Ultra highplex spatial proteomic and transcriptomic analysis of the head and neck cancer tumour microenvironment

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Introduction

Head and neck cancers are the 6*th* most common tumour type globally and account for ~900,000 new cases a year and 450,000 deaths. Arising from multiple anatomical sites, such as the oral cavity, pharynx, and larynx, HNSCC tumours present complex tumour microenvironments. In this study, we sought to understand the tumour and stromal properties of tongue, lip, oral cavity and pharyngeal cancers using cutting-edge, high-dimensional spatial tools.

We profiled a tissue microarray consisting of 85 patient biopsy samples, sampled from the tongue (n=15), oral cavity (n=15), pharynx (n=28), and lip (n=27) using the Nanostring Technologies IO Proteome Atlas (570-plex) to liberate tumour- and stromal- compartment specific proteins associated with clinical endpoints, DFS (disease free survival) and OS (overall survival).

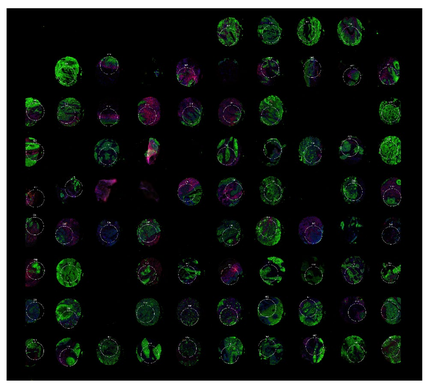

Figure 1. TMA stained for (green) panCK, (red) CD45 and (blue) DAPI, with selected ROIs.

In this cohort study, tongue cancers had the worst overall survival when compared with lip and oral cavity cancers, which had a more favourable prognosis. Differential expression of tumour/stroma-compartments between tongue and lip cancers indicated lower expression of EpCAM, Cytokeratin 19 and CA9 in the lip tumour compartment while the tongue microenvironment has higher expression of fibronectin, alpha smooth muscle actin, osteopontin and proteins associated with higher metastatic potential. When comparing between tongue and pharyngeal samples, a higher expression of PD-L1 proteins and lower histone modifications were found in the tongue. In patients with a better prognosis, Wnt associated SFRP elevated in the tumour microenvironment, while ISG15 proteins associated with poorer prognosis in the tumour compartment.

Further looking at the individual cancers based on prognosis outcomes, proteins like S100(s), IFIT1, ISG15 and histone H3 and H2B modifications are noted to be elevate in patients with poor prognosis. MMP8 was found to be elevated in patients with good prognosis for tongue cancers.

Methods

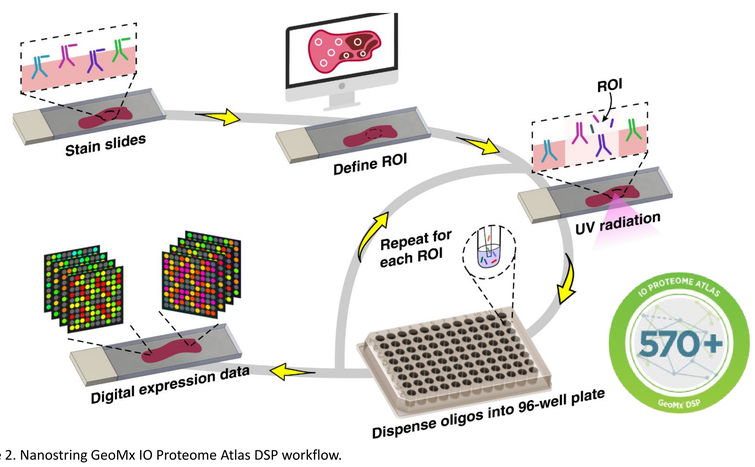

Figure 2. Nanostring GeoMx IO Proteome Atlas DSP workflow.

ROIs were selected based on and sampled in accordance with the Nanostring GeoMx IO proteome workflow and analysis suite. The resulting GeoMx IPA data were processed and QC-ed using a customized spatial profiling workflow based on the R package standR [1]. Data interrogations were conducted using functions available in standR including RLE and PCA analyses. Differential expression was conducted using the voom-limma with duplicate correlation using the edgeR and limma R packages. Survival analyses were conducted using survminer, survival and ggsurvfit packages. Overall and disease-free survival groupings are determined based on extreme quantiles and death/dfs event status.

Results

Survival Analysis

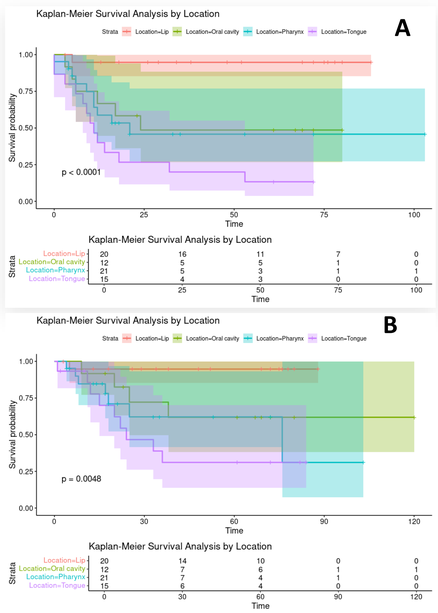

Figure 3. Kaplan-Meier survival curves analyzing (A) disease free survival or (B) overall survival across different cancer locations: lip, oral cavity, and tongue : In this cohort study, tongue cancers had the worst overall survival when compared with lip and oral cavity cancers

Differential Expression Analysis (Cancer subtype/location)

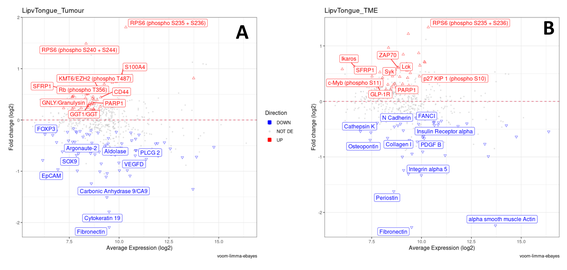

Figure 4. MA plots for differential expression analyses results of either the (A) tumour or (B) stroma compartments between lip and tongue cancers indicated comparatively lower expression of EpCAM (associated with aggressive phenotype of tongue cancer), Cytokeratin 19 and CA9 (tumour markers) in the lip tumour compartments. In the tumour microenvironment (TME), there is a lower expression of fibronectin, alpha smooth muscle actin, osteopontin and proteins associated with higher metastatic potential in the lip.

Figure 5. MA plot for differential expression analysis of the tumour compartments between Pharynx and tongue cancers suggest a higher expression of PD-L1 (associated with early-stage tongue cancers) in the tongue and conversely higher expressions of histone modifications (associated with oral carcinogenesis and aggressive pheonotype) in the pharynx.

Differential Expression Analysis (Overall survival groups and Compartments)

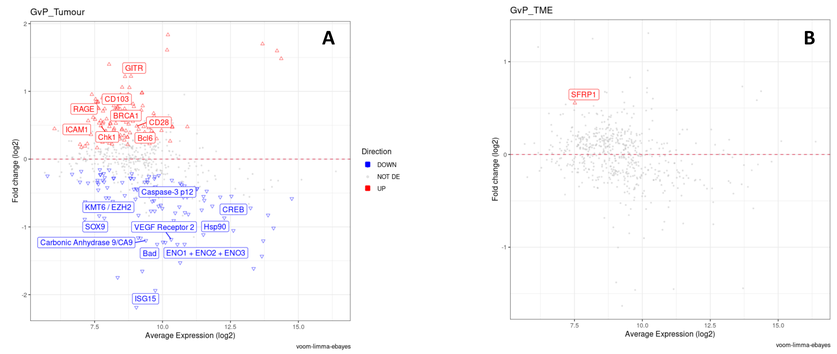

Figure 6. MA plots for differential expression analysis of all samples in either the (A) tumour or (B) Stroma compartments between patients with good or poor prognosis. (A) Higher expression of interferon stimulated gene – ISG15 protein in the tumour of the poor patients. ISG15 found to be elevated in 80% of oral carcinoma. (B) Higher expression of Wnt related SFRP protein (inactivation of which is linked with oral carcinoma) in patients with good prognosis in the microenvironment.

Differential Expression Analysis (Overall survival groups and cancer subtypes)

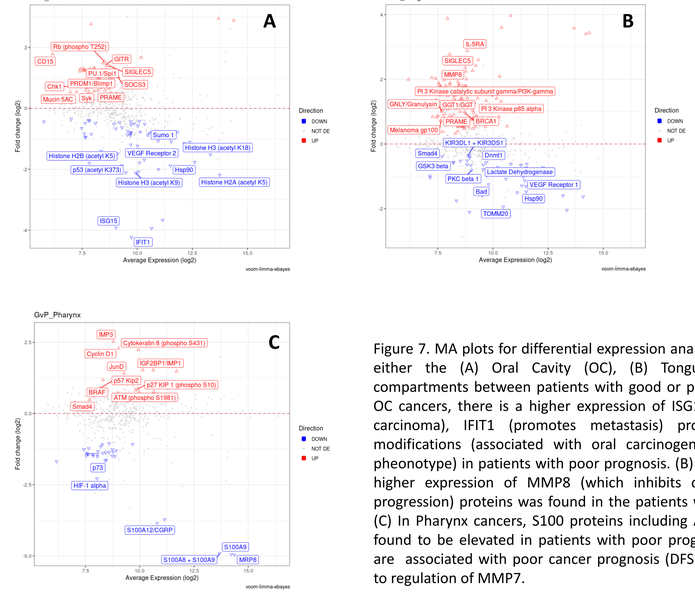

Figure 7. MA plots for differential expression analysis of all samples in either the (A) Oral Cavity (OC), (B) Tongue or (C) Pharynx compartments between patients with good or poor prognosis. (A) In OC cancers, there is a higher expression of ISG15 (elevated in oral carcinoma), IFIT1 (promotes metastasis) proteins and histone modifications (associated with oral carcinogenesis and aggressive pheonotype) in patients with poor prognosis. (B) In Tongue cancers, a higher expression of MMP8 (which inhibits cancer invasion and progression) proteins was found in the patients with good prognosis. (C) In Pharynx cancers, S100 proteins including A8 , A9 and A12 are found to be elevated in patients with poor prognosis. S100 proteins are associated with poor cancer prognosis (DFS) with S100A9 linked to regulation of MMP7.

Conclusions

This study demonstrates that ultra-high plex spatial proteomics provides a lens of biomarker discovery across a head and neck cancer clinical cohort and that anatomy-specific tumour and TME profiles can be contrasted. Moreover, this provides an informed rationale for selecting biomarkers for deeper spatial profiling at single-cell resolution.

Reference:

1. Ning Liu, Dharmesh D Bhuva, Ahmed Mohamed, Micah Bokelund, Arutha Kulasinghe, Chin Wee Tan, Melissa J Davis, standR: spatial transcriptomic analysis for GeoMx DSP data, *Nucleic Acids Research*, Volume 52, Issue 1, 11 January 2024, Page e2, https://doi.org/10.1093/nar/gkad1026

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