

THE ROLE OF VSIG4 AS AN IMMUNO-REGULATORY PROTEIN IN CANCER

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Background V-set and Ig domain-containing 4 (VSIG4; also known as CRIg) is a myeloid-restricted complement receptor indispensable for phagocytosis of C3-opsonized bacteria. Being a B7-family member, Vsig4 has also been described as a negative regulator of T cell activation. Murine Vsig4 expression is restricted to tissue-resident macrophages and notably absent in tumor-infiltrating macrophages. In contrast, human Vsig4 expression is more promiscuous- observed on circulating monocytes, dendritic cells, and macrophages within the ovarian tumor micro-environment (TME). Importantly, Vsig4 expression in the TME correlates with increased tumor burden and poor prognosis. Whether Vsig4 signals through a T cell counter-receptor is unknown. Similarly, the immuno-regulatory effects on tumors by non-infiltrating VSIG4⁺ tissue-resident macrophages have not been studied.

Methods Tumor growth inhibition was assessed *in vivo*. Peritoneal, splenic and intra-tumoral macrophages were analyzed by flow cytometry. Single-cell RNA sequencing was performed on human ovarian tumor tissue and on murine melanoma.

Results Vsig4 was detected on both resting and thioglycolate-elicited peritoneal macrophages as well as liver-resident Kupffer cells with little to no expression in splenic macrophages. *In vitro* differentiation of bone marrow precursors to M1 or M2 macrophages showed no differential expression of respective hallmark genes (NOS2 and Fizz-1) between wild-type and Vsig4^{-/-} precursors. Initially, our *in vivo* studies focused on the highly metastatic 4T1 model of breast cancer implanted orthotopically and found minimal Vsig4 expression (gene and protein) across different stages of tumor growth. However, when we implanted B16-F10 melanoma cells in VSIG4^{-/-} mice, we observed robust tumor growth inhibition relative to wild-type mice. A similar phenotype was seen in bone marrow chimeras (myeloablated wild-type recipients of Vsig4^{-/-} bone marrow), confirming the anti-tumor response was driven by the immune compartment. A dampened anti-tumor response in clodronate liposome-treated Vsig4^{-/-} mice confirmed that the phenotype was dependent on macrophages. Yet, conditional deletion of Vsig4 in macrophages (using a LysM-cre delete and Vsig4^{fl/fl} mice) did not phenocopy global knockouts when implanted with B16-F10 melanoma. *In vivo* Vsig4 expression kinetics revealed presence of Vsig4⁺ myeloid sub-populations in the bone marrow and liver of B16-F10 tumor-bearing mice with no early induction detected in the tumor or spleen.

Conclusions Vsig4 expression dynamics from myeloid progenitor to macrophage/ dendritic cell is being mapped. Transcriptomic/metabolic analyses of Vsig4⁺ myeloid populations from tumor-bearing mice will elucidate the temporal contribution of Vsig4 to immune regulation in cancer. Our goal is to develop Vsig4-targeting modalities targeting Vsig4 potentially in combination with PD-L1/1 and reset T-cell immunity in tumors.

Ethics Approval The study was approved by the Johns Hopkins OHSR-IRB (# IRB00287038). Participants gave informed consent before taking part.

All animal work was approved by the Animal Care and Use Committee (Protocol # MO22M67).

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