6774: Development of a Spatial Metabolic Map of Immunotherapy Sensitive and Resistant Cutaneous Skin Carcinoma

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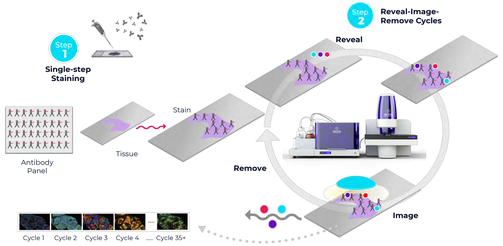
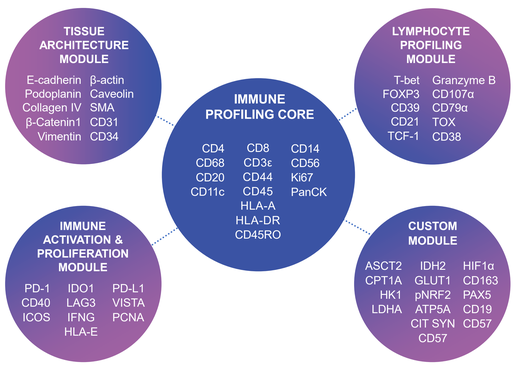
1. Ultrahigh-Plex Spatial Phenotyping

Ultrahigh-Plex Single-cell Spatial Phenotyping has revealed valuable insights into the tumor immune microenvironment (TiME) for biomarker discovery and stratification of clinical responses. Metabolic reprogramming is a key hallmark of cancer and plays an important role in tumor progression, immune activation and metastasis. Here, we investigated the metabolic and immune landscape of cutaneous squamous cell carcinoma (cSCC) with the **PhenoCycler®-Fusion**, an end-to-end integrated spatial biology platform with single-cell resolution and rapid turnaround time.



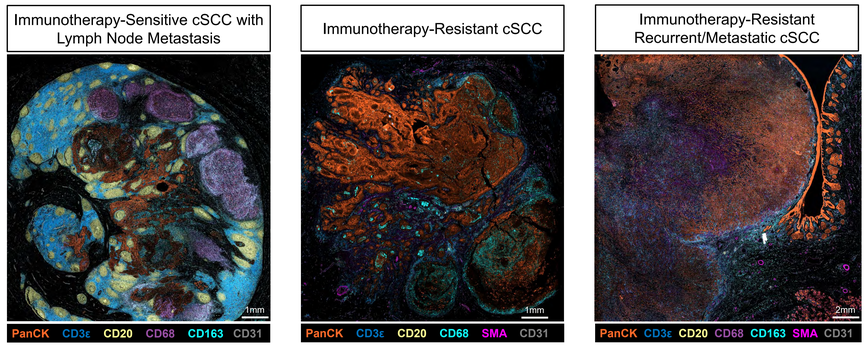
2. PhenoCode™ Discovery Panels

Cutaneous squamous cell carcinoma is the second most common non-melanoma skin cancer. Though prognoses are favorable in most cases, locally advanced and metastatic forms present an emerging health burden. Immunotherapy is a promising solution, however, resistance to immune checkpoint inhibitors (ICI) warrants a deeper investigation into the regulation of the immune response. To that end, we deployed ready-to-use **PhenoCode™ Discovery Panels** to investigate *immune cell lineages, activation states, checkpoints* as well as *tissue structure* in the TiME. In addition, we developed a custom antibody module containing markers of *cellular metabolism* to further elucidate the metabolic regulation of the TiME in three cSCC cases: immune-competent with lymph node metastasis, immune-compromised/resistant and recurrent/metastatic.

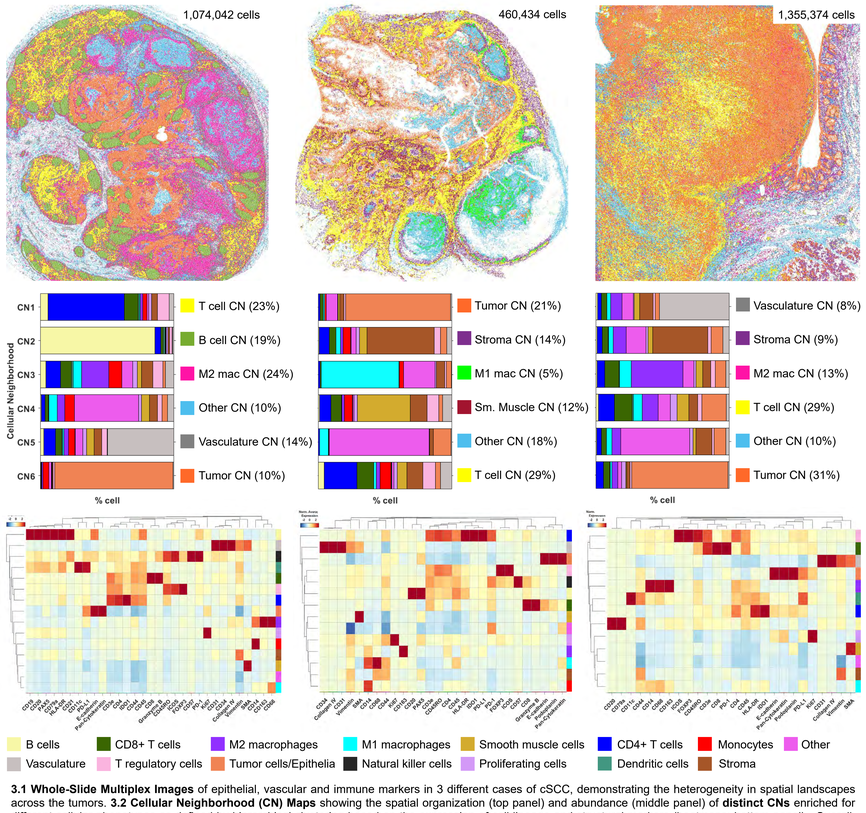


3. Cellular Neighborhood Analyses Reveal Diverse Tumor Immune Microenvironments in Cutaneous Squamous Cell Carcinoma

3.1 Whole-Slide Spatial Landscapes of Immunotherapy Sensitive and Resistant cSCC



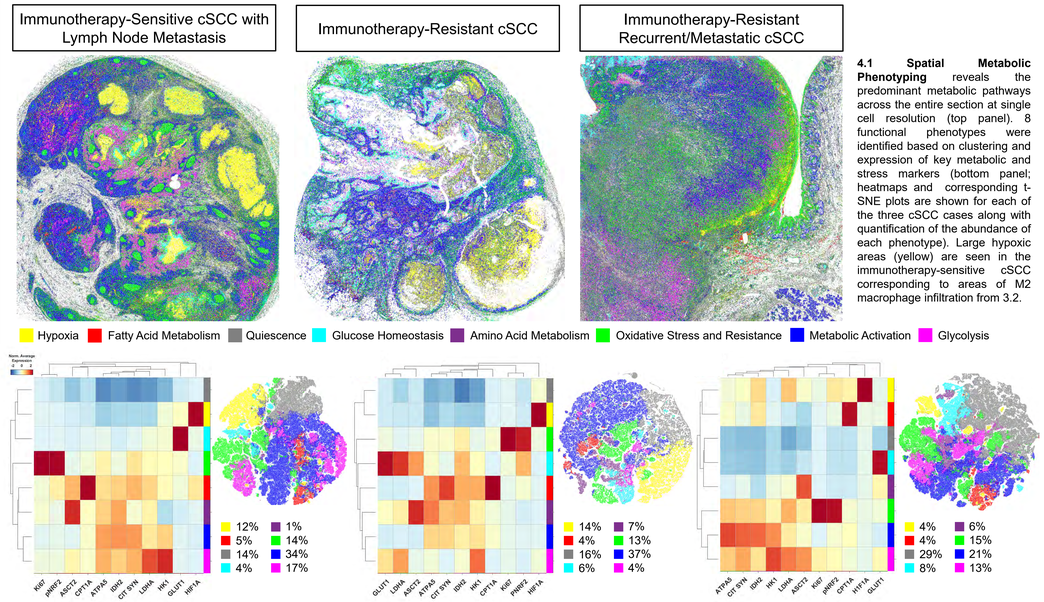
3.2 Cellular Neighborhood Analyses Reveals Differences in Immune Composition and Organization



3.1 Whole-Slide Multiplex Images of epithelial, vascular and immune markers in 3 different cases of cSCC, demonstrating the heterogeneity in spatial landscapes across the tumors. **3.2 Cellular Neighborhood (CN) Maps** showing the spatial organization (top panel) and abundance (middle panel) of **distinct CNs** enriched for different cellular phenotypes as defined by hierarchical clustering based on the expression of cell lineage and structural markers (heatmaps; bottom panel). Overall, the data show an **immune-competent TME** in the immunotherapy-sensitive cSCC and an **immune-compromised TME** in the 2 immunotherapy-resistant cSCCs.

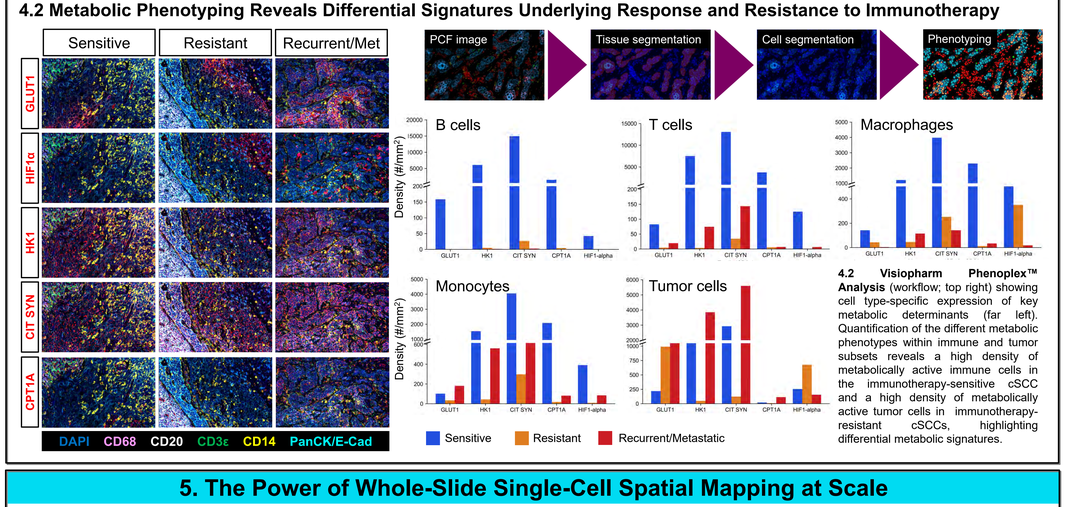
4. Whole-Slide Spatial Metabolic Mapping Combined with Tumor-Immune Phenotyping Reveals Distinct Metabolic Signatures of Response and Resistance

4.1 Spatial Metabolic Phenotyping: Going beyond the Tumor Immune Microenvironment of cSCC



4.1 Spatial Metabolic Phenotyping reveals the predominant metabolic pathways across the entire section at single cell resolution (top panel). 8 functional phenotypes were identified based on clustering and expression of key metabolic and stress markers (bottom panel; heatmaps and corresponding t-SNE plots are shown for each of the three cSCC cases along with quantification of the abundance of each phenotype). Large hypoxic areas (yellow) are seen in the immunotherapy-sensitive cSCC corresponding to areas of M2 macrophage infiltration from 3.2.

4.2 Metabolic Phenotyping Reveals Differential Signatures Underlying Response and Resistance to Immunotherapy



4.2 Visiopharm Phenoplex™ Analysis (workflow; top right) showing cell type-specific expression of key metabolic determinants (far left). Quantification of the different metabolic phenotypes within immune and tumor subsets reveals a high density of metabolically active immune cells in the immunotherapy-sensitive cSCC and a high density of metabolically active tumor cells in immunotherapy-resistant cSCCs, highlighting differential metabolic signatures.

5. The Power of Whole-Slide Single-Cell Spatial Mapping at Scale

This study demonstrates the value of rapid, deep single-cell spatial phenotyping enabled by the PhenoCycler-Fusion system and the PhenoCode Discovery Panels for a comprehensive analysis of the TiME and metabolome. Identifying metabolic signatures in tumor and immune subsets will be crucial to elucidate the pathogenesis of the disease and reveal mechanistic insights underlying clinical response and resistance.

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