The Tumour Immune Microenvironment of Recurrent/Metastatic Nasopharyngeal Carcinoma

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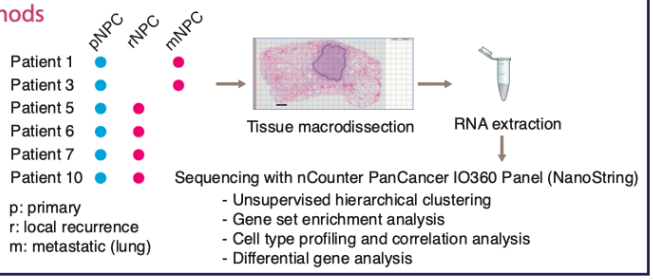
Introduction

The management of recurrent/metastatic (RM) NPC remains a challenge. Over the last decade, targeted and immune therapies have emerged as promising treatment modalities for RM NPC. Particularly, anti-PD1 therapies have demonstrated encouraging effects in multiple clinical trials. However, PD-1-based therapies are limited by patient-specific responsiveness and adaptive resistance after long-term use. To improve the response rate, one approach is to introduce adjunct therapies targeting other dysregulated pathways in RM NPC. However, what contributes to the refractoriness of RM NPC remains to be elucidated.

In this study, we characterised pertinent features of tumour cells, tumour stroma and tumour-infiltrating immune cells in paired primary and RM NPC samples by targeted mRNA sequencing. We found that RM NPC showed significant enrichment of immune cell-related signatures. This was associated with an altered immune cell composition, with enhanced T cell activation and exhaustion coupled with an M2-skewed macrophage population. Together, these data depict a more inflamed yet immunosuppressive microenvironment of RM NPC and highlight macrophages as a potential target to reinvigorate antitumoural immune responses.

Methods



1. pNPC and RM NPC show distinct gene expression profiles

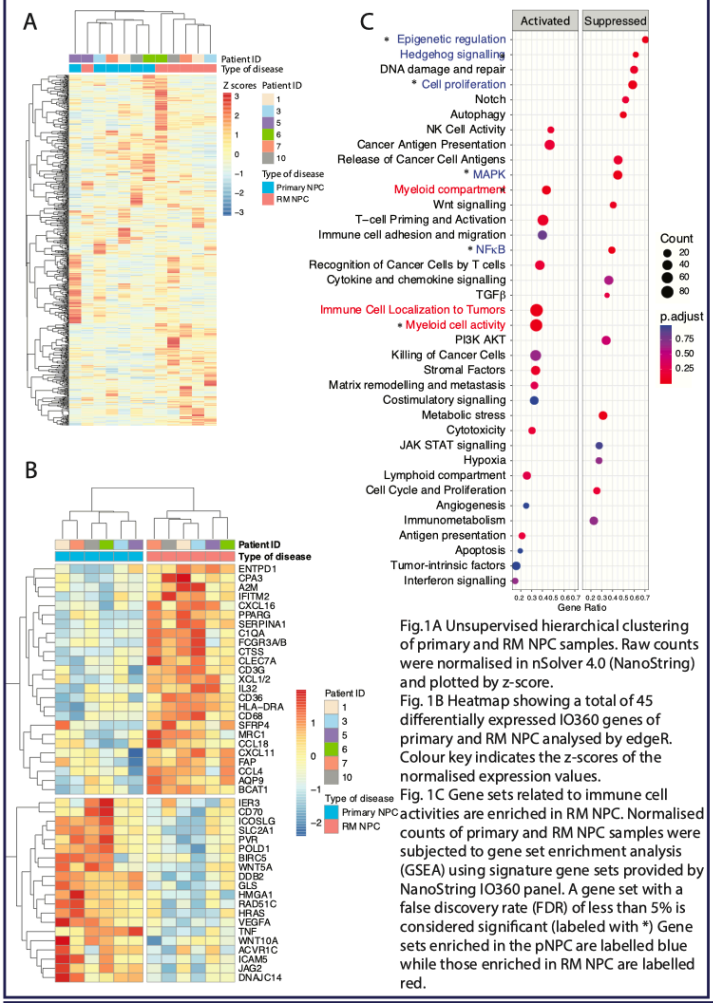


Fig.1A Unsupervised hierarchical clustering of primary and RM NPC samples. Raw counts were normalised in nSolver 4.0 (NanoString) and plotted by z-score.

Fig. 1B Heatmap showing a total of 45 differentially expressed IO360 genes of primary and RM NPC analysed by edgeR. Colour key indicates the z-scores of the normalised expression values.

Fig. 1C Gene sets related to immune cell activities are enriched in RM NPC. Normalised counts of primary and RM NPC samples were subjected to gene set enrichment analysis (GSEA) using signature gene sets provided by NanoString IO360 panel. A gene set with a false discovery rate (FDR) of less than 5% is considered significant (labeled with *) Gene sets enriched in the pNPC are labelled blue while those enriched in RM NPC are labelled red.

2. RM NPC samples exhibit an altered tumour-infiltrating immune cell compositions

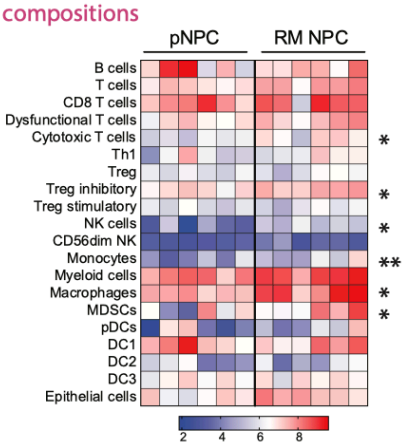


Fig.2 Heatmap showing abundance of 20 types of cells. Cell abundance was represented by the average expression level of sigtnature genes of the indicated cell type. Expression level was indicated by the log2 transformed normalised count. Signature genes were obtained from the nCounter IO360 gene panel and the single cell sequencing data of NPC samples by Chen et al, 2020. Cell types with significant enrichment in RM NPC were labelled with * (p<0.05) or ** (p<0.01, paired t-test).

3. T cell dysfunction is associated with the abundance of macrophages

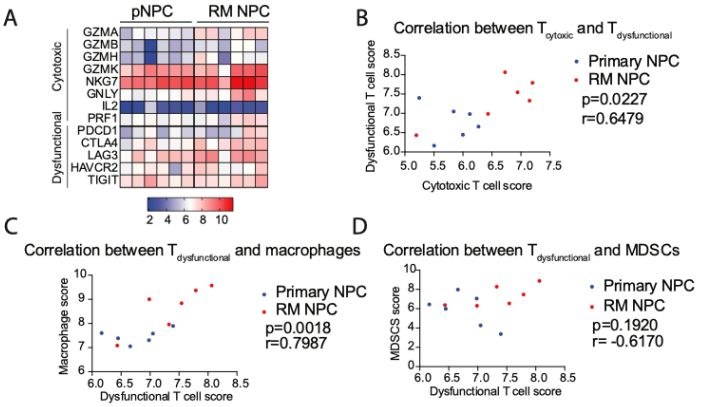


Fig. 3A Heatmap showing the logCPM values of cytotoxic and dysfunctional T cell markers in primary and RM NPC.

Fig. 3B Correlation analysis of meta gene expression levels of cytotoxic T cells and dysfunctional T cells.

Fig. 4C&D Correlation analysis of meta gene expression levels of dysfunctional T cells and macrophages (C) and myeloid-derived suppressor cells (MDSCs) (D).

4. Macrophages in RM NPC adopt an M2-skewed phenotype

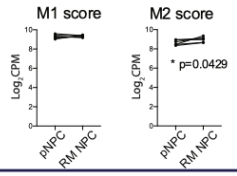
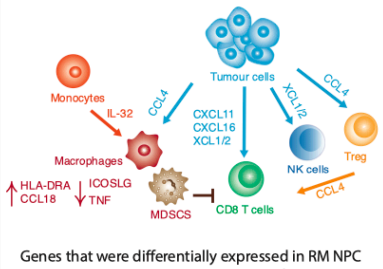


Fig. 4 M1 and M2 macrophage signature gene sets were obtained from the study by Azizi et al (2018). M1 and M2 macrophage scores were calculated by the mean expression level of genes in the corresponding signature gene set. The expression level of each gene was calculated by the mean of log2 transformed counts per million (CPM).

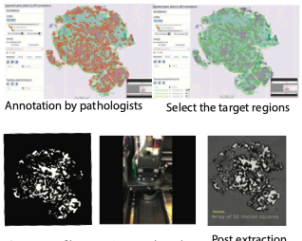
Schema of immunosuppression mechanisms observed in RM NPC



Genes that were differentially expressed in RM NPC (FDR<0.05, Log2FC>1 or <-1) were used to annotate immune cell functions based on previous findings including single cell studies of NPC samples (Zhao et al, 2020; Liu et al, 2020; Chen et al, 2020; Gong et al, 2021; Tay et al, 2022).

Future directions

To identify the driving force of the enhanced immunosuppression in RM NPC by analysing the expression profiles of tumour and immune cells separately



Workflow of tissue microdissection with QuantumCyte CytoMask technology

QuantumCyte

References

Azizi, Elham, et al. "Single-cell map of diverse immune phenotypes in the breast tumor microenvironment." Cell 174.5 (2018): 1293-1308.
Liu, Yang, et al. "Tumour heterogeneity and intercellular networks of nasopharyngeal carcinoma at single cell resolution." Nature communications 12.1 (2021): 1-18; Chen, Yu-Pei, et al. "Single-cell transcriptomics reveals regulators underlying immune cell diversity and immune subtypes associated with prognosis in nasopharyngeal carcinoma." Cell research 30.11 (2020): 1024-1042; Gong, Lanqi, et al. "Comprehensive single-cell sequencing reveals the stromal dynamics and tumor-specific characteristics in the microenvironment of nasopharyngeal carcinoma." Nature communications 12.1 (2021): 1-18; Tay, Joshua K., et al. "The microdissected gene expression landscape of nasopharyngeal cancer reveals vulnerabilities in FGF and noncanonical NF-κB signaling." Science Advances 8.14 (2022): eabh2445; Zhao, Jin, et al. "Single cell RNA-seq reveals the landscape of tumor and infiltrating immune cells in nasopharyngeal carcinoma." Cancer letters 477 (2020): 131-143.