CP6*: Melanoma biology and pigmentation: David Fisher, Massachusetts General Hospital
Funding Source and Period: NIAMS 4R01AR043369-20, 1996-2018
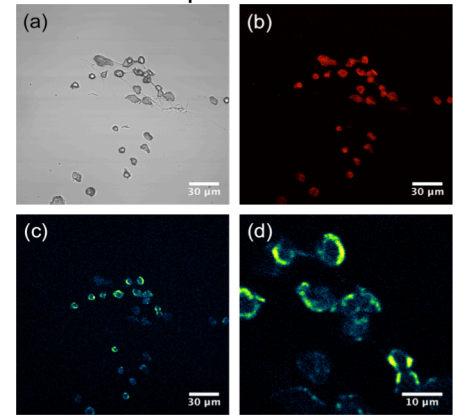**Associated With:** TRD1,2,3
Significance: Melanoma is the cause of death of roughly 10,000 patients in the United States every year and over 230,000 patients worldwide.[1] Melanoma lesions are often identified as abnormally dark skin regions with irregular pigmented patterns. Their color arises from melanin pigments, of which there are two varieties: eumelanin, a dark and easily identifiable pigment, and pheomelanin, a poorly visible red/blonde pigment seen easier in hair where it is highly concentrated. Across the spectrum of human skin pigmentation, redhaired individuals have the palest skin due to their inability to dermally synthesize eumelanin. Redheads also have the highest probability of developing melanoma. A recent landmark study carried out by Dr. David Fisher, Chief of Dermatology at Massachusetts General Hospital, generated a redhead mouse model of melanoma[2] which revealed a UV independent carcinogenic activity of red/pheomelanin pigment—a finding that was recently confirmed in humans.[3] Importantly, the redhead mouse model found that red/blond pigmented melanoma precursor lesions were "invisible" to the naked eye, and could not be detected until the formation of invasive melanoma nodules.

Figure 1 Melanocytes isolated from red-haired C57BL/6 (Mc1re/e, Tyr+/+) mice exhibit strong CARS signal at $\omega_P - \omega_S$ = 2000 cm-1. (a) Trans-illumination image acquired with the pump beam, where the overall shape of the cells can be well visualized. (b) Confocal fluorescence image of tdTomato. (c) False color ("Green fire blue" color in ImageJ) CARS image mapping intracellular pheomelanin distribution. (d) 4X-zoomed view of (c) showing a perinuclear distribution of signal intensity, consistent with the known biology of protective melanin caps.

Though the majority of human melanoma lesions are dark and contain brown/black eumelanin, approximately 2-8% of melanoma lesions lack dark pigment, instead appearing red or pink. These so-call "amelanotic" melanomas are difficult to diagnose, and have been shown to be associated with more advanced depth at the time of clinical detection, as well as higher mortality.[4] Pigment variability within brown/black melanomas can also produce zones lacking dark pigment, which may similarly contain "invisible" pheomelanin—an issue of importance in determining optimal surgical margins. Recent studies in both animal models and human melanomas cells strongly suggest that these amelanotic lesions or foci are in fact pheomelanotic. Taken together, these findings suggest that the detection of pheomelanin is important for the study, diagnosis, and care of melanoma, but there are no clinical instruments capable of visualizing and quantifying melanins on the cellular scale *in situ.*

Approach: Coherent Raman and multiphoton absorption microscopy toolkits have recently gained special consideration for the study and diagnosis of melanoma. In a collaboration between the Fisher and Evans laboratories, coherent anti-Stokes Raman scattering (CARS) was found capable of detecting pheomelanin in cells, mouse models, and human skin. (Figure 1) When sum-frequency absorption (SFA) microscopy was additionally utilized, both pheomelanin and eumelanin could be distinguished and quantified. Using the portable CARS/SFA system being developed in TRD3.3, the Fisher lab will investigate both the presence of invisible nevi in the skin of redheaded mouse models as well as their malignant transformation of these nevi. This will be accomplished using redheaded mouse models engineered to contain a CRE-inducible BRAF[V600E] mutation, the most common mutation found in nevi. Following topical application of tamoxifen, skin will be monitored to track the appearance of pheomelanin-rich dysplastic cells that serve as precursors to melanoma.

Following initial animal studies, next steps will involve a transition to human patients for the identification and tracking of pheomelanotic nevi. The portable CARS system will be used to revisit sites of interest over the course of weeks to follow individual nevi over time. Additional studies will focus on the clinical imaging of amelanotic lesions to identify a set of image-based biomarkers which can be used for clinical diagnostics.

Push-Pull Relationship: The Fisher lab is investigating the fundamental drivers of melanoma, and believes that the natural pigment pheomelanin is oncogenic. The LBRC **pushes** a new portable CARS and SFA microscope for both animal and human imaging *in vivo* to enable real-time imaging of nevi and melanoma lesions. The Fisher lab, in its efforts to translate their findings to the clinic **pulls** the LBRC to develop and provide a turn-key, clinical imaging system for the detection and quantification of melanin pigments in patients for both short and long term studies in the biology of melanomagenesis via TRD3.3. Wide-field two-photon and second harmonic generation deep tissue imaging technologies (TRD1.1) may be **pushed** for complementary *in vivo* investigation allowing the quantification of cellular metabolism and the extracellular matrix environment. Wide-field transient absorption and stimulated Raman imaging (TRD2.3) may be **pushed** for higher throughput quantification of relevant cell culture and tissue section specimens. Preliminary studies with the Fisher lab will in term **pull** both TRD1.1 and TRD3.3 aims toward optimizing imaging protocols for melanoma specimens.

Literature Cited

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