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SPATIAL ANALYSIS OF THE TUMOR MICROENVIRONMENT USING MACHINE LEARNING-ENABLED INTEGRATED MORPHOLOGY-TRANSCRIPTOMIC CELL PHENOTYPES

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Background In recent years, immunotherapy has advanced the cancer field by achieving more durable patient response with a relatively tolerable toxicity. However, the overall response rates remain low (< 20%),¹ mainly due to a lack of understanding of the underlying tumor-immune interactions that determine patient responsiveness. Existing tissue-based assays are restricted to dozens of known transcriptomic or proteomic markers, limiting their usefulness in generating new immunological insights. While recently emerged spatial transcriptomics (ST) technology enables an unbiased whole-transcriptomic profiling, it does not provide single-cell resolution read-outs,² leading to potential masking of rare cell signals and loss of cell-to-cell spatial information. Here, we propose a machine learning (ML) approach to characterize single-cell phenotypic traits, through integrating haematoxylin and eosin (H&E) histology and ST data.

Methods One tumor and one adjacent-normal tissue section collected from a hepatocellular carcinoma (HCC) patient were profiled using 10× Visium ST platform. Using the companion H&E image, tissue regions were categorized as tumor epithelium and stroma, and individual cells were segmented (StarDist) with 53 histological features extracted (QuPath v0.3.2). Unsupervised cell clustering and cluster-specific gene signatures were determined simultaneously whereby genetic algorithm was used for feature selection and gene signatures were obtained by deconvoluting ST. Optimal clustering results were determined through maximizing clustering quality and cell-type specificity for individual clusters, where single-sample gene set enrichment on PanglaoDB database was used for cell-type annotation.

Results More immune cells infiltrated into epithelium in the adjacent-normal tissue (18.2% of total 17,480 stromal cells) as compared to that of the tumor tissue (11.6% of total 10,807 stromal cells). Cell clusters with strong T-cell and B-cell signatures were detected in both tissues (figure 1), with higher tumor infiltration in the adjacent-normal tissue (~16% of the total T or B cells, respectively; figure 2) than in the tumor tissue (~5% of the total T or B cells, respectively). Despite the same morphological feature, i.e., nucleus-to-cell area ratio, was selected in both tissues, comparable values were observed in T and B-cells (~0.56) within the adjacent-normal tissue, whereas an average higher area ratio of B-cells (~0.52) than T-cells (~0.47) within the tumor tissue (figure 3).

Conclusions Our data suggests that, during tumorigenesis, T-cells might undergo morphological changes, and reduced T and B-cells infiltration into the epithelium. Our proposed ML approach not only allows single-cell spatial analysis of tumor-immune interactions, but also provides more refined cell phenotypes defined by both morphology and transcriptomic features.

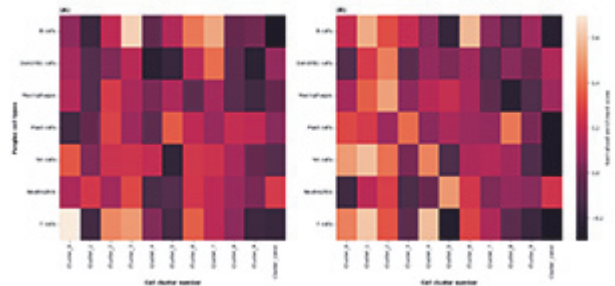
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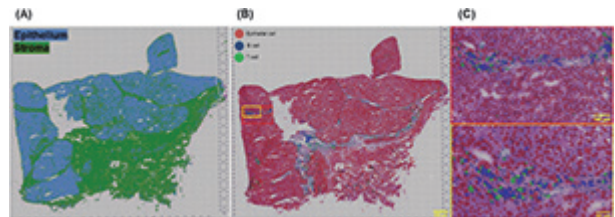
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Ethics Approval This study was approved by the SingHealth Centralized Institutional Review Board (reference numbers: 2018/3045 and 2019/2653)

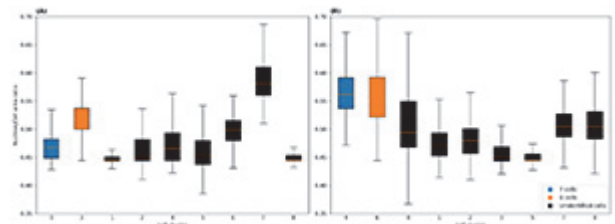
Consent The patients provided their written informed consent to participate in this study.



Abstract 1302 Figure 1 Heat maps of single-sample gene set enrichment (ssGSEA) scores of cell clusters identified at the optimal setting. (A) In the HCC tumor tissue, cluster 0 and cluster 3 demonstrated strong T-cell and B-cell signatures, respectively. (B) in the HCC adjacent-normal tissue, cluster 4 and cluster 6 demonstrated moderate T-cell and B-cell signatures, respectively. Other clusters demonstrated mixed signatures of multiple cell-types



Abstract 1302 Figure 2 Spatial localization of morphology-transcriptome-defined T cells, B cells and epithelial cells in the HCC adjacent-normal tissue. (A) ML-classified epithelial and stromal regions. (B-C) Cellular location of the epithelial, T, and B cells within the tissue space



Abstract 1302 Figure 3 Distribution of morphological feature, nucleus-to-cell area ratio, across the identified cell clusters in (A) the tumor tissue, and (B) adjacent-normal tissue

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