Background Medulloblastoma, the most common embryonal brain tumor of childhood, has a cold tumor immune microenvironment, harboring low lymphocytic infiltration and a paucity of tumoral PD-L1 expression. We and others have shown that this tumor is highly infiltrated with IBA-1 positive tumor associated microglia/macrophages that more frequently express PD-L1 than tumor cells. Despite this, agents disrupting the PD-1 pathway have been ineffective in treating patients with this tumor, suggesting that medulloblastoma may utilize alternate pathways to maintain a suppressive microenvironment.

Methods NanoString GeoMx digital spatial profiling was used to isolate IBA-1 expressing cells infiltrating 12 medulloblastoma tumor samples from a tumor issue microarray that were previously profiled for molecular subgroup. IBA-1+ cells versus other were evaluated for expression of 77 immunomodulatory proteins. mCB DNp53 MYC murine medulloblastoma cell line, which mimics recurrent/refractory disease, was implanted orthotopically into BL6/J mice and evaluated for immune cell infiltration by multicolor flow cytometry and immunohistochemistry. Lenticsrprv2 containing gRNA targeting B7-H3 was used to knock out B7-H3 in vitro. These cells were subsequently evaluated using Incucyte spheroid assays, flow cytometry, western blot and bulk RNA sequencing analyses.

Results Proteomic profiling of IBA-1+ fractions was significantly different than that of other cells, primarily consisting of synaptophysin positive tumor cells. In line with other studies, we find the immune checkpoint molecule B7-H3 to be enriched in the non-IBA-1+ fraction. IBA-1+ cells infiltrating SHH versus non-WNT/non-SHH molecular subgroups cluster separately, with differential protein expression between subgroups, suggesting influence of the molecular drivers on the immunobiology of these tumors. In addition to PD-L1, we find increased expression of CTLA-4, TIM-3, and VISTA within the IBA-1+ component. Similar to human tumors, our syngeneic murine medulloblastoma cell line, mCB DNp53 MYC has a paucity of infiltrating lymphocytes and increased infiltration of microglia/macrophages. Both lymphoid and myeloid cells express VISTA in the microenvironment of medulloblastoma tumor-bearing mice. This model also demonstrates tumoral expression of B7-H3 in vitro and in vivo. To further investigate the influence of B7-H3 on both medulloblastoma and its microenvironment, we used CRISP/Cas9 to remove B7-H3 in this model. VSIG3, a VISTA binding partner, transcript increases when B7-H3 is knocked out.

Conclusions The finding of VISTA in the human and murine tumor microenvironment as well as the up-regulation of VSIG3 in the absence of B7-H3 could represent a unique immunosuppressive mechanism in high-risk medulloblastoma. Ongoing in vivo studies will investigate the interplay of tumoral B7-H3 with VISTA in the brain tumor microenvironment.

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REFERENCES

Ethics Approval Human data is fully de-identified and does not fall under Federal Regulations and is classified as ‘exempt’ but was performed under Einstein IRB 291217, ‘Staining of embryonal tumor tissue microarrays’. Informed consent was not required.

Animal work performed under Einstein IACUC approved protocol 0001069, ‘Evaluating Immune Checkpoints in Medulloblastoma’

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