High content dissection of human melanoma tumor heterogeneity during treatment using mass cytometry

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Background: Tumors are composed of diverse cell types, including cancer cells that can differentially evade therapy and stromal cells that may aid or impede treatment. The emergence of therapy resistant cell subsets is a primary cause of treatment failure in aggressive malignancies, but most high content approaches in cancer research do not achieve single cell resolution and rely on culture-adapted cell lines as model systems. Quantitative fluorescent studies of melanoma can also be confounded by variable intrinsic fluorescence of primary melanoma cells.

Methods: To address these challenges, a 28-antibody mass cytometry (CyTOF) panel including markers of immune cells, endothelial cells, and neural origin melanoma cells was created to characterize melanoma tumor biopsies. A key protein measured in the panel was Nestin, an intermediate filament protein expressed in melanoma cells. In healthy cells, Nestin is expressed embryonically and in rare adult cells, including neural stem cells. Primary tumors from 16 individual patients and 7 melanoma cell lines were characterized by mass cytometry. For 4 patients, the comparison included biopsies obtained before, during, and after treatment as part of a clinical study targeting mutant BRAF V600 and MEK signaling proteins. All specimens were obtained in accordance with the Declaration of Helsinki following protocols approved by the Vanderbilt University Institutional Review Board. Single cell suspensions for mass cytometry analysis were prepared using collagenase-based disaggregation. Data analysis was performed on Cytobank using unsupervised tools (SPADE and viSNE) and traditional directed analysis.

Results: Within primary human tumors, infiltrating leukocytes were distinguished by high CD45, comprised 14.3% ± 25.3% of tumor cells, and were dominated by CD8 T cells expressing CD45R0. Endothelial cells were rare (<5% of CD45⁺ cells) and expressed CD31 and CD61. The remaining CD45⁻ tumor cells were >98% cancer cells distinguished by hallmark melanoma proteins (e.g. Nestin, MCAM, NGFR). In vivo changes in intra-tumor cellular heterogeneity were quantified following therapy and used to identify phenotypically distinct melanoma cell populations that persisted despite treatment. Lower median Nestin protein expression distinguished melanoma cells that persisted following treatment from those that regressed (p < 0.0001, Bonferroni corrected α = 0.002). Within 6 established melanoma cell lines, cells with this melanoma persister phenotype were present, but rare, and constituted on average 7.0% +/- 3.9% of cells.

Highlights: This study establishes a high content single cell mass cytometry approach for characterizing human melanoma tumors. Loss of Nestin protein expression was a significant feature of melanoma cells that persisted in vivo, despite therapy targeting BRAF and MEK signaling. Clinically significant melanoma cell subsets observed in human tumors were uncommon in established cell lines.