Functional characterization of the inhibitory activity and identification of novel T-cell receptors for the tumor-associated macrophage receptor VSIG4

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Abstract

Background: Tumor associated macrophages (TAMs) are important regulators of immunosuppression in the tumor microenvironment (TME) and are often associated with poor clinical responses in patients. VSIG4 (V-Set and Immunoglobulin domain containing 4) is a negative checkpoint regulator (NCR) and its expression has been well established on both tissue resident macrophages and TAM populations [1-3]. However, the inhibitory molecular mechanism of VSIG4 remains largely unknown [1, 2] and many therapeutic strategies targeting TAMs in general have been limited either by a lack of TME-specificity or high target abundance leading to target-mediated drug disposition (TMDD) [4,5]. The development of immunomodulatory antibodies that selectively target antigens in acidic environments, such as the TME, has the potential to increase tumor exposure and reduce toxicity [6].

We have characterized endogenous expression patterns of VSIG4 and a significant induction on differentiated primary macrophages under conditions similar to the TME. Additionally, we have performed a proteomics screen for potentially novel T-cell receptor(s) that interact with VSIG4.

Methods: Endogenous expression patterns of VSIG4 were characterized in polarized macrophage populations. The induction of VSIG4 expression in polarized macrophage populations was demonstrated by both flow cytometry and RNA expression analysis. A Ligand Receptor Capture-Trifunctional Chemoproteomic Reagents (LRC-TriCEPS)-based proteomics strategy [7] was used to identify receptors on primary human T-cells that interact with recombinant VSIG4 protein.

Results: Our results show a robust upregulation of VSIG4 expression in polarized human macrophage populations. Additionally, we established multiple functional assays demonstrating VSIG4-mediated suppression of primary human T-cells. Finally, the LRC-TriCEPS-based proteomics screen yielded novel candidate receptors that interact with VSIG4.

Conclusions: VSIG4 inhibits human T-cell activation in multiple assay formats and through numerous functional measurements. A group of T-cell receptors was found to be involved in novel interactions with VSIG4, and their potential roles in VSIG4-mediated regulation are currently being validated. We believe our work will lead to an enhanced mechanistic understanding of how VSIG4 suppresses T-cell activation and provide a strategy and tools for discovery of therapeutic relevant anti-VSIG4 antibodies.

Background and Methodology

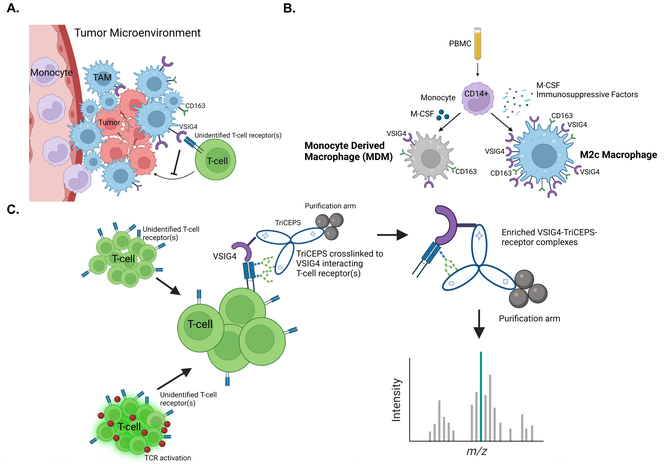

Figure 1. VSIG4 is a TAM-associated antigen that suppresses anti-tumor T cell responses. (A) VSIG4 expression on TAMs and suppression of anti-tumor T-cell responses. **(B)** VSIG4 expression is endogenously upregulated in human macrophages polarized by immunosuppressive stimuli. **(C)** Ligand Receptor Capture-Trifunctional Chemoproteomics (LRC-TriCEPS) strategy to identify human T-cell receptor(s) that interact with VSIG4.

Results

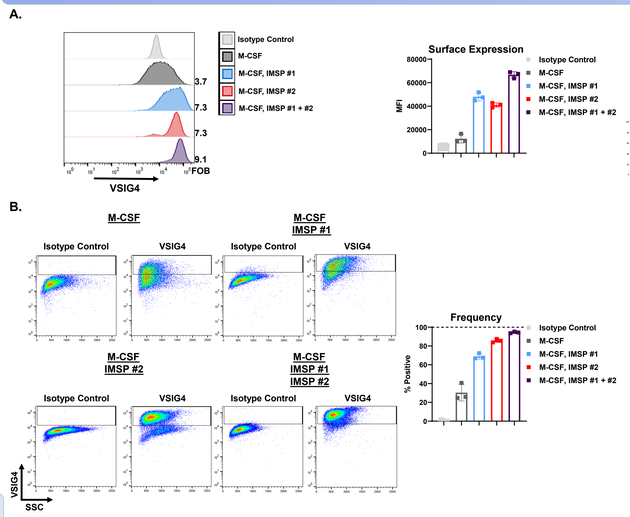

Figure 2. Induction of VSIG4 expression in polarized human macrophage populations. Primary monocytes were purified from PBMCs and differentiated into macrophages under indicated conditions. **(A)** Flow cytometry analysis demonstrated VSIG4 surface expression in macrophage populations treated with M-CSF and IMSP (Immunosuppressive Stimulation). **(B)** Frequency of VSIG4 positive cells in macrophage populations treated with M-CSF and IMSP (Immunosuppressive Stimulation). Analysis represent 3 healthy donors. FOB = Fluorescence Over Background. MFI = Mean Fluorescence Intensity. M-CSF = Macrophage Colony Stimulating Factor. Error bars represent SEM.

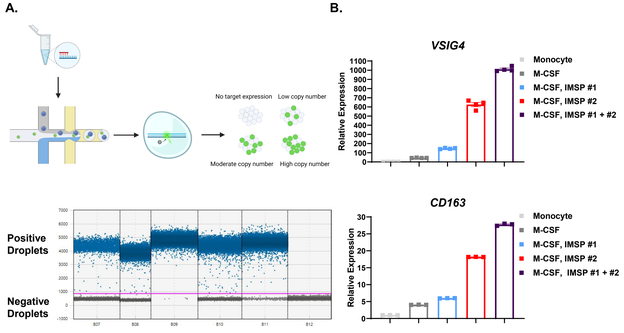

Figure 3. Induction of *VSIG4* and the M2c-marker *CD163* expression in polarized human macrophage populations. (A) Principles, workflow and analysis of droplet digital PCR (ddPCR). ddPCR is a methodology based on the partitioning of water-oil emulsions into individual, nanoliter-sized droplets. A fluorescent probe is then incorporated within each individual droplet through PCR amplification. The partitioning allows for the measurement of thousands of independent amplification events within a sample. Measurements of positive and negative droplets in two optical channels are used to determine target concentration. **(B)** Multiplexed ddPCR analysis revealed gene expression of *VSIG4* and *CD163* in polarized macrophage populations. Similar to surface expression of VSIG4 (Figure 2), the combination of multiple immunosuppressive stimulations led to a robust induction of *VSIG4*. There was also an induction of *CD163* in the polarized macrophage populations. Graphs represent fold induction relative to monocyte sample. Analysis represents results from 2 healthy donors. Error bars represent SEM.

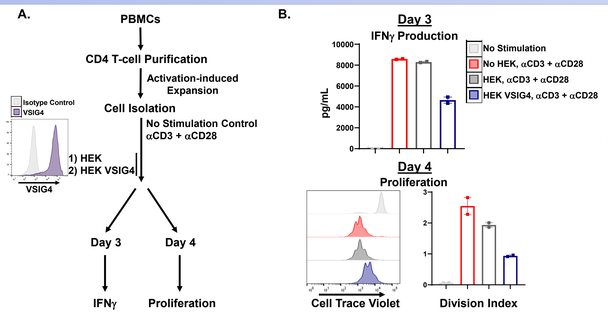

Figure 4. Cell surfaced expressed VSIG4 suppresses primary human T-cell activation. (A) Primary CD4 T-cells were subjected to activation-induced expansion. Human Embryonic Kidney (HEK) cell line over-expressing VSIG4 was internally generated and cocultured with the T-cell blasts. HEK cells have limited to no endogenous expression of VSIG4. **(B)** Functional measurements of T-cell suppression. Analysis represented 3 healthy donors. Error bars represent SEM.

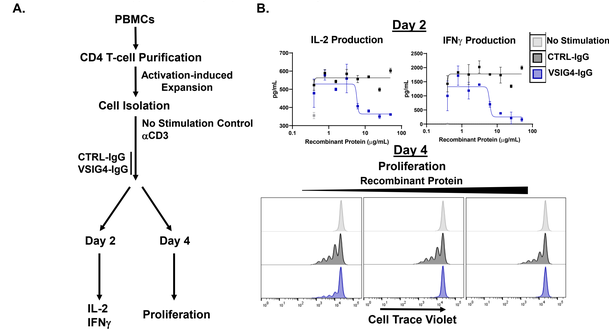

Figure 5. Immobilized VSIG4 suppresses primary human T-cell activation. (A) Primary CD4 T-cells were expanded and subjected to inhibition by immobilized VSIG4-IgG. **(B)** Functional measurements of T-cell suppression. Analysis represented 3 healthy donors. Error bars represent SEM.

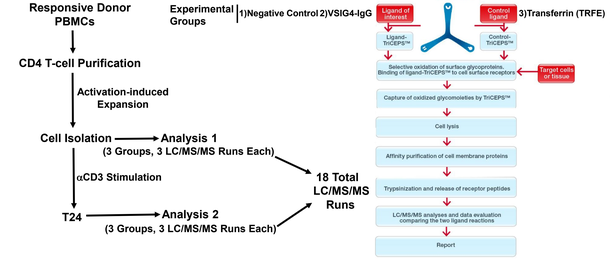

Figure 6. VSIG4 Ligand Receptor Capture (LRC)-TriCEPS experimental design. (A) Primary T-cell blasts from a donor responsive to immobilized VSIG4-IgG-mediated inhibition (Figure 5) was subjected to an LRC-TriCEPS based proteomics screen in the presence and absence of αCD3 stimulation. **(B)** Experimental groups and LRC-TriCEPS workflow.

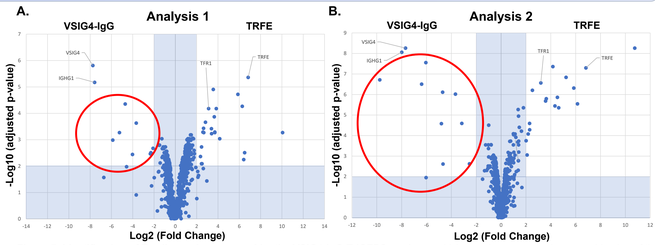

Figure 7. Identification of T-cell proteins captured by the VSIG4-IgG-TriCEPS conjugate. Volcano plots representing a combination of the magnitude of protein enrichment and statistical significance. Each point indicated a different protein. In both sets of analysis, there was significant enrichment of peptides that represented the VSIG4-IgG ligand. There was also significant enrichment of peptides that represented the positive control ligand sample, TRFE. TRFE-TriCEPS conjugate identified Transferrin Receptor 1 (TFR1), a well-established receptor for TRFE. Red circles indicated proteins that were significantly enriched in the VSIG4-TriCEPS samples.

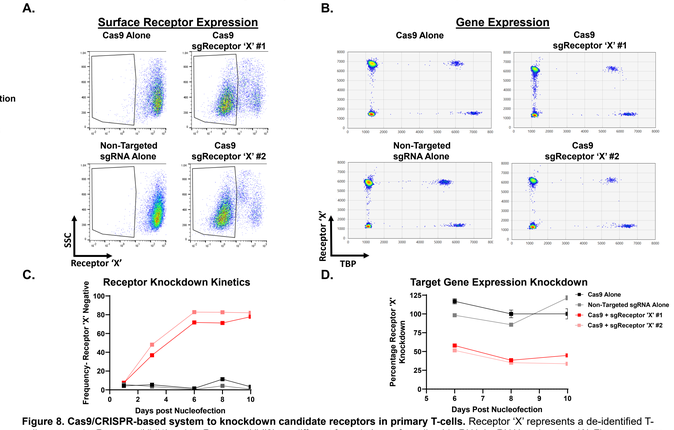

Figure 8. Cas9/CRISPR-based system to knockdown candidate receptors in primary T-cells. Receptor 'X' represents a de-identified T-cell receptor. 'sgReceptor 'X' #1' and 'sgReceptor 'X' #2' are different formulations of small guide RNA (sgRNA) molecules. **(A)** Flow cytometry analysis demonstrated knockdown of receptor surface expression. Representative scatter plots are from day 6 post nucleofection. **(B)** Multiplexed ddPCR analysis revealed knockdown of receptor 'X' mRNA transcript. TATA-Binding Protein (TBP) is a manufacturer-recommended house keeping gene. **(C)** Kinetics and time course of the surface knockdown of receptor 'X'. **(D)** Time course of receptor 'X' mRNA transcript knockdown. 'Percentage Receptor 'X' Knockdown' represents the ratio of receptor mRNA transcript levels in indicated groups relative to the Cas9 Alone control. Error bars represent SEM.

Summary

- **VSIG4 is a TAM-expressed antigen that is robustly upregulated by immunosuppressive polarization.**
- **VSIG4 suppresses primary human T-cell activation as both an immobilized recombinant protein and cell surface expressed receptor.**
- **An LRC-TriCEPS-based proteomics screen was performed in a primary human T-cell population that was responsive to VSIG4-mediated inhibition. The screen was technically successful and identified a statistically significant set of VSIG4-interacting T-cell receptor candidates.**
- **A Cas9/CRISPR-based methodology target gene knockdown was developed in primary human T-cells. This strategy will be employed for validation of VSIG4-interacting T-cell receptor candidates.**
- **As part of the SNS-102 program, pH-selective anti-VSIG4 antibodies have been identified. A lead antibody set is currently undergoing further optimization.**

1) Liao et al. *Laboratory Investigation.* 94. (2014) 2) Zang et al. *J Clin Invest.* 116. (2006) 3) Helmy et al. *Cell.* 5. (2006) 4) Ries et al. *Cancer Cell.* 252. (2014) 5) Graversen et al. *Mol Ther.* 20. (2012) 6) Johnston et al. *Nature.* 574. (2019) 7) Frei et al. *Nat Biotechnol.* 30. (2012) § Created with BioRender