High-plex spatial proteomic profiling of immunotherapy response groups in head and neck cancer identifies tissues signatures associated with therapy response – 117

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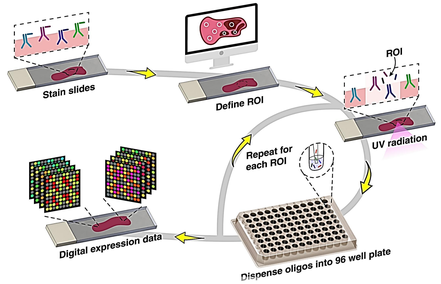
Background

Head and neck squamous cell carcinoma (HNSCC) frequently presents with advanced disease and a poor prognosis. Immunotherapy has shown promising results in patients with metastatic or recurrent (M/R) disease; however, it is only effective in a subset of individuals. Recently, spatial profiling of the tumour microenvironment in HNSCC provided valuable information and new insights into various immune subsets as well as cellular and molecular interactions involved in immunotherapy response or resistance.

Methods



The study was conducted at the Royal Brisbane and Women's Hospital (RBWH, LNR/2020/QRBW/66744) and the study has University of Queensland ratification. Formalin-Fixed Paraffin-Embedded (FFPE) tissues were collected from n=26 HNSCC patients prior to immunotherapy (discovery arm). Validation for this study was carried out in an independent cohort of n=30 HNSCC patient samples collected from the Princess Alexandra Hospital. Pathology Queensland prepared the tissue samples, H&E staining and pathology review to demarcate tumour/stromal regions and GeoMx Digital Spatial Profiling performed using a targeted panel of n=68 antibodies targeting immune cell phenotyping, contexture, activation and drug targets.

Results

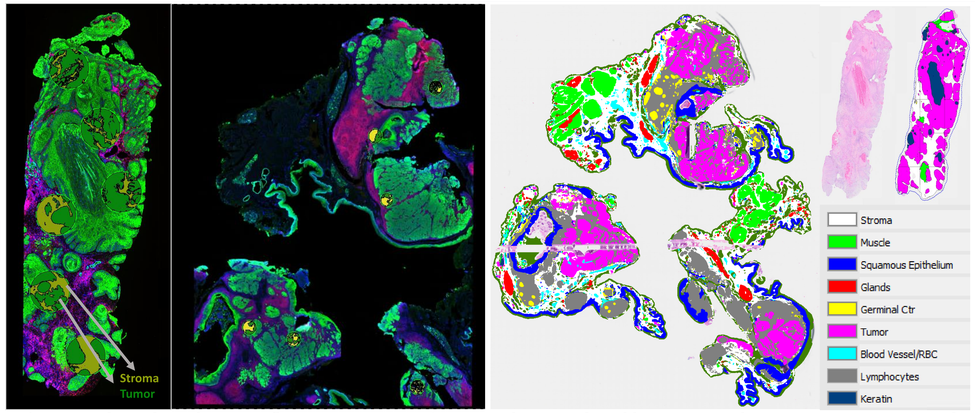


Figure 1. Three-channel immunofluorescence images (FITC, nuclear signal, in blue, Cy3, cytokeratin, in green, Texas Red, CD45, in red) of the two HNC sections showing the regions of interest (ROIs) and areas of illumination (AOIs) from which the protein signatures were acquired. There are 6 ROIs on each section. Within each ROI, one AOI was acquired using a cytokeratin mask ('tumor') and another AOI for the rest of the ROI ('stroma'). Note, the two images are not to the same scale. Oncotopix® Discovery was used to analyze the whole-slide IF and serial-section H&E images for both tissue area (tumor and stroma for IF; 8 classes for H&E) and number and phenotype of cells. The number of each type of cell are shown below in Table 1.

Figure 2: GeoMx analysis workflow

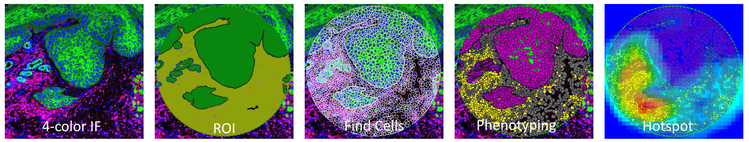


Table 1: GeoMx per-AOI analysis results

Sample Name	ROI	Count (Immune Cells in Stroma)	Count (Immune Cells in Tumor)	Count (Negative cells in Stroma)	Count (Negative cells in Tumor)	Count (Tumor Cells in Stroma)	Count (Tumor Cell in Tumor)	Count total cells Tumor AOI	Count total cells Stroma AOI
RB19P13033	Whole-slide	12989	1598	14603	218	0	31446	33262	27592
RB19P13033	1	156	10	355	0	0	826	836	511
RB19P13033	2	228	20	217	1	0	1547	1568	445
RB19P13033	3	82	2	338	0	0	886	888	420
RB19P13033	4	619	5	927	1	0	907	913	1546
RB19P13033	5	211	9	787	0	0	957	966	998
RB19P13033	6	328	30	854	0	0	810	840	1182
RB15P48811	Whole-slide	691867	250676	151659	9063	0	316639	576378	843526
RB15P48811	1	3206	809	187	4	0	892	1705	3393
RB15P48811	2	2397	755	32	21	0	1410	2186	2429
RB15P48811	3	3301	723	64	8	0	844	1575	3365
RB15P48811	4	2882	536	9	6	0	728	1270	2891
RB15P48811	5	3431	151	128	10	0	1069	1230	3559
RB15P48811	6	2809	2189	12	3	0	642	2834	2821

The workflow for analysis of GeoMx images follows several steps, as shown in Fig 2. First, reading the IF images and importing the ROI/AOI regions, then performing cell segmentation to find cells, then phenotyping those cells based on the IF channels, then data export for GeoMx transcriptome/proteome analysis and further spatial/hotspot analysis.

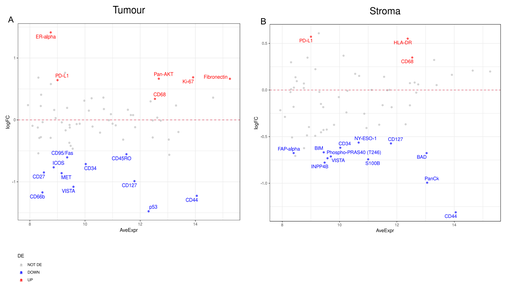


Figure 3. Differential protein expression in the tumour and stromal compartments between patients with partial response (PR) (n=6) versus patients with progressive disease (PD) (n=9). (A) Limma-voom MA plot indicating tumoral expression of protein biomarkers in patients with PR compared to patients with PD, ranked by fold change (logFC). **(B)** Limma-voom MA plot indicating stromal expression of protein biomarkers in patients with PR compared to patients with PD, ranked by fold change (logFC).

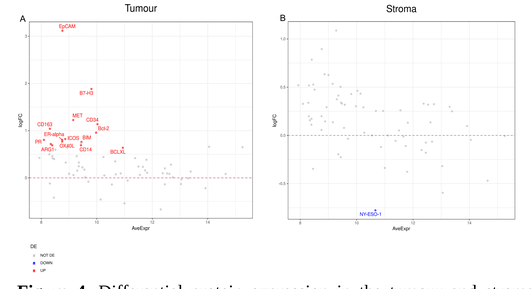


Figure 4. Differential protein expression in the tumour and stromal compartments between patients with partial response (PR) (n=6) versus patients with stable disease (SD) (n=5). (A) Limma-voom MA plot demonstrating tumoral expression of protein biomarkers in patients with PR compared to patients with SD, ranked by fold change (logFC). **(B)** Limma-voom MA plot demonstrating stromal expression of protein biomarkers in patients with PR compared to patients with SD, ranked by fold change (logFC).

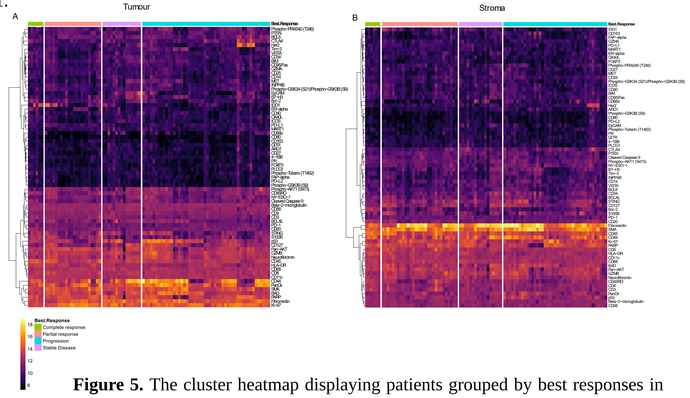


Figure 5. The cluster heatmap displaying patients grouped by best responses in rows and protein biomarker expression in columns. (A) The cluster heatmap of tumoral protein enrichment from patients with different best responses. **(B)** The cluster heatmap of stromal protein enrichment from patients with different best responses.

Conclusion

Better predictive biomarkers of response to immunotherapy are currently needed for Head and Neck Cancers. This study demonstrated informed 'Region of Interest (ROI)' capture using the Oncotopix Discovery to analyze whole slides to demarcate tumour/stroma and gross tissue and cellular structures upstream of ROI selection on both H&E and multiplex IF. Absolute counts for cell types In the tumour and stroma were obtained for quantification and comparison with the GeoMx DSP results. Tumour and stromal compartment specific protein profiles were obtained which associated with response to therapy metrics. Validation of these findings is currently ongoing.