CHARACTERIZATION OF MOLECULAR AND SPATIAL DIVERSITY OF MACROPHAGES IN HEPATOCELLULAR CARCINOMA

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Background Hepatocellular carcinoma (HCC) has a dismal prognosis, and though checkpoint blocking antibodies have significantly improved patient outcome, many patients remain left out, highlighting the need to identify additional immune target to enhance therapeutic immunity. Macrophages (MF) are an abundant and heterogeneous population in the tumor microenvironment (TME), and are associated with a poor prognosis in multiple tumor types, including HCC, however, their molecular and functional diversity is still poorly understood.

Methods We analyzed the molecular and spatial organization patterns of immune cells within the TME and adjacent tissue of 26 resected HCC lesions using single-cell RNA sequencing and multiplex immunohistochemistry (IHC).

Results We found that Kupffer cells, the self-renewing tissue-resident macrophages in liver tissue, are lacking from the TME, which is dominated by monocyte-derived macrophages. ScRNAseq followed by high-resolution clustering identified distinct MF molecular programs within the monocyte-derived macrophage compartment. One MF subset expressed a shared signature with monocytes including FCN1, S100A8 and T cell activation genes like CXCL9 and IL32. Conversely, one subset of MF expressed FOLR2, SEPP1 and genes of the complement (C1Qs), a program shared with KC in the adjacent tissue, and include another intratumoral subset enriched for the expression of TREM2 and GPNMB. Guided by these results, we are developing an IHC antibody panel that allows to visualize distinct MF localization in the TME. Intratumoral MF interface with, and potentially regulate, the T cell compartment within the TME. We are analyzing HCC tumor lesions in our treatment-naïve cohort and in patients treated with neoadjuvant anti-PD-1 therapy (NCT03916627) to study co-localization and direct interaction of MF and T cells using physically-interacting cell sequencing (PICseq). This analysis enables us to identify MF with direct cell-cell contact with T cells, and our preliminary analysis demonstrates an enrichment in MF with an immunosuppressive phenotype. We are also using spatial transcriptomic to map molecular programs of MF in the TME, that we will corroborated with additional patients.

Conclusions Taken together, our data provide a new understanding of intratumoral MF diversity and highlight the presence of specific immunoregulatory MF programs unique to tumor lesions, with subsets of these MF found to be directly interacting with T cells, potentially modulating anti-tumor responsiveness. Our analysis of resected tumor from anti-PD-1 treated patients, will allow us to correlate MF programs, and direct T cell interaction, with clinical response, and will inform therapeutic trials targeting specific MF populations so as to improve clinical efficacy of cancer immunotherapy.

Trial Registration NCT03916627

Ethics Approval Samples of tumor and non-involved liver were obtained from surgical specimens of patients undergoing resection at Mount Sinai Hospital (New York, NY) after obtaining

informed consent in accordance with a protocol reviewed and approved by the Institutional Review Board at the Icahn School of Medicine at Mount Sinai (RUTH Human Subjects Electronic Submission System 18-00407 and 20-04150) and in collaboration with the Biorepository and Department of Pathology.

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