Structure and Functional Binding Epitopes of V-domain Ig Suppressor of T-Cell Activation (VISTA)

V-domain Ig Suppressor of T-cell Activation (VISTA) is an immune checkpoint protein involved in the regulation of T cell activity. Checkpoint proteins are overexpressed by cancer cells or surrounding immune cells and prevent anti-tumor activity by co-opting natural regulation mechanisms to escape immune clearance. Compared to healthy tissues, VISTA is upregulated on tumor infiltrating leukocytes, including high expression on myeloid-derived suppressor cells (MDSCs). Through VISTA signaling, these inhibitory immune cells prevent effective antigen presentation and indirectly promote tumor growth. VISTA is implicated in a number of human cancers including skin (melanoma), prostate, colon, pancreatic, ovarian, endometrial, and non-small cell lung. VISTA is a known member of the B7 protein family but the mechanism of action is still unclear as VISTA has been shown to function as both a ligand[1,2] and a receptor[3]. In the model of VISTA as a receptor, the proposed ligand of interaction is V-set and immunoglobulin domain containing 3 (VSIG3)[4,5].

Antibodies against VISTA have shown anti-tumor efficacy in multiple syngeneic mouse models[1]. Therapeutic development has progressed to human clinical trials with the development of an anti-human VISTA antibody (VSTB11 called 'VSTB' in this study). VSTB inhibits VISTA signaling *in vitro* and shows tumor regression in a murine model of bladder cancer. Putative regions of interaction between VSTB and VISTA have been proposed, but a specific binding epitope has not been identified. The advancement of targeted biologics and small molecule compounds are hampered by the absence of three-dimensional structural information for VISTA.

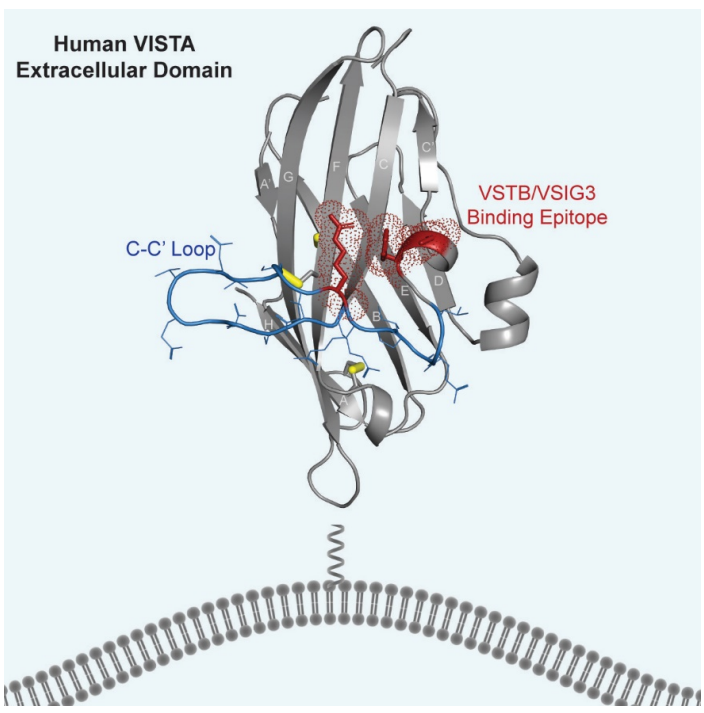

Figure 1. Structure of human VISTA with extended C-C' loop (blue), mapped VSTB/VSIG3 binding epitope (red), and disulfide bonds (yellow).

To better understand the mechanisms of action and further development of VISTA therapeutics, a research team brought together by Stanford Bioengineering graduate student Nishant Mehta and led by Profs. Jennifer Cochran and Po-Ssu Huang determined the 1.85 Å crystal structure of the elusive human VISTA extracellular domain. The work was performed in collaboration with staff scientist Irimpan Mathews at SSRL, with contributions from Bioengineering undergraduate student Sainiteesh Maddineni and graduate student Andres Parra Sperberg. After attempting molecular replacement with 10,000 VISTA models, the structure was solved with a combinatorial MR-Rosetta approach and the incorporation of highly redundant sulfur SAD data. The structure represents a common Ig-like fold, but closer examination reveals important differences that make VISTA unique among B7 family proteins. VISTA consists of ten beta strands, instead of the canonical nine, and three alpha helices arranged in a beta-sandwich formation (Figure 1). The protein fragment between

strands C and C′ is comprised of a unique 21 residue turn that form an extended loop. The conventional fold of the B7 family is comprised of two distinct domains, an IgV domain with nine beta strands and an IgC domain with seven beta strands. Of the seven B7 family proteins that have been crystallized, VISTA is the only family member that lacks an IgC domain. VISTA also contains an extra two disulfide bonds, one of which holds the extended C-C′ loop in a unique, protruding position which may play a role in dimerization or protein-protein interaction within the cell membrane.

An epitope-mapping technology known as yeast surface display was used to determine the binding interface of the VSTB antibody, a known inhibitor of VISTA signaling, on human VISTA. A library of VISTA mutants displayed on yeast was screened to determine the individual residues of VISTA that underlie binding to VSTB. Residues R54, F62 and Q63 were found to drastically affect binding to VSTB without altering structure of VISTA significantly. The three residues found to dictate VSTB binding were also tested for their influence on the purported VISTA binding partner VSIG3 using an ELISA-based assay. Wild-type VISTA bound to VSIG3 with an apparent affinity constant of ~2 μM while the 54A/62A/63A triple mutant bound with a significantly weaker apparent affinity of >20 μM. This result suggests that VISTA binding to VSIG3 is highly dependent on three of the same mutations that comprise the VSTB binding epitope.

In this study, the authors highlight features that make the VISTA IgV-like fold unique among B7 family members, isolate the binding epitope to an inhibitory anti-VISTA antibody, and propose overlap of this antibody-binding region with the VSIG3 binding epitope. The structure and functional epitope presented here will help guide future drug development efforts against this important checkpoint target.

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