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EDITORIAL

Toward a Better Understanding of Hepatocellular Carcinoma Immune Infiltrates

The immune system has an extremely potent antitumoral activity; indeed, it has become clear in recent years that this antitumoral activity can be effectively used to treat tumors. Accordingly, immunotherapies have dramatically changed oncologic therapy. The purpose of immunotherapy with checkpoint inhibitors (CPIs) is the enrichment of tumor-specific CD8+ effector-/CD4+ helper T cells at the site of the tumor and the reinvigoration of exhausted T cells. Although some impressive clinical responses have been reported with CPI therapy, these immunotherapies are not equally effective in all patients and cancer entities. Immunotherapy has also been suggested for the treatment of hepatocellular carcinoma (HCC)[1]; however, liver cancer is subject to great molecular complexity and heterogeneity.[2] Thus, immunotherapy only leads to objective clinical responses in a small number of patients. Of note, a subset of HCC patients carry an infiltrate of tumor-infiltrating lymphocytes (TILs) that seems to be correlated with a positive prognosis.[3] Nevertheless, immunohistochemistry only provides us with rough data on the number of immune cells and their localization in and around the tumors. Numerous other factors such as mutation load, the expression of inhibitory markers such as CTLA-4, PD-1, and PD-L1, or defects in the DNA repair machine all correlate with clinical response to CPI therapy.[4] Gene expression data or analysis of the transcriptome at bulk tissue level may provide us with information about the presence or absence of immune cells in tumors.[5] However, these analyses inherently cannot provide insights about the composition, proportions, heterogeneity, or deeper spatial distribution of the immune cells. A deeper understanding of tumor-resident immune cells is crucial to understand which immune cells respond to CPI therapy and why some tumors are accessible for immunotherapy.

The recent study by Di Blasi et al[6] in this issue has set out to gain a deeper understanding of the different immune cell populations in HCC. For this purpose, the TILs of 36 HCC patients were characterized by multidimensional flow cytometry and were compared with the immunophenotype of T cells from healthy liver (NTILs) or from peripheral blood mononuclear cells (PBMCs) of the same donors, and by performing t-Distributed Stochastic Neighbor Embedding analysis, different clusters were identified. Notably, TILs were primarily composed of CD4+ T cells, whereas CD8+ T cells and CD4-CD8-double negative and $\gamma\delta$ cells were found less frequently in tumors. Phenotyping of bulk cells revealed a higher frequency of ICOS and CD137 expressing cells, suggesting that the cells were recently activated by antigen. Within the CD4+ T-cell population, the expression of co-stimulatory ICOS and co-inhibitory TIGIT was increased primarily in tumors. This suggests that these CD4+ cells have been activated but are in an exhausted state and are likely poor antigen responders. However, if these cells were stimulated in vitro, they were able to proliferate but were no longer functional with respect to cytokine production. In line with this, typical exhaustion markers were found to be elevated (FoxP3, Helios, but not T-bet). Within the CD8 T-cell population, CD8+ CD38+ PD-1+ T cells were enriched, and some of these cells were functionally active. When comparing the identified clusters (eg, CD4+ ICOS+ TIGIT+, CD8+ CD38+ PD1+, and CD4-CD8-double negative T cells) with immunohistochemistry staining results of the same samples, an accumulation of activated and tissue resident CD4+ ICOS+ TIGIT+ and CD8+ CD38+ TIGIT+ PD1+ T cells was observed in the immune-inflamed HCCs, possibly suggesting that these have been activated within the tumor. The authors also studied the implications of CPI therapy on TIL phenotype and function in 7 of the 36 patients before and after initiation of therapy. This revealed that CPI induced only minimal changes in the frequency of total T cells and only minimal changes in clusters of PBMCs and NTILs. In contrast, major changes in clusters of TILs were observed. Specifically, in 2 of 7 patients responding to nivolumab therapy, the previously identified group of ICOS+ TIGIT+ CD4+ T cells decreased, and activated CD4+ and interferon-γ-producing $CD38^{+}$ CD8+ T cells were found to be increased. Taken together, these results suggest that the presence of certain TIL clusters (eg, ICOS+ TIGIT+ CD4+ TILs) may serve as a prognostic indicator of CPI therapy response.

Recently, another study has proposed a distinctive functional composition of T cells in HCC.[7] By using deep single-cell RNA sequencing in 6 treatment-naive patients, 11 clusters have been proposed, and their results suggested that the preferential accumulation of both Tregs and exhausted $CD8^{+}$ T cells in HCC might be a result of local expansion of these cells. In contrast, recent findings from patients with basal and squamous cell carcinoma suggest that preexisting tumor-specific T cells have limited reinvigoration capacity, and that the T-cell response to CPI therapy is generated from a different repertoire that has just immigrated into the tumor in patients under CPI therapy.[8] Enriched immune-suppressive Tregs and depleted CD8+ T-cell populations were found in hepatitis B virus–induced HCCs in a combined analysis using next generation sequencing and CyTOF.[9] Notably, with the help of next generation sequencing techniques, increased populations of CD8+ T cells and natural killer cells and decreased levels of CD4+ memory T cells and macrophages were observed in patients with melanoma responding to

CPI therapy.[10] The description of similar immune cell clusters and their alteration during CPI therapy are thus basically in agreement with the study by Di Blasi et al,[6] and it remains to be seen which of the proposed clusters and phenotypic profiles will prove most valuable in terms of prognosis for CPI therapy.

In summary, this study provides important insights into the immune cell composition in HCC. This deeper understanding might contribute to the development of rational combinatorial immunotherapies that specifically target the immune cell clusters of interest within HCC. These preliminary results may indicate that certain lymphocyte populations impaired during the ongoing immune response in HCC may identify patients responding to CPI therapy. Prospective studies should follow in this area to confirm this theory.

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Conflicts of interest
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