CANCER COMMUNICATIONS Open Access

RESEARCH HIGHLIGHT

Nasopharyngeal carcinoma joins the single-cell party

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1 | MAIN TEXT

Next-generation sequencing has revolutionized cancer research, providing large-scale molecular characterizations for all common cancer types and contributing to personalized medicine through understanding of inter-tumor heterogeneity [1,2]. Exemplified by the cancer genome atlas (TCGA), these studies uncovered driver mutations and divided each cancer type into subtypes with distinct molecular features and clinical characteristics, such as survival and treatment response [3]. However, these studies, which largely considered each tumor as an individual "bulk" sample, are insufficient for characterizing the diversity of cells within a tumor. Such diversity includes the various immune and stromal cell types as well as the multitude of cellular states among neoplastic cells, which collectively underlie tumor biology and clinical behavior.

The advent of single-cell technologies, such as single-cell RNA sequencing (scRNA-Seq), overcomes this weakness and heralds a new revolution in cancer research [4-6]. Several cancer types have already been profiled by large-scale scRNA-Seq studies, providing a cellular view of their composition and diversity, and further clarifying previous observations. For example, subtypes identified previously by TCGA are now refined and their underlying basis have been revealed [6]. This is a rapidly developing field, and in the coming years we can expect single-cell atlases to be published for most cancer types, and a cell-centric perspective gradually replacing the historical tumor-centric perspective.

A new study by Chen et al. [7] has applied this scRNA-seq approach to nasopharyngeal carcinoma (NPC). NPC is a relatively uncommon cancer type that is found mainly in East and Southeast Asia and is strongly associated with Epstein-Barr virus (EBV) infection. Treatment consists of combinations of radiotherapy and, depending on stage and location, surgery and/or chemotherapy. NPC tumors generally show high rates of immune infiltration and programmed death ligand 1 (PD-L1) expression, and accordingly, there are several trials of programmed cell death protein 1 (PD-1) inhibitors for recurrent or metastatic NPC. However, these only show 1-year progression-free survival rates around 20%-30% [8]. Bulk studies have subdivided NPC into three molecular subtypes, highly dependent on immune cell infiltration [9].

Chen et al. profiled tumor samples from fifteen NPC patients (including two EBV-negative) by scRNA-Seq with the widely used 10x Chromium system, resulting in high-quality data for almost 50,000 cells. Of these, only 7,581 were neoplastic cells, while the vast majority were immune cells. Surprisingly, almost no fibroblasts nor endothelial cells were identified in the single-cell data, although immunofluorescence analysis of a large FFPE control cohort indicated an expected frequency of 3%-4% for each of these cell types. This highlights a frequently overlooked limitation of the single-cell approach, related to the difficulty of dissociating and profiling certain cell types.

Among the neoplastic cells, variability in gene expression profiles was found to represent primarily epithelial differentiation, proliferation, and cell secretion.

Abbreviations: EBV, Epstein-Barr virus; EMT, epithelial-mesenchymal transition; HNSCC, head and neck squamous cell carcinoma; NPC, nasopharyngeal carcinoma; scRNA-Seq, single-cell RNA sequencing; TCGA, the cancer genome atlas

Interestingly, one of the epithelial differentiation signatures was associated with EBV infection, while the cell secretion signature was associated with immune infiltration. These observations raise interesting hypotheses regarding the influence of cell-to-cell (and virus-host) interactions. For example, it suggests that either lymphocytic infiltration is promoted by the malignant cell secretion signature or, alternatively, that this signature is induced in the malignant cells by the lymphocytes. Further studies would be required to evaluate the cause and effect leading to such associations.

Among the immune cells, Chen et al. provided a much higher resolution of the immune composition in NPC compared to the most comprehensive bulk study [9]. Multiple subsets of monocytes, T-cells, and B-cell were defined. Importantly, several of these subsets of immune cells were correlated with EBV infection and/or survival, and two subsets were of particular interest. CLEC9A-positive dendritic cells were associated with good prognosis and with EBV-negativity. In contrast, a proliferative B-cell subset was associated with poor prognosis. As potential biomarkers, the inhibitory receptors LAG-3 and TIM-3 (HAVCR2) were highly expressed in dysfunctional $CD8^+$ T-cells, and localized to tumor epithelial nests.

Notably, none of the signatures identified as associated with survival in this study were associated with survival when analyzing their expression in the TCGA head and neck squamous cell carcinoma (HNSCC) dataset, which does not include NPC. This highlights potential differences between the subtypes of HNSCC and suggests that either the subsets of cells differ between NPC and other HNSCCs, or that the prognostic significance of these subsets is specific to NPC. More detailed comparisons across single-cell studies of HNSCC would have to be performed to further clarify these observations. When compared to scRNA-Seq of HNSCC tumors of the oral cavity [6], neoplastic cells showed similar, though not identical, signatures of cell cycling and epithelial differentiation in both studies. However, in the oral cavity tumors, a prominent pattern of intra-tumor diversity was linked to partial epithelial-mesenchymal transition (EMT), which was not observed by Chen et al. in NPC. Thus, the diversity of both neoplastic and immune cells appears to differ between NPC and other subtypes of HNSCC.

While deepening our understanding of NPC biology, the clinical impact of the work by Chen et al. is yet to be seen. LAG-3 and TIM-3 serve as potential immunotherapy targets in a range of malignancies, along with other potential targets [10]. This work may help to prioritize LAG-3 and TIM3 over other potential targets in the context of NPC. While neither of these markers was significantly associated with survival in the bulk validation cohort, specifically studying these coinhibitory receptors in immune cells of patients treated with immunotherapies could be more fruitful. Similarly, immune subsets that were shown to correlate with prognosis should also be quantified in relation to specific immunotherapies and considered for patient stratification. Finally, as is the case for TCGA and other single-cell studies, the impressive dataset produced by Chen et al. should be considered as a fundamental resource for the NPC research community, enabling future analysis and the testing of additional hypotheses.

AUTHORSHIP

M.M. and I.T. conceived and wrote the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

CONSENT FOR PUBLICATION

Not applicable

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Not applicable

FUNDING

ACKNOWLEDGMENTS

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