Single Cell Spatial Biology for Precision Cancer Medicine

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In cancer, complex ecosystems of interacting cell types play fundamental roles in tumor development, progression, and response to therapy. However, the cellular organization, community structure, and spatially defined microenvironments of human tumors remain poorly understood. With the emergence of new technologies for high-throughput spatial profiling of complex tissue specimens, it is now possible to identify clinically significant spatial features with high granularity. In this PSB workshop, we will highlight recent advances in this area and explore how single cell spatial profiling can advance precision cancer medicine.

Keywords: Spatial biology, spatial transcriptomics, machine learning, artificial intelligence, cancer biology, precision medicine

1. Introduction, Background, and Motivation

Maps are indispensable tools for understanding and navigating our world. While the earliest maps had limited resolution, in recent decades, we have witnessed an explosion in the scale, scope, and complexity of digital mapping data. Today, large fleets of satellites perform high resolution geospatial surveys at a global scale, while smartphones and wearables provide a nearly "limitless" supply of real-time physiological data with spatial coordinates. Significant advances in spatial mapping technology have permeated other areas as well, including biology – where, for example,

the IMAXT project (one of Cancer Research UK's Grand Challenges) is currently building the first 3D virtual reality map of a tumor.

Within biology in particular, technologies for mapping spatial organization are in the midst of a revolution. In 2020, *Nature Methods* highlighted "Spatially Resolved Transcriptomics" as the method of the year[1]. However, existing platforms for spatial biology are highly heterogeneous. For example, single-cell proteomic assays, such as cyclic immunofluorescence, CODEX, molecular ion beam imaging (MIBI), and imaging mass cytometry (IMC) are capable of cellular, or even sub-cellular, regional analysis but are limited to joint profiling of tens to hundreds of preselected proteins. Likewise, commercially available platforms for profiling single-cell mRNA expression in spatial dimensions, such as MERSCOPE (Vizgen) and CosMx (NanoString), are limited to preselected genes. In contrast, Visium (10x Genomics) and GeoMX (NanoString) can recover the entire transcriptome, but at lower spatial resolution. Clearly, such differences, along with the complexity of the data generated by each assay, require sophisticated analytical solutions. Moreover, while current platforms are predominantly limited to two-dimensional profiles, 3D, 4D (spatiotemporal), and even multiomic analysis capabilities are on the horizon, driving the need for increasingly powerful and scalable computational methods.

Previous PSB workshops have emphasized the importance of translational bioinformatics and precision medicine, however none have focused on the computational and analytical challenges underpinning spatial transcriptomics and proteomics. In this workshop, we will explore and highlight recent advances in this burgeoning arena, with an emphasis on cancer. As one of the major beneficiaries of spatial profiling technologies, cancer research has advanced considerably in recent years through meticulous cell atlasing and spatial profiling efforts[2-13]. For example, using MIBI to analyze 36 proteins in 41 triple negative breast cancers, Keren et al.[6] identified immune-mixed and immune-compartmentalized tumors. In the latter, the immunoregulatory protein PD1 was generally expressed on CD4 T cells, whereas in the former, PD1 was largely expressed on CD8 T cells. Moreover, compartmentalized tumors showed distinct immune structures at the tumor boundary that predicted longer survival time. These findings offer potential insights into why PD1 expression is not a reliable biomarker for response to immune checkpoint inhibition.

This workshop will cover computational aspects of multiplexed imaging, spatial transcriptomics, and platform integration (e.g., alignment of single-cell and spatial transcriptomics), with an emphasis on basic and translational cancer research. Our goal is to stimulate new ideas, foster critical debate, and form new collaborations in this exciting and challenging research area.

2. Speaker Abstracts

Atlas of clinically distinct cell states and ecosystems across human solid tumors

Andrew J. Gentles

Tumors are complex ecosystems consisting of malignant, immune, and stromal elements whose dynamic interactions drive patient survival and response to therapy. A comprehensive understanding of the diversity of cellular states within the tumor microenvironment (TME), and their patterns of co-occurrence, could provide new diagnostic tools for improved disease management and novel targets for therapeutic intervention. To address this challenge, we

developed EcoTyper, a novel machine learning framework for large-scale identification of TME cell states and their co-association patterns from bulk, single-cell, and spatially resolved tumor expression data. Applied to over 6k tumor and adjacent normal samples from solid tumor types profiled by The Cancer Genome Atlas (TCGA), EcoTyper identified robust transcriptional states across 12 major cell types, including epithelial, fibroblast, endothelial, and 9 immune subsets. These states included both known and novel cellular phenotypes, nearly all of which could be validated in a compendium of scRNA-seq tumor atlases. For example, EcoTyper recapitulated the transcriptional profiles of M1 and M2 polarized macrophages, along with 7 other macrophage states. Most cell states were specific to neoplastic tissue, ubiquitous across tumor types, and significantly associated with overall survival, both in TCGA and in over 10k held-out tumor specimens. We found that specific cell states co-occur in distinct cellular communities with characteristic patterns of ligand-receptor interactions, genomic features, clinical outcomes, and spatial organization. One such ecosystem defined a normal-like state that was strongly enriched in non-malignant samples. Others delineated novel pro- and anti-tumor inflammatory environments involving specific fibroblast, endothelial, and immune cell transcriptional programs. In summary, large-scale deconvolution of cell type-specific transcriptomes across thousands of solid tumors revealed a comprehensive atlas of TME cell states and cellular ecosystems. Our results provide a high-resolution portrait of cellular heterogeneity in the TME across multiple solid tumor types, with implications for novel diagnostics and immunotherapeutic targets.

The spatial landscape of progression and immunoediting in primary melanoma at single-cell resolution

Ajit J. Nirmal

Cutaneous melanoma is a highly immunogenic malignancy, surgically curable at early stages, but life-threatening when metastatic. The spatial organization of the tumor ecosystem during early-stage melanoma is not well understood. Here we integrate high-plex imaging, 3D high-resolution microscopy, and spatially resolved micro-region transcriptomics to study immune evasion and immunoediting in primary melanoma. We collected highly multiplexed single-cell data from 70 distinct histological regions from 13 specimens (patients) selected to have multiple progression-associated histologies within a single resection. These histologies range from pre-malignant fields in which melanocytic atypia represents the first steps in cancer initiation to non-invasive (radial growth phase) and invasive (vertical growth phase) primary melanoma that eventually gives rise to disseminated disease. We find that recurrent cellular neighborhoods involving tumor, immune, and stromal cells change significantly along a progression axis involving precursor states, melanoma in situ, and primary invasive tumor. Hallmarks of immunosuppression were detectable as early as the melanoma precursor stage, and when tumors become locally invasive, a consolidated and spatially restricted environment with multiple overlapping immunosuppressive mechanisms forms along the tumor-stromal boundary. This environment is established by cytokine gradients that promote expression of MHC-II and IDO1 and by PDL1-expressing macrophages and dendritic cells engaging activated T cells. However, only a few millimeters away, T cells synapse with melanoma cells in fields of tumor regression. Thus, invasion and immunoediting can co-exist within a few millimeters of each other in a single specimen. Multiplexed single-cell imaging and micro-region mRNA profiling link morphological and molecular features of tumor evolution within and across primary cancer specimens, revealing highly localized programs of immune and tumor cell communication via paracrine cytokine signaling and direct cell-cell contact.

Systems approach to target tumor ecosystem responses for therapeutic benefit
Laura M. Heiser
Breast tumors arise and progress via processes that involve intrinsic deregulation of epithelial cells and that also alter the composition and function of associated stromal and immune cells. Together, these tumor-intrinsic and microenvironmental changes enable malignant epithelial cells in the tumor to acquire key cancer hallmarks, including proliferation, migration, immune evasion and further evolution. The resulting collection of cancer and stromal cells comprise a complex, adaptive tumor ecosystem. Dr Heiser will discuss how multiple tissue imaging was used to test the hypothesis that treatment strategies designed to simultaneously attack cancer cell state vulnerabilities and promote anti-tumor microenvironments may lead to deeper therapeutic responses in patients. To examine therapeutic responses of diverse aspects of the tumor ecosystem, they deployed a novel drug delivery microdevice that enables rapid, high-throughput assessment of the effects of multiple therapies on tumor cells and the surrounding microenvironment. When coupled with multiplex tissue imaging, this platform provides a comprehensive assessment of the state and spatial organization of the tumor ecosystem as it adapts to therapy. These studies demonstrated that many drugs designed to target malignant epithelial cells strongly impact stromal and immune cells, providing new insights into the importance of considering multiple aspects of the tumor ecosystem when designing effective therapeutic strategies. Together, this integrated experimental-computational approaches have provided insights into adaptive responses of diverse components of the tumor ecosystem that can be targeted to improve therapeutic responses.

Mapping the spatiotemporal proteome architecture of human cells
Emma Lundberg
Biological systems are functionally defined by the nature, amount, and spatial location of the totality of their proteins. We have generated an image-based map of the subcellular distribution of the human proteome, showing that there is great complexity to the subcellular organization of the cell. As much as half of all proteins localize to multiple compartments, giving rise to potential pleiotropic effects, and around 20% of the human proteome shows spatiotemporal variability. Their temporal mapping results shows that cell cycle progression explains less than half of all temporal protein variability, and that most cycling proteins are regulated post-translationally, rather than by transcriptomic cycling. This work is critically dependent on computational image analysis, and we will discuss machine learning approaches for classification of spatial subcellular patterns and how such embeddings can be used to build multi-scale models of cell architecture. We will also demonstrate the importance of spatial proteomics data for improved single cell biology and present how the freely available Human Protein Atlas database (www.proteinatlas.org) can be used as a resource for life science.

Robust alignment of single cell and spatial transcriptomes with CytoSPACE
Aaron M. Newman
Spatial transcriptomics is a powerful tool for delineating spatial gene expression in primary tissue specimens. However, commonly used platforms such as 10x Visium currently rely on bulk gene expression measurements, whereas single-cell spatial expression platforms such as Vizgen MERSCOPE have low gene recovery. To overcome these challenges, we developed CytoSPACE, a robust and efficient computational method for optimally aligning single-cell and spatial transcriptomes into a reconstructed tissue specimen at single-cell resolution. Across multiple

benchmarking experiments, CytoSPACE outperforms previous methods with respect to noise tolerance and accuracy. Using diverse examples spanning mouse brain regions, mouse kidney, and human tumors, we illustrate the ability and versatility of CytoSPACE to enable exciting new discoveries that are not obtainable from competing methods or from scRNA-seq or spatial platforms alone.

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