Research Grant Report for Endowments Sub-Committee and BAOMS Website
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Title of Project: Identification of a New Immune Cell in Oral Squamous Cell Carcinoma

Background

Oral squamous cell carcinoma (OSCC) accounts for approximately 95% of oral cancer cases and is responsible for 6500 cases in the UK alone each year. Due to the structurally close nature of the primary tumour location with a large associated network of lymph nodes, metastasis to the neck has a high rate of occurrence, with 50% of patients presenting with clinically detectable metastasis to the neck at diagnosis. Previous breast cancer research has established a positive correlation between a novel immune cell type, ILC3 (innate lymphoid cell type 3), and lymph node metastasis. As the surgical treatment plan for OSCC is often related to the status of the neck, it is important to be able to accurately establish lymph node metastasis. ILC3 evaluation in a new tumour model, OSCC, is therefore an important area of investigation, with possible applications in surgical planning.

Methods

Thirty patient samples of OSCC were stained using fluorescently tagged antibodies to identify CD127, CD3 and RORγt, as well as a Hoechst counterstain to identify cell nuclei. Following staining, all samples were imaged using confocal microscopy. Tumour tissue and margins were identified on Haematoxylin and Eosin (H&E) slides using light microscopy, with input from a histopathologist to distinguish tumour tissue from tumour stroma and any non-malignant tissue. Tumour areas were transferred onto digital confocal microscopy images using FIJI image analysis software. ILC3 cells were identified and quantified within the tumour areas and in two surrounding 25μm and 50μm boundaries (Figure 1). ILC3 cells were identified as those showing a phenotype of $RORγt^{+}CD127^{+}CD3^{-}$. Once ILC3s were quantified, raw counts were used along with total tumour area to calculate ILC3 density (ILC3 cells/mm^2). Lymph node metastasis for each patient was predicted based on these density values (where <1 ILC3/mm^2 was predicted as no metastasis and >1 ILC3/mm^2 predicted metastasis).

Results

Predictions of lymph node metastasis based on ILC3 density alone were correct in 45% of cases. Despite various analyses, no significant difference was found between metastatic and non-metastatic groups. No correlation was found between ILC3 density and lymph node metastasis. ILC3 density was strongly correlated with CTCF values, an indicator of total immune cell infiltration. Patients with high ILC3 density values appeared to have better survival rates than those with lower density values when plotted as a Kaplan-Meier.

Conclusions

Correct staining and identification of ILC3s in OSCC was completed for the first time, proving ILC3 presence in another cancer type. Understanding the mechanisms that underpin ILC3 transit to tumour sites remains essential to be able to identify potential routes for further research. This study reported a significantly strong correlation

between ILC3s and immune cell invasion (CTCF), as well as developing successful protocols for image acquisition and analysis that could prove to be widely applicable for further tissue-based studies.

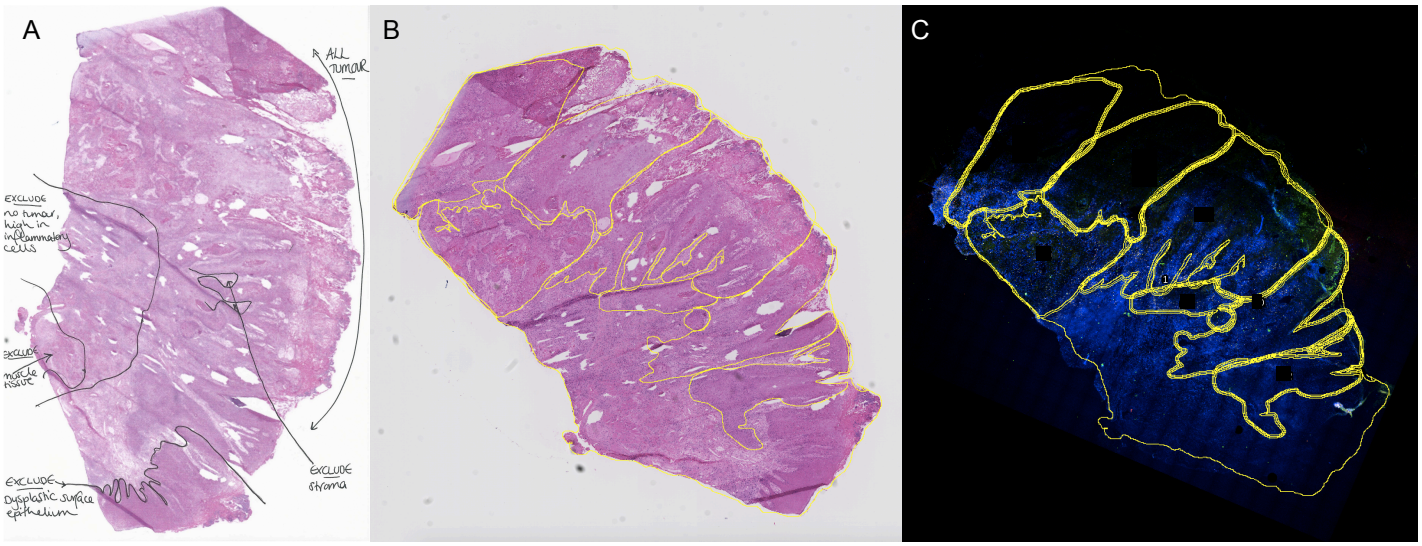

Figure 1 – Main Aspects of Cell Quantification. (A) Annotated H&E image identifying tumour margins and non-malignant tissue to exclude. (B) Digital H&E image with tumour margins in yellow. (C) Digital tumour margins transferred to confocal tile scan image, with 25µm and 50µm boundaries.