma treatment.

XPA-induced autophagy promotes cislain resistance in melanoma cells

Our study aimed to investigate the mechanism of cislain resistance in melanoma cells and determine the role of XPA in autophagy. We found that XPA expression is associated with cislain resistance in melanoma cells, and the inhibition of autophagy could reverse this resistance. These results indicate that targeting XPA may be a potential strategy for overcoming cislain resistance in melanoma cells.

Measurement of skin pigmentation using a chromometer in a 3-dimensional epidermal model containing functional melanocytes

This study aimed to measure skin pigmentation using a chromometer in a 3-dimensional epidermal model containing functional melanocytes. We used a melanocyte line (hMEL) and a keratinocyte line (hKer) to create a fully functional skin model. The chromometer measurements were performed on this model to evaluate the impact of different factors on skin pigmentation. The results showed that the skin color could be accurately measured using this model, suggesting its potential use in skin pigment measurement.

Differences in patient and tumor characteristics in amelanotic vs. pigmented melanomas

This study compared the characteristics of amelanotic and pigmented melanomas to identify differences in patient and tumor factors. We found significant differences in tumor growth, skin depth, and patient age between the two groups. These findings highlight the importance of considering melanoma type when planning treatment.

Tyrosinase targeting self-delivering RNAi compounds

This study aimed to develop tyrosinase-targeting RNAi compounds for the treatment of melanoma. We used a melanoma cell line (A375) and an amelanotic melanoma cell line (A375S) to evaluate the effectiveness of these compounds. The results showed that tyrosinase-targeting RNAi compounds could significantly reduce melanin content, suggesting their potential use in melanoma treatment.

MSX1-induced neural crest-like reprogramming promotes melanoma progression

This study investigated the role of MSX1 in melanoma progression. We found that MSX1 expression was correlated with melanoma progression, and the knockdown of MSX1 inhibited melanoma cell growth and migration. These findings suggest that MSX1 may be an important target for the treatment of melanoma.

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**Strategic use of BCL-2 inhibitors to target melanoma cells and melanoma initiating cells**
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Currently approved drugs for melanoma have produced promising results, but many patients eventually relapse and individuals without the BRAFV600E mutation show limited benefits. Downregulation of BCL-2 family contributes to melanoma’s resistance to treatments; eliminating melanoma initiating cells (MICs) should be part of drug development to prevent relapse. Therefore this study tested the efficacy of BCL-2 inhibitors in killing melanoma cells and MICs of SC-2001 (an oblique dermal melanoma BCL-2 family inhibitor), either alone or in combination with ABT-737 (a small molecule BCL-2/BCL-XL/BCL-W inhibitor). Interestingly, we found SC-2001 alone effectively de-bulked melanoma cells, but only the combination effectively eradicated the MICs. We used in vitro assays and mouse xenograft models to test drug efficacy and mechanistic involvement of BCL-2 inhibitors. Importantly, downstream activation of pro-apoptotic genes was observed in low dose SC-2001 (2 μM) with ABT-737 (3 μM) decreased AICD1, disrupted the cell’s survival properties, and more effectively inhibited the self-renewal of MICs (p < 0.05). Results were similar in multiple melanoma cell lines and in dermal microneedle and microneedle-assisted device for improved delivery of MICs. This combination is a promising treatment strategy for melanoma, regardless of mutation status of BRAF or NRAS.

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**The adverse correlation of primary cilia in melanoma is likely independent of proliferation and cell cycle progression**
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The primary cilium is a cellular organelle that exists in melanocytes and melanoma cells. Recently, frequent depletion of primary cilia was observed in melanoma, suggesting that primary cilia may be used as a biomarker for melanoma. However, the statistical power of using primary cilia as a biomarker remained to be determined. It also remained unclear whether the loss of cilia in melanoma was due to increased proliferation or cell cycle progression. Here, we evaluated primary cilia in 87 cases of melanocytic nevi and melanomas and found highly significant reduction in ciliated melanocytic nevi compared with melanomas (p < 0.01). This trend was similarly observed when only the non-proliferating cells were examined. Furthermore, when paired melanoma cell lines (cell lines derived from primary tumors and metastatic tumors of the same patients) were analyzed, we found that continuous melanocytic cell lines were more easily established from nevi and ciliated melanocytic nevi vs. non-proliferating melanomas. The loss of primary cilia was found in non-tumor and tumor tissue of melanomas. The results suggest that the loss of cilia is adversely correlated with melanoma, which is unlikely caused by increased proliferation or cell cycle progression in melanoma. Further investigation on the molecular mechanism underlying loss of primary cilium and its association to melanoma, and identity novel therapeutic avenues to reduce melanoma-related morbidity and mortality.

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**Switching the enemy: The anti-leprotic clofazimine displays anti-melanoma activity through induction of genotoxic stress**
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Even though the development of molecularly targeted therapeutics has recently revolutionized melanoma therapy, current available agents are urgently needed for patients with previously untreated metastatic melanoma. Currently, a vast array of genotoxic drugs have been identified to recruit leucocytes and “self-elimination” of tumour cells. However, the potential of genotoxic drugs as a mechanism to elicit cancer cell death remains largely unexplored. The objective of this study was to identify and validate the non-genotoxic anti-melanoma activity of the anti-leprosis drug clofazimine (CFZ). We demonstrate here that CFZ induces genotoxic stress through the activation of p53 and p21, and suppression of CDK2 and CDK4, and this in turn induces cell cycle arrest and apoptosis of melanoma cells. CFZ also potentiates the cell cycle arrest activity of BRAF inhibitors in melanoma cell lines and in mice xenografts, reducing tumor growth and tumor burden. These findings suggest that CFZ may be used as an adjuvant drug in the treatment of melanoma.

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**Reregulation of chemotactic signals, leukocyte recruitment, and immunity in segmental vitiligo**
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Anti-cytokine therapies (e.g. anti-IL-12/23) are widely used to treat autoimmune diseases. Their long-term effects is an important issue that has not been thoroughly investigated. Because IL-12 and IL-23 play a critical role in tumor development, their role in the formation of pigmented lesions and their progression to melanoma was evaluated in a murine model. Wild-type (WT) IL-12p35KO, IL-23p19KO, and IL-12/12p35KO mice were treated with dimethylbenz[a]anthracene (DBA) followed by 12-O-tetradecanoyl-phorbol-13-acetate (TPA) in order to produce pigmented dysplastic nevi that evolve into metastatic melanoma. IL-23p19KO mice developed 2-fold more lesions with increased radial growth (p<0.001) compared to WT mice. In contrast, there was a not a statistically significant difference in the size or number of lesions in IL-12p35KO mice compared to WT mice. After 25 weeks, draining lymph nodes (dLNs) were examined histologically for melanocytic foci. An increase in the number and size of melanocytic cell foci was observed in the dLNs from IL-23p19KO compared to IL-12p35KO and WT controls (p<0.01). Nonetheless, continuous melanocytic cell lines were easily established from new and dLN of the IL-23p19KO mice. The lines exhibited attachment independent growth and tumor growth in nude mice. Primary melanocyte cultures from IL-23p19KO mice exhibited delayed DNA repair in response to DBA, while WT melanocytes exhibited enhanced DNA repair when re-lit. IL-23p19KO mice were found to secrete very high levels of IFNγ (shown to promote the development of melanomas in murine models). WT mice treated with neutralizing IFNγ mAb developed significantly fewer lesions (p<0.05) than mice treated with control Abs. These results describe novel roles for IL-23-mediated inhibition of melanoma initiation and progression, through direct and indirect mechanisms.

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**De-regulation of chemotactic signals, leukocyte recruitment, and immunity in segmental vitiligo**
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Generalized non-segmental vitiligo (NSV) was recently re-evaluated as an autoimmune disorder. There also have been few reports suggesting a contribution of the immune system to the progression of segmental vitiligo (SV). With the knowledge that chemokines, small protein molecules that direct trafficking of leukocytes, play an important role in the activation and maintenance of the immune response, we assessed chemotactic molecules in the skin and blood of patients affected by segmental vitiligo at different stages of disease progression. Only minor changes including slightly elevated levels of CCL2 and IL-12p70 were observed. In the blood, higher levels of the intra-cutaneous CCL2, CCL11, CCL24, CCL22, CXCL12 and CCL5 were detected in the vitiliginous skin. Elevated chemokines within perilesional and lesional SV skin were associated with infiltration of the skin with leukocytes, which were more prominent in samples of SV with the recent onset (<6 months) than in those of longer duration. Remarkably, higher levels of de-regulated chemokines was restricted to melanocytes in the perilesional skin. Further characterization of lesional and perilesional skin showed prominent infiltration with CXCL12+ dendritic cells, CR4+ T cells, and eosinophils. All infiltrating cells were clustered at the dermal-epidermal junction of the perilesional skin. Some of the infiltrating cells were also detected within the epidermis. Contrary to perilesional skin, infiltrates were detected mostly in the dermis in SV lesions. This pattern of de-regulated chemotactic molecules in the SV-affected skin was similar to that identified in the NSV-affected skin of recent onset (<6 months). These results suggest that chemokines are key mediators of melanocyte recruitment and cell cycle progression, leukocyte infiltration, and recruitment of immune cells in segmental vitiligo.

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**Evaluation of a topical formulation containing myristyl nicotinate for modulating melanin production in comparison to a formulation containing hydroquinone**
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The objective of this study was to determine the efficacy of a topical formulation containing myristyl nicotinate in comparison with a formulation containing hydroquinone. A methodology was designed to assess the potential of a myristyl nicotinate containing cosmetic formulation (test material) to induce changes in tissue pigmentation using an in-vitro tissue model prepared from human keratinocytes and melanocytes. After obtaining favorable results from the in-vivo study a double blind clinical study on thirty seven panelists aged 32-65 was designed to evaluate the efficacy of the test material for overall skin brightening and skin tone modulation. The 12 week clinical study was designed evaluate the test material and a commercially available formulation containing Hydroquinone (2%). Qualified dermato logical evaluation, image analysis and statistical analysis (Z-test) on consumer perception questions were assessed at week 4, 8 and 12 respectively. The results of the in-vitro study indicate that the test material had a statistically significant reduction in the melanin content compared with the formulation containing hydroquinone. Additionally, the test material had a statistically significant decrease in melanin content compared to the lotion vehicle. While the hydroquinone treated tissues also saw a significant reduction in melanin content, the decrease observed with the test material was statistically better than the tissues treated with hydroquinone. Clinical results (in-vivo) indicate that the test material showed statistically significant improvement in multiple parameters for skin brightening. In conclusion, the test material containing myristyl nicotinate was able to induce a change in the over-expression of melanin with recent onset (less than 4 months) skin. The test material was performing better than hydroquinone in-vitro and comparable to hydroquinone in clinical studies.
4'-Bromo-resveratrol, a new small molecule inhibitor of SIRT3, imparts anti-proliferative effects and causes metabolic reprogramming of human melanoma cells

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Sirtuin-3 (SIRT3), an important member of sirtuin family, is localized to mitochondria where it deacetylates mitochondrial proteins and regulates a variety of cellular functions. Earlier, we have demonstrated that SIRT3 is overexpressed in human melanoma cells and tissues and its genetic knockdown resulted in a significant antiproliferative response in human melanoma cells (JID 134, S121-S131, 2014), suggesting that small molecule inhibitor(s) may be useful for melanoma management. In this study, we determined the effect of a small molecule-SIRT3 inhibitor, 4'-bromo-resveratrol (4BR), in human melanoma cells (G361, SK-MEL-28 and SK-MEL-2). We found that treatment of melanoma cells with 4BR (12.5-200 \times 10^{-3} \text{ mM}; for 24-72 h) resulted in a significant dose- and time-dependent decrease in cell proliferation. Further, 4BR treatment also resulted in a marked decrease in the growth/viability, clonogenic survival and migration ability of melanoma cells. Furthermore, 4BR treatment was found to cause a marked downregulation of proliferating cell nuclear antigen (PCNA) protein and SIRT3 mRNA and protein. In addition, 4BR treatment to melanoma cells caused 1) an induction of apoptosis (PARP cleavage), decrease in pro-caspase-3, pro-caspase-8, and 2) G0/G1 phase cell cycle arrest. These effects were accompanied by an increase in the protein levels of p21Waf1 and decrease in cyclin D1 and cdk2. Interestingly, our data also demonstrated that 4BR resulted in a metabolic reprogramming in human melanoma cells as evident from decrease in the levels of 1) lactate production, 2) glucose uptake, 3) NAD+/NADH ratio, 4) lactate dehydrogenase A (LDHA) and glucose transporter 1 (Glut1) proteins. Taken together, our data suggested that SIRT3 small molecule inhibition via 4BR (or other pharmacological means) could be developed as a novel therapeutic strategy against melanoma. Further research is ongoing in our laboratory to ascertain the mechanism and in vivo significance of our finding.

105F is a novel immunoadaptive treatment candidate for melanoma that induces apoptosis and the secretion of pro-inflammatory IL-6

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We have previously shown that a low molecular weight subfraction (105F) of human cell conditioned media (CCM) from hypoxia-induced multipotent cells grown in a perfusion bioreactor system inhibits tumor cell growth in vitro and in two animal models. We have further studied the mechanism of action as it related to melanoma. Uveal and nRAS melanoma cancer cell growth was inhibited by greater than 90% in in vitro proliferation assays. Immunostaining of Annexin V and immunoblot analysis suggest that the mechanism of action of the 105F is through the induction of apoptosis by the upregulation of Caspase 3 and 9. In the TUMCAM assay, melanoma load was reduced by up to 80% with 105F treatment compared to controls (p < 0.05). To further elucidate the mechanism involved custom kinome array assays were performed on two chemotherapy resistant melanoma cell lines, DO4 and C918. The results indicated that the induction of apoptosis is downstream from Toll-like receptor (TLR) signaling pathways. Results also implicated adaptive immune responses including the secretion of pro-inflammatory cytokines. As such, ELISA analyses showed the release of IL-6 from the DO4 melanoma cells after treatment with 105F. Such an immune “flare” could be critically important in recruiting immune cells to the tumor for cytotoxic attack. To further study the effects of 105F on tumors and the immune system, colony forming units (CFU) assays were done with co-cultures of mesenchymal stem cells (MSC) primed to be pro-inflammatory (MSC1) and DO4 or B16 melanoma. Both human and mouse cancer colonies were diminished in the MSC1 alone treatment, colonies were smaller and tighter after 105F alone treatment, and colonies were synergistically diminished after the combined treatment. These results support a role for 105F in a novel immunoadaptive treatment for melanoma.