DEEP HIGH-PLEX SPATIAL PHENOTYPING OF GLIOBLASTOMA MULTIFORME PROVIDES NEW INSIGHTS INTO THE IMMUNE LANDSCAPE AND TUMOR HETEROGENEITY

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Background Highly multiplexed spatial biology technologies have become key to unveiling unique cellular phenotypes and cellular neighborhoods in healthy and diseased tissues. Glioblastoma (GBM), the most prevalent and aggressive brain tumor in adults, is characterized by high intra- and inter-tumoral heterogeneity. Along with the highly infiltrative nature of these tumors, the immunosuppressive microenvironment leads to poor clinical outcomes with median survival of less than 15 months with current standard-of-care treatments. While immunotherapies have been approved for the treatment of various types of cancer, clinical trials in GBM treatment yielded little to no success.

Methods Using the Phenocycler®-Fusion, an integrated spatial biology system, we performed deep analysis of over 50+ proteins to interrogate the immune, vascular, and tumor landscapes in primary and recurrent GBMs. Antibody panels included markers for cell lineage, immune checkpoints, tissue structure, and cellular activation states which, when analyzed, provided comprehensive information of the tumor microenvironments’ phenotypic constituents as well as their spatial relationship.

Results Most of the tumor tissues exhibited high levels of glial marker GFAP, indicative of their cell-of-origin. In some tumors, GFAP expression overlapped with Vimentin and CD44, two characteristic markers for the mesenchymal subtype of GBM associated with poor prognosis. GBM tumors also showed varying degrees of proliferation and angiogenesis as evidenced by Ki67 and CD31 staining, respectively. MMP9 expression was very common in most tumors and MMP9 positive cells were found to be associated with the vasculature. Infiltrating T cells also varied significantly from almost non-detectable to a large presence in certain tumors. Both resident microglia and infiltrating monocytes/macrophages could be found across most of the tumors. Intriguingly, some GBM samples showed high expression of β-catenin and exhibited a remarkable infiltration of myeloid-derived suppressor cells (MDSCs; CD14high/HLA-DRlow), a large amount of glioma-associated macrophages (CD163+) and a high percentage of FoxP3+ Tregs.

Conclusions Deep spatial phenotyping of GBM tumors revealed a high degree of cellular heterogeneity in both intra and inter-tumoral fashion. This unbiased, whole-slide approach of analyzing multiple proteins simultaneously within complex GBM tissue structures will greatly enhance our understanding of the immune-suppressive tumor microenvironment in GBM. Further investigation of the immune landscape before and after immunotherapy treatment will help us understand the mechanism of therapy resistance and guide new drug development.

Ethics Approval This study was performed in accordance with Cedars-Sinai IRB protocol No STUDY00001892