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Title:

Label-free profiling of melanins in human eye melanomas using 2-photon microscopy techniques

Abstract:

Human eye (uveal) melanoma is the most common intraocular tumour in adults, primarily in Caucasians (Kaliki and Shields, 2017). The predicted incidence of the most frequently occurring form of uveal melanoma, choroidal melanoma, is around 90-100 cases per year in Australia (Reader et al., 1997), a relatively small number when compared to the skin melanoma yearly prevalence (around 14,000 new cases in 2017) (AIHW, 2017). The progression of choroidal melanoma (CM) is complex, involving genetic and immune-related factors (Farquhar et al., 2018). Melanin/pigmentation genes and types (*eu*melanin/dark and *pheo*melanin/light) can also impact on CM progression (Ferguson et al., 2016).

In this study, an optimised 2-photon fluorescence lifetime imaging microscopy (FLIM)-phasor method was used to identify melanin fluorescence lifetime profiles in formalin-fixed, label-free paraffin sections of human CM and surrounding human choroidal melanocytes (HCMs) of varying pigmentation. Sections of 'very dark' to 'very light' pigmented CM (n=9) with 'mixed pigmented' surrounding HCMs were imaged using FLIM (3 sampled regions). Fluorescence lifetimes were measured at every pixel of captured FLIM images and Fourier transformed to phasors presented in a 'fit-free' phasor plot (Digman and Gratton, 2014). These plots were segmented by 7 phasor clusters of linearly increasing fluorescence lifetimes mapped to intracellular melanin in CM and HCM cells. The fraction of FLIM image pixels linked to each melanin-mapped cluster was obtained from all sampled regions to form melanin fluorescence lifetime profiles. The measured sampled regions displayed distinct intracellular melanin (*eu*melanin:*pheo*melanin) profiles with varying dominant melanin-mapped clusters. The 'dark CMs' were mapped to mostly short lifetimes (high *eu:pheo* ratio). The dominant 'highest pixel fraction' cluster measured from 'light CMs' was mapped to long lifetimes. This implied that the sampled CMs have low *eu*:*pheo* mixture. The 'mixed pigmented HCMs around the CMs' showed a mixed *eu*:*pheo* content based on the dominant mid-valued lifetime cluster and distinct from the CM melanin FL histogram profiles. The application of a FLIM-phasor method provides a fast 'model-free' way to unmix melanin fluorescence lifetimes in CMs and HCMs, and provides a basis for exploring the role of melanin forms in eye melanoma pathogenesis.

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