964 **SINGLE-CELL SPATIAL CHARACTERIZATION OF MICROENVIRONMENTAL IMPACTS ON IMMUNOTHERAPY DISCREPANCIES BETWEEN ADULT AND PEDIATRIC NASOPHARYNGEAL CARCINOMA PATIENTS**

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Background Nasopharyngeal carcinoma (NPC) is an EBV-related and highly inflamed malignancy of strategic importance in Asia and Africa. Only 0.1–1% NPC incidences are diagnosed in children, and pediatric NPC exhibits superior prognosis and immunotherapy outcomes. Microenvironmental characteristics might contribute to such discrepancies between adult and pediatric NPC. Thus, we apply scRNA-seq and Visium spatial RNA-seq to primary pediatric NPC with paired blood, and incorporate our data with multi-central NPC cohorts. We develop a computational framework to infer spatial cellular constitutes and signaling. We report low lipid metabolism, weak interaction between NPC cells and Tregs/CD8+ T cells, and enriched T memory stem cells (Tscm) collectively result in stronger and long-lasting immunity in pediatric NPC and as new immunotherapeutic vulnerabilities (figure 1).

Methods We applied scRNA-seq to 5 primary pediatric NPC tissues with paired blood samples, and spatial-seq to 4 paired pediatric and 7 adult NPC tissues. We established a multi-central NPC cohort with 69 patient samples by integrating our scRNA-seq data with three adult NPC cohorts.[1–3] A personalized pipeline incorporated with Seurat,[4] Spacexr[5] and SpaCET[6] was used to analyze single-cell spatial data. Key findings were validated by multiplex staining, flow cytometry, and blood tests on patient samples.

Results We established a systematic NPC scRNA-seq and spatial-seq cohort containing 503,021 cells from 69 samples, and 15,222 spatial spots from 11 samples. We developed a personalized computational framework to characterize spatial co-localization and regionally enriched signaling. We unveiled that fatty acid (FA)/cholesterol metabolism were elevated in the tumor-T cell core in adult NPC ($p=2.12e-5$), and such metabolic aberrance was validated by Oil Red O staining and blood cholesterol/FA tests ($p=0.012$). Strong co-localizations between NPC cells and Tregs ($r=0.47$, $p<2.2e-22$)/exhausted T cells ($r=0.29$, $p<2.2e-22$) were found in adult NPC, with spatially enriched CD70-CD27, LGALS9-TIM3 and Nectin-4 interactions. This finding was corroborated by multiplex staining of KRT19+ NPC cells, CD4+/FOXP3+ Tregs, and CD8+/PD-1/TIM-3+ exhausted T cells. We identified novel IL7R+/ANXA1+ Tscm populations in pediatric NPC, with strong resilience to immunosuppressive cues, including TGF-β, PEG2 and adenosine, and had a positive impact on long-term immunity and immunotherapeutic outcomes ($p=7.49e-9$).

Conclusions We demonstrate that higher immunosuppression and exhaustion caused by lipid metabolism and tumor-intrinsic interactions, and a lower age-associated Tscm pool, are the vital drivers of immunotherapeutic discrepancies in adult and pediatric NPC patients. Pre-treatment screening of Tscm abundance in NPC patients might help stratify immunotherapy responders, and targeting metabolic and interacting vulnerabilities might generate therapeutic benefits.

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Ethics Approval The study was approved by the ethics committee at the University of Hong Kong. We complied with all related ethical regulations. Written informed consent was obtained from adult and pediatric NPC patients for their tissues and blood samples to be used in the study.

Consent Written informed consent was obtained from adult and pediatric NPC patients for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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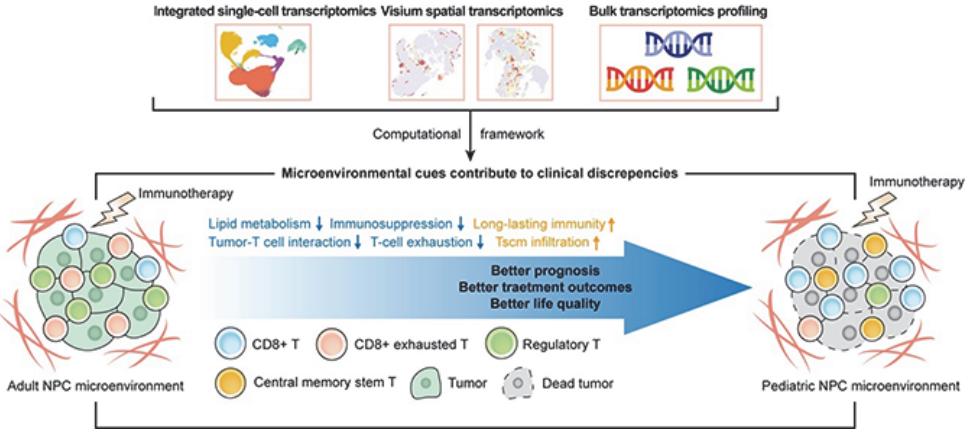

Abstract 964 Figure 1