DECRYPTING OF RADIOTHERAPY-INDUCED IMMUNOMODULATION EFFECT SYNERGIZED WITH IMMUNOTHERAPY IN HEPATOCELLULAR CARCINOMA BY SPATIAL MULTI-OMICS PROFILING

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Background Multiple clinical trials have shown an overall improved response rate in hepatocellular carcinoma (HCC) by combining radiotherapy (RT) with immunotherapy (IO), however, treatment rates remain low and unpredictable. The suboptimal outcomes are largely due to the lack of knowledge of the underlying immunomodulation effect and an effective treatment-response biomarker. Previous studies using tissue-based assays like multiplexed immunofluorescence (mIF) have demonstrated that cellular spatial organization within the tumor microenvironment represents a critical factor influencing anti-tumor immunity. Hence, we sought to characterize the in-situ molecular immune response of RT-treated HCC tissues, to advance our understanding of RT-induced immunomodulation effect and its synergistic benefits with IO.

Methods Using an HCC cohort treated with Yttrium-90 (Y90)-radioembolization (locoregional RT) and anti-PD-1 combination therapy, we profiled the FFPE tissues collected at baseline and post-Y90 from 4 responders and 8 non-responders using NanoString’s Digital Spatial Profiler (DSP), 10× Genomics Visium technology, and mIF. In DSP profiling, two types of regions of interest (ROIs) were selected by a pathologist: (1) geometric ROIs far from Y90 beads, and (2) continuously micro-dissected ROIs contouring the Y90 beads; Visium data contains spatially barcoded spots (figure 1).

Results Tumor Immune Dysfunction and Exclusion (TIDE) analysis using DSP geometric ROIs showed a counter-intuitively decrease of IO-responsiveness (i.e., higher TIDE scores) in responders post-RT. Immune analysis using DSP micro-dissected ROIs revealed that RT might have induced a systemic increase in T-cell exclusion and decrease in T-cell dysfunction in the non-responders, but opposite trends in the responders (figure 2). A possibly RT-induced systemic increase in CD274 (ligand of PD-1) expression was seen in the responders (P = 0.001; \( P_{\text{distance-to-Y90-beads}} = 0.04 \)). Analysis using the higher-spatial-resolution Visium data showed a systemic increase in cytotoxic T-cell abundance in the non-responders post-RT where the cells were spread over the tissues (figure 3). mIF analysis showed a specific subset of T-cells, CD38+CD8+, in the responders preferentially interacted with the tumor cells (P = 0.001; \( P_{\text{cell-to-tumor distance}} = 0.08 \)), an effect possibly induced by RT (P = 0.001; \( P_{\text{cell-to-Y90-bead distance}} = 0.001 \)) (figure 4).

Conclusions TIDE, a transcriptomic-based IO-biomarker mainly tested in melanoma, is not directly applicable to HCC. Spatial multi-omics analysis enables identification of RT-specific immunomodulation effects that synergized with IO in HCC, including the systemic increase in T-cell dysfunction and CD274 expression. To explain for the systemic increase of cytotoxic T-cells in non-responders, further investigation on the tumor-specificity by cell-to-cell proximity analysis is needed.

Trial Registration NCT03033446
Abstract 867 Figure 2

Abstract 867 Figure 3

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