CHARACTERIZATION INTER INTRA TUMOR HETEROGENEITY PRIMARY MELANOMA MELANOMA BRAIN METASTASES

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Background Melanoma often metastasizes to the brain,1 usually with a lethal outcome. Recent studies have reported that systemic immunotherapies (IOT) are effective in patients (pts) with melanoma brain metastases (MBM).2 3 Nevertheless, failure of IOT in nearly half of patients with MBM leads to the urgency to deeper investigate mechanisms of intrinsic and acquired resistance. The aim of this study is to deeply characterize the tumor microenvironment (TME) of primary melanoma (PM) and MBM and to assess inter- and intra-tumor heterogeneity in order to identify potential strategies able to increase the success of IOT in pts with MBM.

Methods Formalin-fixed, paraffin-embedded (FFPE) tumor biopsies were derived from 7 PM and 14 MBM biopsies from different pts. RNA was isolated from tumor regions and subjected to whole gene expression profiling (GEP). Ingenuity Pathway Analysis (IPA) was performed for enrichment assessment, and Microenvironment Cell Populations-counter (MCP-counter) method was used to estimate the abundance of immune and stromal infiltrated cell subpopulations. Selected transcripts including mRNA for CD163, CD45 and CD20 were evaluated by immunohistochemistry (IHC). Inter- and intra-tumor immune heterogeneity of n=59 selected immune protein was also determined in PM and MBM by digital spatial profiling (DSP) using Nanostring GeoMx technology.

Results Whole GEP revealed 888 transcripts differentially expressed between PM and MBM (p ≤ 0.01). We observed an increased expression of genes involved in glycolysis (i.e. ALDOA, ENO2, PKM), immune checkpoint signaling (i.e. TIM3), macrophage activity (i.e MARCO, CD14), complement signaling (i.e C1QA/B/C) and chemotaxis (i.e CCL3) in PM vs. MBM. Conversely, overexpression of genes involved in epithelial signaling (i.e. KRT1), wound healing (i.e. WNT3), stem cell proliferation (i.e. YAP, TP63) and immunosuppressive cytokines and chemokines (i.e. CXCL21, CXCL19) was found in MBM vs. PM. Interestingly, by evaluating the protein expression of immunotherapy drug targets, pan-tumor targets, myeloid targets and immune activation targets using IHC and DSP, we found important inter- and intra-tumor immune heterogeneity in PM and MBM tumors.

Conclusions If confirmed in a larger cohort and correlated with therapy outcomes, our results might lead to the development of novel therapeutic strategies able to increase the success of systemic IOT therapy in patients with advanced melanoma who develop metastases at sites including, but not limited to, the brain.

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REFERENCES

Ethics Approval Samples were procured under studies approved by Providence Saint Joseph Health Institutional Review Board or Western Copernicus Group Institutional Review Board. All specimens evaluated were derived from consenting patients.