MELANOMA AND MELANOCYTIC NAEVI

BEHAVIOR OF MELANOMA CELLS UNDER HIGH CONFINEMENT, INDUCED HIGH ACTIN CONTRACTILITY AND LOW SUBSTRATE ADHESION MEDIATING LEADER BLEB BASED MOTILITY

G Adams Jr [1] - M Preciado-lopez [2] - R Fischer [2] - J Logue [3] - C Waterman [2]

National Institute Of Health; Howard Univeristy College Of Medicine, National Heart Lung Blood Institute; Dermatology, Bethesda; Washington, United States [1] - National Institute Of Health, National Heart Lung Blood Institute, Bethesda, United States [2] - Albany Medical College, Regenerative And Cancer Cell Biology (rccb), Albany, United States [3]

Introduction: Melanoma, a skin related disease likely to undergo metastasis without early detection. Metastasis involves spreading of cells from benign nevi from epidermis-to-dermis, invading blood vessels, extravasating, and populating distant sites. High-resolution microscopy of melanoma cells adjacent-to-tumors in mouse skin or migrating in-vitro in confined, non-adhesive 3D environments mimicking the tissue microenvironment take on a highly stereotypical morphology with a single-large bleb leading fast, persistent movement termed ‘leader bleb-based migration’ (LBBM). LBBM was shown to be driven by polarized actin cytoskeletal and myosin II contractility driving cortical retrograde flow along the large bleb coupled to non-specific friction with the microenvironment.

Objective: Characterize localizations of organelles and actin-regulatory proteins and dissect the contribution of actin-regulatory proteins to drive LBBM in metastatic human melanoma cells that could serve as specific drug targets for blocking LBBM inhibiting melanoma metastasis.

Materials and Methods: Fluorescent fusion proteins, such as organelle markers or actin-regulatory proteins, were over-expressed in A375 melanoma cells confined to a 3 µm space under a polydimethylsiloxane (PDMS) pad imaged by time-lapse high-resolution spinning-disk confocal microscopy analyzing spatial distribution of proteins in LBBM and the effect of their overexpression on cell morphology and LBBM motility parameters.

Results: Florescence markers for organelle pathways, including secretory, excretory, and metabolic revealed that most membranous organelles were localized in the cell body. However, secretory organelles were also found in the bleb. Actin nucleating factors localized

towards the leader bleb tip, while the cross-linking proteins, filamin and fascin, were found in the bleb. Morphology and motility parameters in cells overexpressing cross-linkers revealed increased bleb size and cell speeds.

Conclusion: This is the first description of the cellular “anatomy” of organelle distribution in cells undergoing LBBM and suggests an important role of actin crosslinkers in regulating leader bleb size and speed in modulating LBBM rendering new potential therapeutic targets.