Background: Programmed cell death (PD)-1 is an important inhibitory receptor in T cells. Antibodies targeting PD-1 elicited clinical responses in multiple tumors. Nevertheless, response is limited to a fraction of patients and thus, understanding PD-1 signaling should provide novel biomarkers of response and new therapeutic targets.

Methods: Here we used mass spectrometry to uncover proteins that interact with the cytoplasmic tail of PD-1. PD-1 ligation is known to induce binding of the phosphatase SHP2 to the cytoplasmic tail of PD-1, which dephosphorylates signaling proximal components of the T cell receptor, hence preventing T cell activation. We found several novel PD-1-interacting proteins. Two of them, EFHD2 (EF-Hand-domain-family-member-D2) and SAP (SLAM associated protein), were analyzed for their contribution to PD-1 inhibitory functions.

Results: SAP over-expression blocked PD-1 effects by inhibiting SHP2 activity. In contrast, silencing EFHD2 abrogated PD-1 effects, including elimination of the ability of PD-1 to inhibit cytokine secretion and cell proliferation. EFHD2 co-localized with PD-1 in the immunological synapse where it contributed to PD-1 clustering.

Conclusions: Studying PD-1 interactome has identified new proteins that can modulate PD-1 function in opposite ways. Additional proteomics-based analysis will be utilized in Sheba Medical Center to discover novel immunotherapy targets.

The approach: GST affinity purification

Schematic of the different versions of the GST-PD-1 tails. The phospho-deficient tail has two mutations at positions 223 and 248 (the tyrosine was switch to phenylalanine).

Subtractive analysis of the results

Venn diagrams schematic of the subtractive analysis. GST-PD-1 pulldown was performed in triplicates on pervanadate-treated Jurkat T cells and the interacting proteins were analyzed by mass spectrometry.

EFHD2: 239 AA. EFHD2 KO mice had an augmented inflammatory response to parasitic stimuli (Eur. J. Immunol. 2014, 44: 3206–3219) and also develop spontaneously anti ds-DNA antibodies

EFHD2 is necessary for PD-1 inhibitory effect on IL-2 secretion. Overexpression of EFHD2 rescues PD-1 inhibitory effect on IL-2 secretion. EFHD2 is co-localized with PD-1 at the plasma membrane and in the immunological synapse (Raji-Jurkat conjugates). EFHD2 KD reduced PD-1 clustering at the immunological synapse. PTC: primary T cells.

SAP blocks PD-1 functions (PNAS 2018)

A. Jurkat T cells expressing PD-1-GFP and treated with pervanadate, followed by lysis and immunoprecipitation with anti-GFP antibodies. WCL = whole cell lysate. IB: immunoprecipitation. IB: immunoblotting. SAP was immunoprecipitated in GFP-PD1 expressing cells but not in GFP expressing cells. B. SHP2 and SAP tested for their interaction with different versions of PD-1 tails. Both SHP2 and SAP interact with the ITSM (248) but not with ITIM (223). C. SAP is not necessary for PD-1 inhibitory functions (IL-2 secretion). D. Overexpression of SAP blocks PD-1 inhibitory effect on IL-2 secretion.

Conclusions: Studying PD-1 interactome has identified new proteins that can modulate PD-1 function in opposite ways. Additional proteomics-based analysis will be utilized in Sheba Medical Center to discover novel immunotherapy targets.

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