MULTIMODAL IMAGING OF CELLULAR SENESCENCE – OXIDIZED LIPIDS AND ENZYMATIC ADAPTATIONS IN AGING SKIN AT THE SINGLE CELL LEVEL

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Changes in carbohydrate metabolism are a key feature of aging which also manifest in the epidermis. Furthermore, the synthesis and distribution of epidermal lipids changes with age. Both these parameters cannot be investigated with immunohistochemistry, as neither serves as useful epitope. We developed a multimodal analytical histocytometry approach combining modalities that localize lipids and enzymatic activities with immunofluorescent imaging of the skin to localize changes that are correlated with appearance of senescent cells. The activities of key metabolic enzymes were determined on tissue sections of aged and juvenile skin with a formazan-based assay. Lipids were localized and quantified using FTICR MALDI - mass spectrometric imaging. We correlated those modalities with immunofluorescent imaging and analyzed the intensities of the respective signals at single cell level, using Strataquest tissue cytometry. We analyzed skin from donors of young (< 30 y) versus advanced (> 67 y) ages and we investigated epidermal equivalent models containing labeled UV-damaged or senescent keratinocytes. Enzymatic activities displayed specific patterns across the stratifying epidermis, and had diverging trajectories in aging, with a marked decrease in suprabasal glucose-6-phosphate dehydrogenase (G6PD) activity. G6PD, the rate limiting enzyme of the pentose phosphate pathway was also identified as a rapid response pathway activated upon UV damage in the epidermis. The lipid molecular imaging identified differentiation- and age-related changes of polar lipids in skin biopsies and epidermal equivalents, and pro-senescent stress dependent reactive aldehydophospholipid species in the basal epidermal layers. While these methodologies are still in development, it is evident that correlative analytical imaging – with the aid of AI driven histocytometry – will continue to yield novel insights into skin and epidermal biology by localizing previously undetectable parameters within the epidermis in the context of aging.