Single-cell genomics identifies immune response to neoadjuvant chemoradiotherapy

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Esophageal carcinoma is the 7th most common cancer worldwide and has the 6th highest mortality rate.[1] As the major subtype, esophageal squamous cell carcinoma (ESCC) is highly lethal with its patients suffering a dismal five-year survival rate of <20%.[2] For locally advanced, resectable ESCCs, preoperative neoadjuvant chemoradiotherapy (neoCRT) is commonly employed. However, just like most other cancer therapies, clinical responses to neoCRT vary substantially among patients, largely owing to the lack of predictive biomarkers for patient selection. Undoubtedly, advanced characterization and understanding of cellular mechanisms underlying the responsiveness to neoCRT is required for the development of biomarker-based precision treatment.

Tumor microenvironment is a complex ecosystem of diverse cell types, together shaping cancer biology and impacting the sensitivity to therapy. In ESCC, multiple stromal cell populations correlate strongly with patient survival and therapeutic efficacy.[3,4] Recently, the stromal heterogeneity of ESCC tumor ecosystem has been analyzed by single-cell RNA-Seq approaches.[3–6] However, cellular and molecular changes of ESCC tumor microenvironment in response to CRT have not hitherto been explored in detail. In the recent issue of eBiomedicine, Wen et al. address this critical question using single-cell transcriptomic profiling of 111,784 cells from 8 pre- and 7 post-neoCRT ESCC samples.[7] Extensive computational analyses of this rich dataset have provided new insights into complex alterations of immune cells induced by neoCRT.

T cells are footman soldiers carrying out the anti-tumor immune response. The authors found that the fraction of tumor-infiltrating $CD8^+$ T cells was significantly increased by neoCRT. This observation was validated by multiplexed fluorescent immunohistochemistry (mfIHC) in an independent cohort of pre- and post-neoCRT patients who received the same regimen as those profiled by single-cell analysis. Notably, RNA-velocity analysis revealed a transition from proliferative, intermediately active states to more exhausted states after neoCRT. Indeed, mfIHC showed that the frequency of cytotoxic T cells was reduced following neoCRT. These results suggest that neoCRT promotes $CD8^+$ T cell tumor-trafficking but also its exhaustion, in line with a recent report of elevated levels of exhausted $CD8^+$ T cells in post-neoCT ESCC samples.[8] In an analogous manner, dynamic changes of $CD4^+$ T cells were analyzed. Interestingly, the activity of FOXP3, the master regulator of Treg cells, was reduced strongly in major responders compared with poor responders after neoCRT. Consistently, mfIHC revealed higher Treg to Th ratio in poor responders than that in major responders in post-neoCRT samples.

In the myeloid compartment, attention was first given to conventional dendritic cells (cDCs), which have two well-recognized subsets, cDC1 and cDC2. The primary functions of cDC1 and cDC2 include acquiring tumor antigen, migrating into lymph nodes, and priming respectively $CD8^+$ and $CD4^+$ T cells. The investigators noted that both cDC2 and cDC1 could potentially differentiate to $LAMP3^+$ cDCs in pre-neoCRT samples, congruent with findings from other types of primary tumors.[9] In contrast, from post-neoCRT samples, $LAMP3^+$ cDCs were found to be originated predominantly from cDC1s. MfIHC staining also demonstrated that the cDC2 subset was decreased after neoCRT, suggesting that neoCRT promotes the maturation of cDC1s but inhibits the infiltration of cDC2s. These changes of cDCs possibly contribute to the aforementioned alterations in $CD8^+$ T infiltration/exhaustion and $CD4^+$ T differentiation after neoCRT, given the central role of cDCs in presenting tumor antigens upon neoCRT-induced tumor cell death.

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The impact of neoCRT on cell–cell crosstalk among various immune subsets was also investigated. Notably, the number of inferred interactions between intermediate activated/exhausted $CD8^+$ T cells and cDCs/macrophages was decreased after neoCRT. This was highly specific to this particular subset of $CD8^+$ T cells since other T cell clusters did not display any changes. These interactions notably involved cytokines and immune checkpoints, such as PDCD1/CD274, PDCD1/FAM3C, TIGIT/NECTIN2. Considering the importance of these immune checkpoint proteins, their reduced interactions may affect substantially anti-tumor immune response, which might also impact the sensitivity to neoCRT.

While the sample size was limited, the above results have potentially significant translational implications. For example, neoCRT boosted the infiltration of exhausted $CD8^+$ T cells while weakened the cell–cell interactions involving inhibitory checkpoints between $CD8^+$ T cell subsets and cDCs/macrophages, creating an opportunity for these $CD8^+$ T cells to be reinvigorated by the anti-PD-1 regimen. In fact, in the neoadjuvant setting, anti-PD-1 therapy has already been shown to improve neoCRT for ESCC patients.[10] In addition, the observation that Treg cell activity is higher in post-neoCRT poor responders indicate that targeting Treg cells in conjunction with neoCRT may enhance treatment efficacy. Moving forward, it is important to functionally test these phonotypical changes using animal models or patient-derived tumor organoids. Furthermore, considering the complex cell–cell interactions and communications, direct and indirect effects of neoCRT on immune cells need to be carefully dissected in a well-controlled system. Finally, although numerous molecular alterations were demonstrated, specific biomarkers associated with tumor responses to neoCRT remain to be identified. Nevertheless, the present study has generated a high-resolution landscape of cellular and molecular alterations of ESCC immune ecosystem induced by neoCRT, representing a significant step forward in understanding the immune response to CRT while identifying potential new avenues to enhance treatment efficacy for this deadly disease.

Contributors

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Declaration of interests

The authors disclose no conflicts.

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