The College of Arts and Sciences / Department of Molecular Genetics

Defining how BRAF and NRAS mutations cooperate with UVA and UVB light to initiate melanoma

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ABSTRACT

Melanoma is the malignant transformation of pigment-producing melanocytes in the body. The majority of skin cancer-related deaths are attributed to melanoma, even though this disease only makes up about one percent of all skin cancers (National Cancer Institute, 2017). While the most common forms of melanoma contain either a *BRAF* or *NRAS* mutation, it has been shown that these "driver mutations" are insufficient to initiate melanoma and are not caused by damage from ultraviolet (UV) light. Our lab has developed genetically engineered mouse models with endogenous, melanocyte-specific expression of either $BRaf^{V600E}$ or $NRas^{Q61R}$, allowing us to accurately model the genetics of human melanoma. Using these murine models we aim to determine if UVA, a wavelength found commonly in tanning beds, can enhance melanoma formation and progression, and if this process is dependent of skin pigmentation. Further, we will use these models to test the ability of sunscreen to reduce the risk of developing melanoma. Sunscreen provides a certain level of protection from sunlight, which contains UVA and UVB light, which can travel past the ozone layer and are linked to human melanomas. While sunscreen has been demonstrated to protect one from sunburns, caused predominately by UVB radiation, limited research has been performed on the ability of sunscreen to reduce melanoma risk. Therefore, we will use our mouse models to determine the efficacy of common sunscreen filters in preventing melanoma. Our findings will improve understanding of the mechanisms behind UVA-induced melanoma formation and aid in the formulation of effective melanoma preventatives.

Hypothesis

We hypothesize that the exposure of *TpB* and *TpN* mice to UVA will cause a reduction in Melanoma Free Survival (MFS) and Overall Survival (OS) as well as an increase in Tumor Burden (TB) and Tumor Growth Rate (TGR). We also hypothesize that mice treated with 4.8% octinoxate prior to UVB exposure will exhibit longer OS and MFS and lower TB and TGR compared to mice treated with vehicle controls (acrylates or mineral oil) or 10% octocrylene.

METHODS

Mouse Models

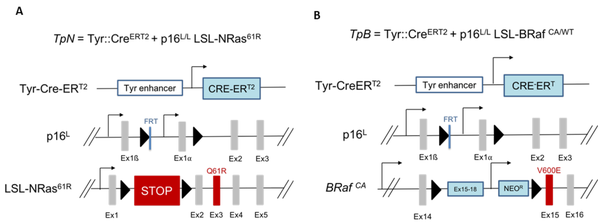

Figure 1.1 The *TpN* and *TpB* mouse models A. *TpN* mice contain a transgenic melanocyte-specific, tamoxifen-inducible CRE allele (*Tyr::CreER^{T2}*), and are homozygous for a conditional p16 knockout allele (*p16^{L}*) and a conditional allele encoding oncogenic $NRas^{61R}$ (*LSL-NRas^{61R}*). Painting the mice with 4-hydroxytamoxofien (4-OHT) deletes *p16* and turns on *NRas^{61R}* expression in melanocytes. **B.** *TpB* mice also contain the transgenic *Tyr-CreER^{T2}* and are homozygous for the *p16^{L}* allele. These animals are also heterozygous for a conditional allele encoding $BRaf^{V600E}$ ($BRaf^{CA}$). Painting the mice with 4-OHT deletes *p16* and turns on $BRaf^{CA}$ expression in melanocytes.

UV Procedure

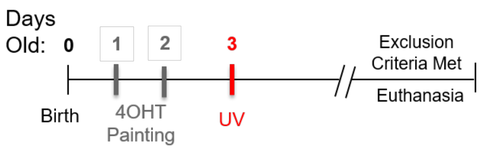

Figure 1.2 Pups are painted on post-natal days 1 and 2 with 20mM 4-OHT. On day 3, half the pups are exposed to either 4.5 kJ/m^2 of UVB or 70 kJ/m^2 of UVA. After UV exposure the mice are monitored and checked two times a week for melanoma–development. Any tumors are measured three times a week, and the total number of tumors per mouse recorded upon euthanasia.

Sunscreen Procedure

All pups are treated for the first three days as described in Figure 1.2. On day 3, before exposure to 4.5 kJ/m^2 of UVB, the pups are sprayed with a sunscreen containing either 4.8% octinoxate, 10% octocrylene, 2% acrylates or 0.1% mineral oil. After UVB exposure, the animals are monitored as described in Figure 1.2.

RESULTS

UVB

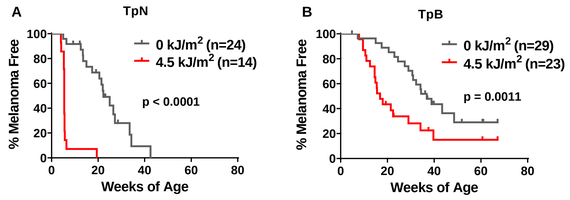

Figure 2.1 A and **B** show that after treatment with 4.5 kJ/m^2 of UVB, both the *TpN* and *TpB* mice exhibit a decrease in Melanoma Free Survival when compared to the mice receiving no UV treatment. P-values determined using Gehan-Breslow-Wilcoxon tests.

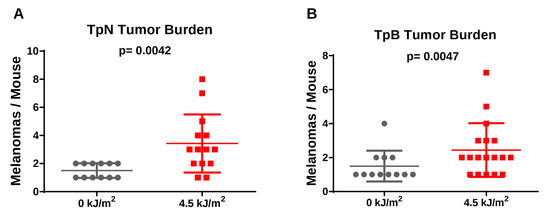

Figure 2.2 A and **B** show that after treatment with the 4.5 kJ/m^2 of UVB the *TpN* and *TpB* mouse models have an increase in Tumor Burden when compared to the mice that receive no UV treatment. P-values determined using Gehan-Breslow-Wilcoxon tests.

UVA

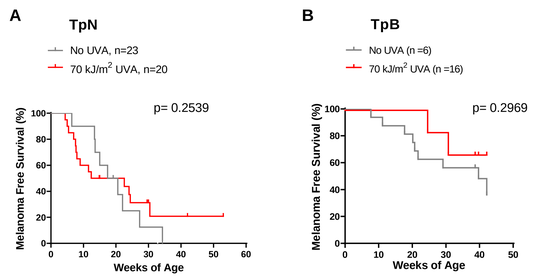

Figure 2.3 A and **B** show that after exposure to UVA, Melanoma Free Survival is not significantly decreased in either *TpN* or *TpB* mouse models when compared to mice who received no UV treatment.

RESULTS

Single Filter Sunscreens

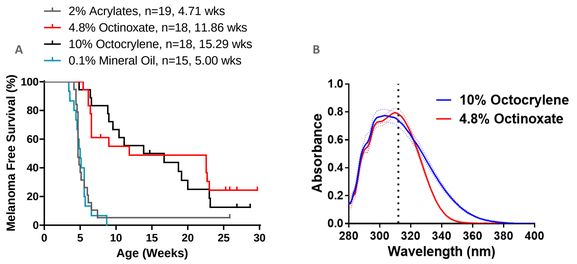

Figure 2.4 A Mice treated with 10% octocrylene had a similar average Melanoma Free Survival as those treated with 4.8% octinoxate. Acrylates and mineral oil did not protect the mice from UVB-accelerated melanoma formation. **B,** Absorbance data showing that 10% octocrylene and 4.8% octinoxate have a similar ability to absorb UVB light.

CONCLUSIONS

- UVB radiation accelerates melanoma in the *TpN* and *TpB* mouse models.
- UVA radiation does not significantly accelerate melanoma in *TpN* or *TpB* mouse models.
- The UVB filters, octocrylene and octinoxate did not show a difference in ability to block UVB but did show an increased ability compared to the vehicle controls.

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