973 PHENOTYPICAL DIFFERENCES OF NEUTROPHILS IN TUMOUR-DRAINING LYMPH NODES IN HEAD AND NECK CANCER

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Background Tumor-draining lymph nodes (TDLNs) play a crucial role in coordinating immune responses. It has been established that neutrophils accumulate in TDLNs across various cancer types, and their presence in TDLNs has been associated with a poor prognosis. Despite accumulating evidence supporting the significance of neutrophils in the development of anticancer immunity, the specific role of neutrophils in TDLNs, particularly in human samples, remains largely unexplored. In this study, we aim to investigate whether there are any phenotypic differences between neutrophils in TDLNs, non-TDLNs, and healthy lymph nodes.

Methods We analyzed TDLNs and non-TDLNs from 40 patients with oral squamous cell carcinoma (SCC) and 16 lymph nodes from healthy individuals using flow cytometry. The expression of the following markers was examined: CD45, CD15, CD16, CD62L, CD26, CD47, CD11b, CD24.

Results In our study, neutrophils were classified into different subsets based on their expression of CD16 and CD62L, with each subset representing a distinct stage of maturity and activity. The analyzed compartments exhibited significant differences in the phenotypes of neutrophils. Healthy lymph nodes and non-TDLNs were enriched with $CD16^{high}CD62L^{high}$ neutrophils, which indicate mature non-activated neutrophils. On the other hand, TDLNs showed high infiltration of $CD16^{high}CD62L^{dim}$ neutrophils, indicating mature and hyperactivated neutrophils. Cluster analysis revealed differential expression of CD36, a molecule involved in the recognition and phagocytosis of apoptotic neutrophils by macrophages. TDLNs exhibited lower levels of CD36 compared to non-TDLNs and healthy lymph nodes, suggesting enhanced survival of neutrophils in TDLNs.

Conclusions Our findings demonstrate that neutrophils in healthy lymph nodes, non-TDLNs, and TDLNs in oral SCC exhibit distinct phenotypes based on the expression of activation and survival molecules. The presence of hyperactivated and long-lived neutrophils in TDLNs may potentially influence the response to novel checkpoint inhibitors.

Ethics Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. Ethics Committee Approvals: 2015/1650–31/2 and 2019–03518.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0973