Defining and targeting the HTLV-1 Tax and PDZ proteins interactome.

Karim Blibek, Naoaki Fujii, Sebastien Legros, Mathieu Boxus, Jean-Francois Dewulf, Pascale Zimmermann, Richard Kettmann, Jean-Paul Borg, Franck Dequiedt and Jean-Claude Twizere.

GIGA-Research, Signal Transduction Unit, Protein Signaling & Interactions Lab, University of Liege. Belgium.
Email: jean-claude.twizere@ulg.ac.be

Summary

Primate T-lymphotropic virus species comprise four members (HTLV-1 to -4) that have been discovered in human. Only the HTLV-1 infection leads to adult T-cell leukemia/lymphoma (ATLL) and tropical spastic paraparesis (TSP), an immune degenerative neurologic syndrome. All the four viruses share a similar genomic organization and encode transforming Tax oncoproteins. In contrast to HTLV-2 and 4, HTLV-1 and 3 Tax proteins contain a PSD-95/Drosophila Discs Large/Zona Occludens-I (PDZ) binding motif at their C-terminal that has been shown to play crucial roles in the distinct transforming properties of the Tax proteins. To systematically investigate PDZ-containing proteins roles in HTLV-1 biology, we initiated a global interactome network analysis of Tax and associated human PDZ-containing proteins. This was accomplished through the use of our framework of binary interactome mapping that includes stringent yeast two hybrid and pulldown screening, systematic retesting by protein complementation assay and evaluation of PDZ gene expression in T lymphocytes.

Results

Fig. 1

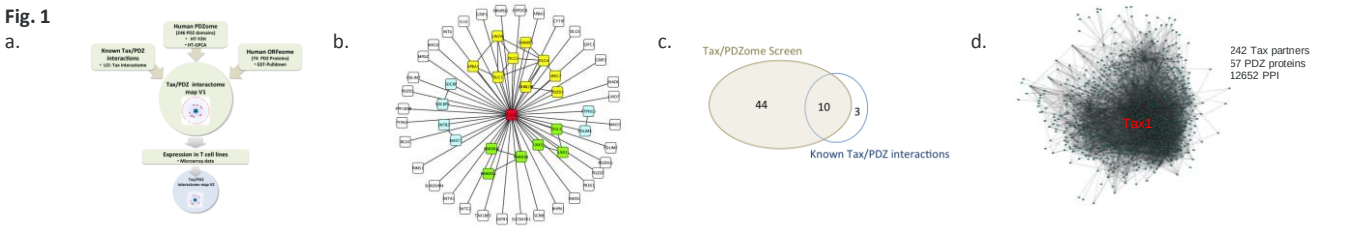

Fig. 1. Tax/PDZ protein Interactome. a) Experimental pipeline: a protein array containing 96% of the human PDZ domains was screened by HT-Y2H and interactions were retested by protein complementation assay in mammalian cells (HT-GPCA). Known Tax/PDZ interactions were manually curated from the literature (LCI). The human ORFeome is a collection of 15000 cloned human genes. This collection contains 74 PDZ ORFs that were tested for their association with Tax1 using GST-pulldown. We combined all the data in our Tax/PDZ interactome version 1. Expression of PDZ genes was examined in 24 different T cell lines and a pool of 5 HTLV infected T cells using microarray, resulting in dynamic Tax/PDZ interactomes in T cells. **b)** A physical Tax/PDZ interactome map : small networks of 2 (light blue), 3 (light green) and more than 3 (yellow) PDZ partners are shown. **c)** Comparison to reported Tax/PDZ interactions. 77% of reported interactions were found in our screen. **d)** A Tax-1/human interactome : We combined our novel Tax/PDZ interactions with other reported Tax-1 interactioons and, as protein do not act alone but as complexes, we also included first degree common interactors of Tax partners to obtain a network of 12652 interactions (PPI) between 841 proteins. Tax/PDZ associations represent 7% of the whole network, suggesting a crucial role of PDZ proteins in multi-protein Tax complexes assemply.

Fig. 2

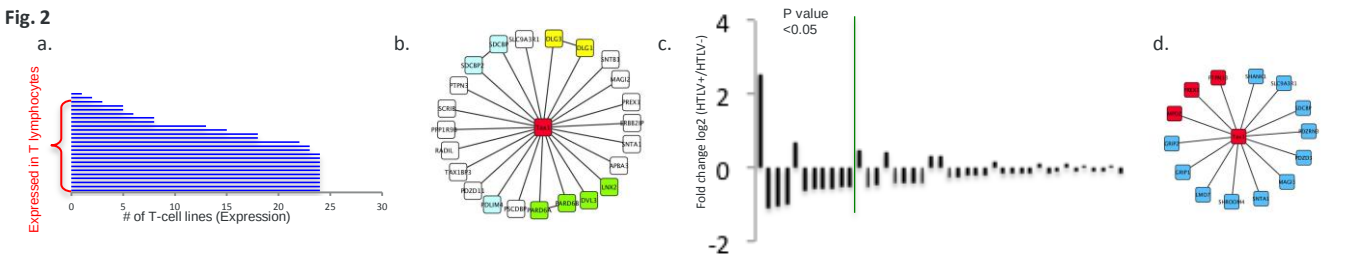

Fig. 2. PDZ proteins expression on T cells. a) Expression of PDZ genes was examined in 24 different T cell lines by microarray. The graphs represent PDZ genes and the number of T cell lines with significant expression intensities. **b)** A dynamic Tax/PDZ interactome map : Nodes represent PDZ genes significanty expressed in T cell lines. **c)** Comparative expression of PDZ genes was examined in 5 HTLV+ and 5 HTLV- cell lines by microarray. The graph represents PDZ genes and their fold change expression in HTLV+ versus HTLV- cell lines. **d)** A dynamic Tax/PDZ interactome map : Nodes represent PDZ genes differentially expressed in HTLV+ compared to HTLV- cell lines. Red and blue nodes correspond to overexpressed and underexpressed genes, respectively.

Fig. 3

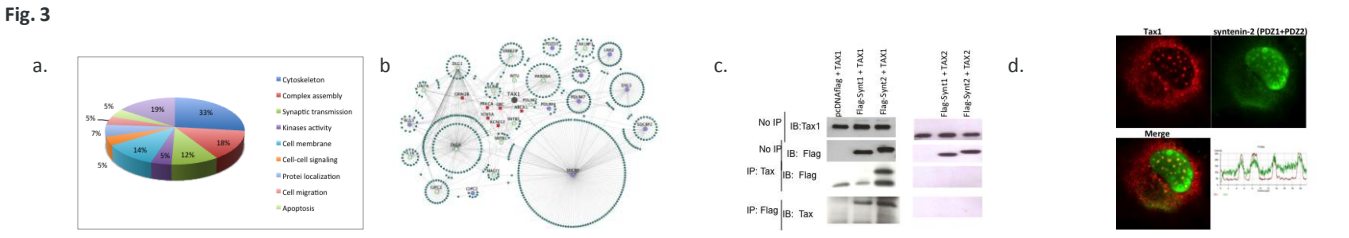

Fig. 3. Detecting of the syntenin 1 and 2 as potential important Tax partner. a) Distribution of GO terms annotations across 57 PDZ genes in the Tax interactome. **b)** The cytoskeleton subnetwork of Tax/PDZ interactome. Light green nodes represent PDZ proteins known to interact with Tax. Blue nodes represent PDZ proteins identified in our screen. Red nodes are common interactors for different PDZ proteins. This subnetwork identifies syntenin (SDCBP) as an important « hub » in the Tax/PDZ cytoskeleton organization. **c)** HEK 293T cells were transfected with Flag-Synt1, Flag-Synt2, HTLV-1 andHTLV-2b Tax expression constructs, as indicated. Forty eight hours post-transfection, cells were lysed and protein expression verified by immunoblotting using anti-Tax-1, anti-Tax-2 and anti-flag-specific antibodies. The lysates were immunoprecipitated using Tax or Flag specific antibodies. Immunoprecipitated proteins were analysed by SDS-PAGE and immunoblotting using an anti-Flag, anti- Tax antibodies. **d)** HeLa cells were transfected with Flag-syntenin-2 and HTLV-1 Tax-expressing constructs. Twenty-four hours post-transfection, cells were fixed, permeabilized and stained with anti-Flag or anti-Tax-1 antibodies and Alexa 488 or Alexa 633 -conjugated secondary antibodies. Cells then were analyzed using a confocal microscope.

Fig. 4

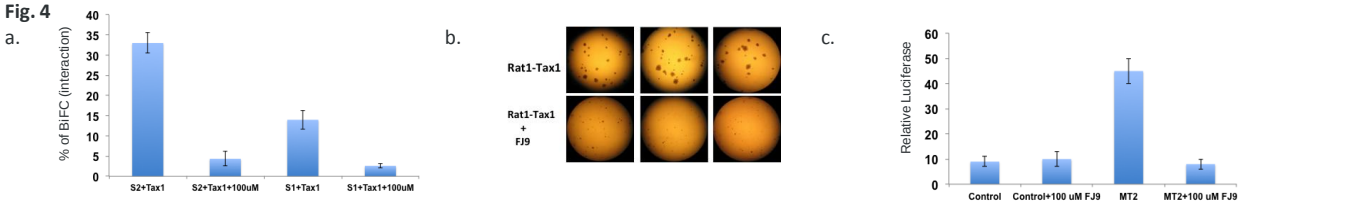

Fig. 4. Fj9 molecule could antagonize Tax-transformation activity and cell-to-cell transmission of HTLV-1. a) HEK 293T cells were transfected with the BiFC C-terminal fragment fused to syntenin-1 or –syntenin-2, and BiFC N-terminal fragment fused HTLV-1 Tax expression constructs, as indicated. Twenty four hours post-transfection, cells were treated with 100 uM of FJ9 or DMSO and cultured for an additional 24H-period. Cells were analysed by flow cytometry. Results are mean and SD of three independent experiment. **b)** Rat-1 cells were stably infected with pTax-IB lentivirus, and pools of resistant cells (5×10^3 cells) were seeded in 0.33% agarose-containing DMEM-10% FCS, 100 uM of FJ9 or vehicle (DMSO) overlaid on 0.5% agarose in a 24-well plate. After 3 weeks of culture, colonies were examined under light microscope. **c)** Jurkat cells harboring a luciferase gene under the control of the viral LTR promoter were co-cultured with a HTLV1 producing cell line (MT2), in the presence or not of 100 uM of FJ9 small molecule. Twenty four hours post-coculture, the activation of the luciferase reporter and cell viability were examined. Results are mean of three independent experiments.

Conclusion

We identified 57 PDZ proteins physically associated with Tax-1, representing 38% of the human PDZome and 23% of the current version of Tax interactome. We performed a clustering analysis to define biological functions associated with Tax/PDZ interactions. PDZ Proteins involved in cytoskeleton organization, protein complex assembly, synaptic transmission, cell migration and apoptosis were overrepresented. We finally demonstrated that a small molecule able to disrupt Tax/PDZ interactions could antagonize Tax-transformation activity and cell-to-cell transmission of HTLV-1.