FUNCTIONAL AND TRANSCRIPTOMIC PROFILING OF MYELOID CELLS IN PEDIATRIC SOLID TUMORS TO INFORM NEXT GENERATION IMMUNOTHERAPY

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Background Monocytes are innate immune cells recognized for their ability to play both tumor permissive and surveillant roles in cancer. The heterogeneity of monocyte function can be guided by tumor-derived factors. Phenotypic and transcriptional alterations in circulating monocytes and other myeloid-derived cells in patients with solid tumors have been reported and associated with clinical outcomes. However, specific monocyte functions and their perturbations in the setting of solid malignancies have not been well explored.

Methods Primary monocytes isolated from healthy donors and pediatric patients with sarcoma were used to examine the production of reactive oxygen species (ROS) via staining with 2′,7′-dichlorofluorescin diacetate (DCFDA). Monocyte-mediated phagocytosis of tumor cells was tested by co-culture with the HuO9-H3 human osteosarcoma cell line. Functional populations from healthy donor monocytes were sorted by FACS and bulk RNA-seq was performed on the sorted samples for transcriptomic profiling.

Results We found that monocytes are functionally heterogeneous and identified populations of low and high ROS production as well as phagocytic and non-phagocytic cells. Phagocytosis was dysregulated in patients when compared to healthy donors. In addition, phagocytosis significantly increased when tumor cells were pre-incubated with anti-CD47, a blocker of the “do not eat me” signal on tumor cells (53.16% vs 12.06%, p=0.0456). We successfully enriched for these functional populations from three healthy donors by FACS. Pathway enrichment analysis revealed key inflammatory processes increased in phagocytic cells such as GM-CSF, VEGF and PDGF signaling. Of note, phagocytic monocytes of tumor cells blocked with anti-CD47 had downregulated inflammatory signaling and increased anti-inflammatory PPAR signaling, compared to those from the isotype control. In addition, phagocytic cells had higher expression of MHC class II surface receptors than nonphagocytic cells suggesting an activation of antigen presentation.

Conclusions Myeloid ROS production and phagocytosis can provide circulating markers of tumor microenvironment activity. Phagocytosis of tumor cells triggers transcriptional changes in monocytes related to inflammatory and antigen presentation responses. Such changes are dampened when levels of phagocytosis were increased by the addition of anti-CD47, highlighting the importance of evaluating the delicate balance between pro- and anti-tumoral myeloid function. The incorporation of functional selection with -omic characterization provides insights into our understanding of monocyte-function in solid malignancies, which will in turn inform the design of myeloid-mediated therapies.

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Ethics Approval This study is exempt research as determined by NIH Institutional Review Board.