An interaction map for HTLV-1 Tax and PDZ-containing proteins.

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Summary

Human T-cell leukemia virus type 1 (HTLV-1) retrovirus encodes for the Tax protein, which has a transforming capacity *in vitro*. Tax contains at its C-terminus a binding motif for PDZ domain-containing proteins (PSD95-DLG1-ZO1). It has been shown that the C-terminal motif of Tax is involved in Tax oncogenic capacity. Ten different PDZ domain-containing proteins have been reported to interact with Tax, but the specificity of Tax-human PDZome interactions has not been investigated. The objective of this study is to obtain a comprehensive interactome map for Tax and the human PDZome and to determine a global role of Tax-PDZ interactions in HTLV-1 biology.

Results

The Tax/PDZ interactome map

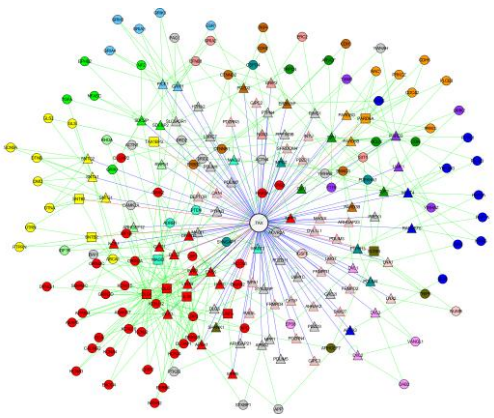


Fig. 1: Several protein-protein interaction methods were used to generate this network: predicting interaction *in silico*, yeast two-hybrid (Y2H) and GST pulldown. In addition to Tax, there are 212 proteins in this network: 100 Tax partners (n) and 112 partners of Tax partners (n+1). The squares (with red edges) represent the Tax partners already known in the literature (10 proteins). The triangles (with blue edges) represent novel Tax partners that we found (90 proteins). The circles represent the partners of Tax partners (112 proteins) reported in databases. The nodes with the same color denote a cluster (the gray nodes don't belong to any cluster: 56 proteins). Proteins surrounded by a red outline don't have any known interactor in the literature (22 proteins).

2. Clustering analysis of Tax/PDZome network

Table 1: In this table, we have reported the most important biological functions associated with each cluster of the network. Interestingly Several other clusters in our network are also composed of protein involved in membrane polarization, shape or traffic. This is consistent with known roles of some PDZ containing proteins such as DLG or SCRIB implicated in immunological synapse formation.

Cluster	Number of nodes	term ID	Biological function
1	49	GO:0006812	cation transport
		GO:0007268	synaptic transmission
		GO:0007610	behavior
		GO:0022607	cellular component assembly
		GO:0030030	directional locomotion
		GO:0051899	membrane depolarization
		GO:0007163	establishment or maintenance of cell polarity
2	15	GO:0060048	muscle contraction
		GO:0019698	Gbi42 protein signal transduction
		GO:0006541	glutamine metabolic process
3	12	GO:0002089	lens morphogenesis in camera-type eye
		GO:0007267	cell-cell signaling
		GO:0043230	cell junction organization
4	10	GO:0007155	cell adhesion
		GO:0022603	regulation of anatomical structure morphogenesis
		GO:0022610	biological adhesion
		GO:0071681	cellular response to indole-3-methanol
		GO:0045216	cell-cell junction organization
5	9	GO:0045216	cell-cell junction organization
		GO:0035088	establishment or maintenance of apical/basal cell polarity
		GO:0043552	positive regulation of phosphatidylinositol 3-kinase activity
		GO:0007163	establishment or maintenance of cell polarity
		GO:0030029	actin filament-based process
		GO:0051301	cell division
6	7	GO:0030031	cellular component disassembly involved in apoptotic process
7	7	GO:0035640	regulation of cell communication
8	7	GO:0007215	glutamate receptor signaling pathway
9	6	GO:0042307	apical junction assembly
10	6	GO:0042995	cell projection
		GO:0031256	leading edge membrane
		GO:0031252	cell leading edge
		GO:0044463	cell projection part
11	6	GO:0007164	establishment of tissue polarity
		GO:0016055	Wnt receptor signaling pathway
12	6	GO:0007277	regulation of G-protein coupled receptor protein signaling pathway
13	5	GO:0019717	synaptosome
		GO:0030054	cell junction
14	4	GO:0043615	astrocyte cell migration
15	4	GO:0021849	neuroblast division in subventricular zone
		GO:0051260	protein homooligomerization
16	4	GO:0007409	axonogenesis
		GO:0010741	negative regulation of intracellular protein kinase cascade

3. Comparison of different protein-protein interaction assays

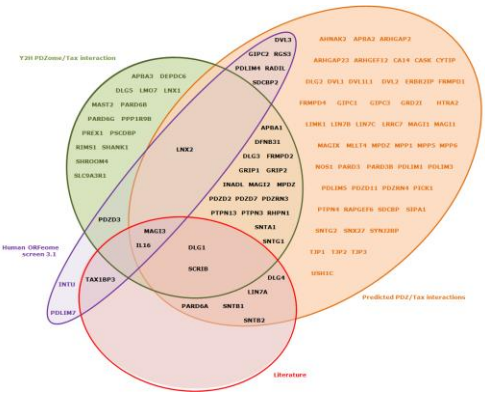
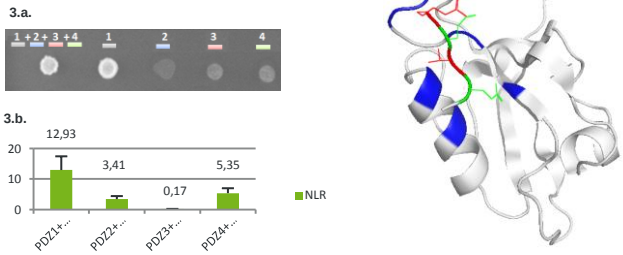


Fig. 2: Among the 100 PDZ proteins, IL16, MAGI3 and LNX2 are found by the three methods used, but only LNX2 is not characterized in the biology of HTLV-1. The advantage of combining several methods in this analysis is illustrated by the validation of all Tax/PDZ interactions previously characterized in the literature.

4. PDZ domain 1 of LNX2 is responsible for the interaction with Tax



PDZ1 248 251 255
TIEIHRSNPYIQLGI S IV G GNE TPLINIVIQEVYRDGVIARDGR LLAGDQILQVNNYNISNVS H NYA R AVLSQPCNTLHLTVLRER
254 296 300

Tax Ct 349 351 353
R E T E V
350 352

Fig. 3: We tested the interaction of LNX2 mutants with Tax by Y2H (3.a.) and Luciferase Complementary Assay (3.b.) and determined that the PDZ1 is responsible for the interaction. We also identified by "docking" (3.c.) 6 amino acids at the PDZ1 as potentially responsible for the interaction with Tax.

Conclusions

- By using different protein-protein interaction methods we have generated a Tax/human PDZome interaction map. We then performed a clustering analysis to define biological functions associated with Tax/PDZ interactions. PDZ Proteins involved in cell shape, cytoskeleton organization and membrane polarization and traffic were overrepresented.

- We then focused on LNX2 protein and tested its individual PDZ domains. We found that the first PDZ domain of LNX2 is responsible for the interaction with Tax. Furthermore, we have demonstrated by "docking" analysis that 6 amino acids from PDZ1 could be implicated in the interaction with Tax.

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