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\textsuperscript{TM}-Fusion, an end-to-end integrated spatial biology platform with single-cell resolution and rapid turnaround time.

2. PhenoCode\textsuperscript{TM} 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\textsuperscript{TM} 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

Immunotherapy-Sensitive cSCC

Immunotherapy-Resistant cSCC

3.2 Cellular Neighborhood Analyses Reveals Differences in Immune Composition and Organization

3.3 Whole-Slide Multiplex Images of optimal, vascular and immune markers in three different cases of cSCC, demonstrating the heterogeneity in spatial landscapes across the lesions. 3.3 Cellular Neighborhood (369 Maps) showing the spatial organization of the panel and downstream panels of plates fixed by TAD (www.visiopharm.com). Heatmaps of the TAD dye are shown in the middle panel. The data show that immune-competent TME in the immunotherapy-sensitive cSCC and an immune-competent resistant TME in the immunotherapy-resistant cSCC.

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

Immunotherapy-Sensitive cSCC

Immunotherapy-Resistant cSCC

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

4.3 Metabolic Signatures Associated with Response and Resistance

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 metabolism. 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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