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**P02.02** SINGLE-CELL RNA SEQUENCING OF NEUROBLASTOMA TUMORS REVEALS IMMUNOREGULATORY INTERACTIONS AS NOVEL TARGETS FOR IMMUNOTHERAPY

J Wienke*, WM Kholosy, LL Visser, KM Keller, P Lijnzaad, T Margaritis, KPS Langenberg, RR De Kriger, FCP Holstege, JJ Molenaar. Princess Maxima Center for Pediatric Oncology, Utrecht, Netherlands

Materials and Methods

Samples were enzymatically digested, single-cell FACS sorted and sequenced by Cel-Seq2 protocol. Samples were enzymatically digested, single-cell FACS sorted and sequenced by Cel-Seq2 protocol.

Results

Lymphoid cells in the TME consisted of αβ-, γδ-T cells, NK cells and B cells. Among αβ-T cells we identified CD8+ T cells, two functionally distinct clusters of CD4+ T cells, naïve-like T cells and FOXP3+ regulatory T cells (Tregs). CD8+ T cells had reduced cytotoxic capacity compared to blood-derived T cells from a reference group. Tregs expressed high levels of PRDM1, LAYN and ICOS, suggesting an effect functions. Treg profile, which is associated with increased inhibitory capacity. Although NK cells expressed the cytotoxic genes NKG7, KLRF1, GNL1, GZMB and PRF1, their expression was significantly lower than in blood-derived reference NK cells. Gene set enrichment analysis (GSEA) confirmed a reduced cytotoxic capacity of tumoral NK cells, which correlated with a decreased expression of activating receptors (r=0.41, p<0.001) and increased TGFβ signaling (r=-0.45, p<0.001). In addition, NK cells highly expressed the heterodimeric receptor KLRC1:KLRD1, which can inhibit NK cell function through HLA-E binding. High HLA-E expression by endothelial, immune and mesenchymal cells confirmed its inhibitory activity in the TME. Within the myeloid component we identified various immunosuppressive populations, comprising a cluster of IL10 and VEGFA expressing macrophages, three clusters of M2 differentiated macrophages expressing MMP9 and LGALS3, and dendritic cells with intact antigen presenting capacity, but high expression of numerous genes encoding immunosuppressive molecules such as IDO1, LGALS1, LGALS2, CCL22 and NECTIN2. In MYCN amplified tumors, we observed even lower cytotoxic capacity of CD8+ T and NK cells. We identified increased TGFβ1 expression and defective antigen presentation by myeloid and tumor cells as potential causes for reduced cytotoxicity in MYCN amplified tumors. To identify relevant targets for immunotherapy we constructed an unbiased interaction network, which revealed NECTIN1=CD96 and MIF=CD74 as active immunoregulatory interactions between tumor and T/NK cells, and CD80/CD86=CTLA4, CLEC2D=KLRB1, HLA-E=KLRC1/KLRC2, CD99=PILR1, LGALS9=HAVCR2, and NECTIN2=TIGIT between myeloid and T/NK cells.

Conclusions

Cytotoxic lymphocytes in the neuroblastoma TME show reduced cytotoxic capacity, likely due to highly immunosuppressive myeloid cells, Tregs and numerous immunoregulatory interactions, which may serve as novel targets for immunotherapy in neuroblastoma.

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**P02.03** AUTOMATED CELL TYPE SPECIFIC PD-L1 QUANTIFICATION BY ARTIFICIAL INTELLIGENCE USING HIGH THROUGHPUT BLEACH & STAIN 15-MARKER MULTIPLEX FLUORESCENCE IMMUNOHISTOCHEMISTRY IN HUMAN CANCERS

NC Blessin*, E Bady, T Mandelkow, C Yang, J Raedler, R Simon, C Fraune, M Lennartz, S Minner, E Burandt, D Hoffmaier, G Sauter, SA Weidemann. University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Materials and Methods

The quantification of PD-L1 (programmed cell death ligand 1) has been used to predict patient’s survival, to characterize the tumor immune microenvironment, and to predict response to immune checkpoint therapies. However, a framework to assess the PD-L1 status with high interobserver reproducibility on tumor cells and different types of immune cells has yet to be established.

Materials and Methods

To study the impact of PD-L1 expression on the tumor immune microenvironment and patient outcome, we used a fully automated PD-L1 quantification on tumor cells and immune cells was established and validated. Automated PD-L1 quantification was facilitated by incorporating three different deep learning steps for the analysis of more than 80 different neoplasms from more than 10,000 tumor specimens using a bleach & stain 15-marker multiplex fluorescence immunohistochemistry panel (i.e., PD-L1, PD-1, CTLA-4, panCK, CD68, CD163, CD11c, iNOS, CD3, CD8, CD4, FOXP3, CD20, Ki67, CD31). Clinopathological parameter were available for more than 30 tumor entities and overall survival data were available for 1,517 breast cancer specimens.

Results

Comparing the automated deep-learning based PD-L1 quantification with conventional brightfield PD-L1 data revealed a high concordance in tumor cells (p<0.0001) as well as immune cells (p<0.0001) and an accuracy of the automated PD-L1 quantification ranging from 90% to 95.2%. Across all tumor entities, the PD-L1 expression level was significantly higher in distinct macrophage/dendritic cell (DC) subsets (identified by CD68, CD163, CD11c, iNOS; p<0.0001) and in macrophages/DCs located in the Stroma (p<0.0001) as compared to intratumoral macrophages/DC subsets. Across all different tumor entities, the PD-L1