INTEGRATING MICROSCOPY TECHNOLOGIES FOR UNDERSTANDING AND MODELING INFLAMMATION INDUCED IMMUNO-SUPPRESSION IN TUMORS

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Inflammation promotes tumorigenesis through immune-suppression and is mediated by intercellular mechanisms between immune cells, host stroma, vasculature, extra-cellular matrix and tumor cells. Thus, we are integrating sample preparation (*in vitro* and *ex vivo*), image acquisition and analysis to investigate these phenomena from cell to tissue scales. Microscopic imaging of multiplex immunofluorescence (MIF) labeled thin (≈ 10 µm) tissue sections is the choice technique for elucidating these mechanisms, because 20+ labels can be applied to the same section while preserving the spatial relationships of cells to each other and to extracellular structures. However, drawbacks exist. Thin sections contain arbitrary fractions of each cell. This results in inaccurate quantification of cellular morphology, the level of labels per cell and the 3D spatial relationships between cells. Therefore, we have advanced MIF to label to 50 µm depth in tissue and optically cleared tissue to image to 0.5 mm depth. Furthermore, we developed software to automatically detect tissue regions with round versus elongated cell nuclei as an indicator of epithelial versus mesenchymal cells, and to interactively segment whole cells from 3D tissue images. Since samples are fixed, assays are restricted to one time point. To overcome this, we model tumor-immune cell interactions *in vitro* by imaging live co-cultured cells in a restricted exchange environment chamber (REEC). Cells in the chamber consume oxygen and nutrients diffusing from a single source, generating radial gradients of these molecules, which in turn generates distinct cell phenotypes depending on radial position.

We applied these technologies to extend our current understanding of proinflammatory signaling as it relates to the identification of novel therapeutic targets associated with inducible nitric oxide synthase (Nos2) and cyclooxygenase (Cox2) in the 4T1 syngeneic mouse model used to study triple-negative human cancer. High expression of both proteins strongly predicts poor survival in patients with ER- breast cancer. We discovered that Nos2 and Cox2 expression is spatially distinct in 4T1 mouse tissue, in spheroids grown from 4T1 cells and amongst 4T1 cell cultured in the REEC, revealing that the Nos2 / Cox2 feed-forward loop is an intercellular mechanism. Moreover, in the latter two environments Nos2 cells were localized in hypoxic regions, as anticipated since hypoxia and nutrient deprivation drive Nos2 expression. Furthermore, by live coculture, 4T1 cells and macrophages segregated into distinct regions, with 4T1 cells migrating to the source of oxygen and nutrients.

Ongoing studies are: 1) evaluating tissue expansion for improved antibody penetration and increased effective spatial resolution, 2) investigating how different immune cell types interact in the tumor microenvironments before and after treatment with irradiation and anti-inflammatory drugs and 3) discovering the cellular organization of cancer stem-like cell niches.

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