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

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Abstract

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 is highly sensitive, it may provide limited information about tumor cell phenotype, cell-to-cell interactions, and the functional state of immune cells. Immunohistochemistry provides a more detailed picture of immune cell localization and infiltration, but requires huge amounts of high-quality tissue and is limited by the limited number of analytes that can be assessed in a single analyte. Here, we report a more comprehensive approach using an automated multiplexed method, MultiOmyx, to characterize immune cell infiltrates in single cancer tissue sections.

Methods: Fluorescent dye-conjugated antibodies targeting 45 specific proteins of epithelial cells, T-lymphocytes, B-lymphocytes, macrophages and neutrophils were applied to tissue sections and imaged by fluorescence microscopy through ten nanometer wavelength intervals. Massively parallel automated image analysis was conducted to segment different cell types and analyze their spatial and quantitative distributions. Using a proprietary computational biology toolkit, CytoViva\(R\), we derived immune cell infiltration scores as well as population-level distributions. We tested the utility of this method in colorectal cancer, where immune cell infiltrates have been shown to be correlated with patient survival.

Results: Diverse immune cell profiles were evident across all subjects. We observed cases ranging from very few immune cells of any type, to high levels of immune cell infiltration involving dense inflammatory infiltrate. Correlation analysis between traditional histological examination of immune cell infiltrates and derived immune cell infiltration scores revealed a high level of concordance. In addition, we evaluated several clinical outcomes in a retrospective cohort of colorectal cancer patients. Our analyses suggested that high levels of immune cell infiltration were associated with improved patient survival, both subject-level and population-level.

Conclusions: Our multi-plexed approach provides 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. Given the established role of the tumor microenvironment in cancer outcomes, we expect our analytical approaches will find utility in cancer research and clinical diagnostics.

Immune Cell Infiltration in Lung Cancer

Comparison of two stage I lung cancers

Immune Cell Infiltration in Colorectal Cancer

Comparison of two stage III, grade III colorectal cancers

Immune Cell stromal distribution metrics by stage and grade in 747 stage I-III colorectal cancer subjects

Population level immune cell characterstics: All data are presented as violin plots – a vertical representation of a smoothed histogram visualized as a mirror image. The top row depicts mean of the single cell median stromal immune cell marker expression. The bottom row shows percentage of cells positive in the stromal region of the image.

Immunofluorescence Targets

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Future directions and working model

- Multivariate model building – are immune cell features additive or synergistic in predicting prognosis?
- What is the relationship between immnosurveillance and major CRC subtypes (CIMP, MSI, H?)
- Are there associations between immunoproteins and other colorectal cancer hallmarks (e.g., hypermethylation) in this study? e.g., p53 overexpression, signal transduction phenotypes, morphological characteristics, etc.

Conclusions

- MultiOmyx analysis allowed for the quantification of diverse immune cell types in single specimens of colon and lung cancers.
- Epithelial and stromal reactivity of immune cells was determined, allowing localization specific analysis of immune cell population metrics.
- The overall distribution of T-lymphocytes, B-lymphocytes, macrophages and neutrophils was highly variable between different subjects in lung and colon cancers.
- In immune cell positive colon cancers, T-lymphocytes were the most common in general, followed by macrophages and neutrophils. Outside of gut associated lymphoid regions, B-lymphocytes were very rare, but were present in low numbers in a minority of cases.
- Intraepithelial and stromal localization and quantity of immune cell infiltration may provide additional resolution into well recognized tumor immunology relationships with colon cancer prognosis.
- T-lymphocyte associated prognostic relationships are in research with previously reported findings, and innate immune cell populations may add information to these prognostic models.

References

- Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer cell 2012; 21:399-332