

Characterization of diverse immune cell types in single cancer tissue specimens using hyper-plexed in situ immunofluorescence staining technology

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Background: The immune system and tumor microenvironment are strongly implicated in cancer pathophysiology and patient outcome. Immune cell analysis methods include flow cytometry for blood and aspirate samples, and immunohistochemistry and gene expression for solid tissues. While flow cytometry and gene expression provide hyper-plexed analysis, protein co-localization and/or spatial analysis of tumor and stroma are not possible. Protein analysis in formalin-fixed, paraffin-embedded tissues is typically limited to 1-2 markers per tissue slide using chromogenic stains, or up to 4-5 markers using immunofluorescence. We recently developed a multiplexed fluorescence microscopy method (MultiOmyxTM) for the quantitative characterization of multiple analytes in routinely collected, formalin-fixed paraffin-embedded tissue (Gerdes et al., PNAS 2013). We applied this platform to human lung and colorectal cancer samples to determine the expression and co-expression of up to 8 immune cell proteins at a single-cell level in samples from 747 colorectal cancer patients and 144 lung cancer patients on tissue microarrays.

Methods: Fluorescent dye-labeled antibodies targeting lineage specific proteins of epithelial cells, T lymphocytes, B lymphocytes, macrophages and neutrophils were applied to tissue samples and imaged by fluorescence microscopy. Novel dye inactivation chemistry and image analysis algorithms enabled iterative staining and image analysis of seven and eight immune cell targets on single colorectal and lung cancer tissue microarray slides, respectively. A proprietary computational biology toolkit (LayersTM) enabled single cell analysis and visualization capabilities. Cell lineage specific protein expression in each cell was used to enumerate immune cells and define epithelial or stromal residency status in each image. Automated analysis was compared with manual cell counting to confirm if the results are consistent with visual characterization. Quantitative features of immune cell infiltration were used to develop Cox proportional hazards models of disease recurrence.

Results: Diverse immune cell profiles were evident across all subjects. We observed cases ranging from very few immune cells of any lineage, to high levels of immune cell infiltration including dense intraepithelial infiltration. Our quantitative approach facilitated enumeration of immune cell numbers and type in both epithelial and stromal tumor regions. Quantitative features of immune cell infiltration were independent risk factors in multivariate Cox proportional hazards modeling of colorectal cancer recurrence.

Conclusions: Our analytical approaches provide a means to rapidly quantify immune cell populations in epithelial cancers, including the delineation of tumor infiltrating cells and cells residing in the tumor stroma. Immune cell features derived in this analysis were successful in multivariate modeling of colorectal cancer recurrence. Given the growing understanding of prognostic implications of the immune system and tumor microenvironment in common cancers, we expect our analytical approaches will find broad utility in cancer research and clinical diagnostics.