SUCCESSFUL IMMUNE-CHECKPOINT THERAPY PROMOTES SPATIAL REORGANIZATION OF THE LYMPHOID AND MYELOID CELLULAR POPULATIONS

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Background We previously used scRNASeq and CyTOF to define some of the cellular and molecular changes that occur during immune checkpoint therapy (ICT)-induced rejection of our well-characterized mouse T3 MCA sarcoma model in syngeneic mice. Here, we used the Phenocycler (CODEX) multiplex imaging system with a 35-plex antibody panel to characterize the spatial changes in the lymphoid and myeloid cell populations that result in either tumor outgrowth in mice treated with control antibody (cmAb) or successful tumor rejection in mice treated with the combination of αPD-1 and αCLA-4 (ICT).

Methods T3 tumor-bearing mice were treated with cmAb or combo ICT, tumors were harvested at different time points, fresh frozen, sectioned, and subjected to CODEX multiplex imaging. Large-field images encompassing the entire tumor sections were processed using an Akoya-developed pipeline. A hierarchical cell clustering approach was used to profile 4,051,156 cells resulting in the identification of 12 cell types.

Results When analyzed at day 10 (one day before ICT-induced rejection becomes detectable) tumors from combo ICT-treated mice displayed a marked increase in the percentage of iNOS+ macrophages, Ly6G+ neutrophils, and a corresponding decrease of CX3CR1+ macrophages, Tregs and tumor cells compared to matched tumors from cmAb treated mice. Although the overall frequencies of CD4+ and CD8+ T cells were not significantly altered at this time point by combo-ICT, they were found in areas of higher density compared to tumors from control mice. Interestingly, whereas KI67+ T cells localized to collagen-rich areas of the tumor stroma, Granzyme B+ T cells accumulated inside the tumor region. Nearest neighbor analysis revealed that combo ICT changed the landscape of heterotypic cellular interactions. Specifically, whereas in cmAb-treated mice, tumor cells and CX3CR1+ macrophages were the two main cell types interacting with one another, in combo ICT-treated tumors, interactions between CD4+ T cells, cDC2, CD206+ macrophages, iNOS+ macrophages, and CD8+ T cells predominated. Cellular neighborhood (CN) mapping indicated the formation of 8 distinct spatially organized CNs across the tumor tissue. Among these, we identified CN-1 that showed increases in frequency after combo ICT and was enriched for T cells, NK cells, and cDC1. Longitudinal images from tumors harvested on days 6 to 13 showed the expansion of the lymphoid-rich CN-1 during tumor rejection.

Conclusions These results not only confirm our previous findings of lymphoid and myeloid compartment remodeling during successful ICT but also now provide a detailed view of the dynamic spatial changes leading to successful ICT.

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