

EXPLORING THE SPATIAL HETEROGENEITY OF IMMUNE CELLS IN NASOPHARYNGEAL TUMOURS

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Background Nasopharyngeal cancer (NPC) is a squamous cell carcinoma of the upper pharynx, strongly associated with Epstein-Barr virus (EBV), and with varying incidences in the world. We have previously investigated the presence and distribution of CD8⁺ T cells in NPC and found that an ‘inflamed profile’, with CD8⁺ T cells in and around cancer cell areas, was associated with better disease-free survival (*cf.* non-inflamed ‘desert’ tumours). In this study, we explore immune-related biomarkers in a spatial context to assess distribution of cell types and quantify selected biomarkers within the NPC.

Methods Using the GeoMx Digital Spatial Profiler (DSP), 49 target proteins were digitally quantified on an NPC tissue microarray consisting of 30 unique biopsies. DSP combines spatial and molecular profiling technologies and enable assessment of different features of intratumoral structures in a cell type-specific manner while simultaneously performing protein expression analysis on selected regions-of-interest (ROIs). A total of 96 ROIs were obtained based on morphological staining using CD45, CD8, and PanCK antibodies, and a quantitative analysis was performed between ROIs within the tumour area and outside/stroma.

Results To evaluate unbiased grouping of segments, principal component analysis and k-means clustering were conducted. Protein targets CD56, CD20, CD68, PD-1, FOXP3, and Ki-67 were the most differentially expressed in the CD45⁺ segments within the tumour area, suggesting a presence of B cells, NK cells, macrophages, and regulatory T cells in the tumour area. In contrast, B7-H3, fibronectin, CD163, CD4, VISTA, LAG3, and TIM3 were higher in the CD45⁺ segments outside the tumour barrier, indicating a presence of suppressive populations of myeloid cells and T cells. Additionally, the expression of B7-H3 correlated best with a presence of CD14, fibronectin, and CD163. Interestingly, the presence of PD-1 was limited to within tumour-rich regions, in contrast to other ‘immune regulatory’ proteins like LAG3, VISTA, and TIM3, which were found on the tumour border and in the stroma. The detected proteins were validated with multiplexed staining.

Conclusions This study considers the spatial heterogeneity of the NPC immune microenvironment and demonstrates a variable expression of immune checkpoint markers in NPC. The identified immune profiles and highlighted biomarkers may be of relevance as a prognostic tool and for therapeutic targeting.

Ethics Approval The collection of biobank samples from Lund University was approved by ethics committee Dnr 2014/117.

Consent In accordance with the ethics approval, informed consent was not required, but the study was advertised in print media with the option to opt-out

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