

CHARACTERIZATION OF INTRACELLULAR INTERACTOME OF CD81 AND EWI-2 IN HUMAN T-LYMPHOCYTES

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Tetraspanin-enriched microdomains (TEM) are organized platforms at the plasma membrane, playing a crucial role in adhesion processes, migration and invasion of immune or tumor cells. The extracellular interactions of TEM proteins are well characterized, but their intracellular interactions are poorly known.

In previous pull-down experiments of total extracts of human T-Lymphocytes using several TEM proteins as baits, we demonstrated the intracellular interactions of EWI-2 with ribosomal proteins, of CD81 with tubulins, and of both baits with proteins involved in cytoskeletal activity regulation.

In this work, we made a deeper insight into the proximal cytosolic interactome of EWI-2 and CD81 by analyzing the interactions of these proteins in human exosomes, which are known to be enriched in TEM proteins. 27 pull-down experiments from 6 different donors were performed using the biotinylated cytosolic C-terminal domains from EWI-2 and CD81 as baits, followed by high-throughput mass spectrometry identification of the interacting partners. The number of identified peptides per protein in each interactome was assessed and compared to a control pull-down experiment performed with beads without bait. More than 270 specific interactions were characterized from a total of 2880 identified proteins. These interacting partners were differentially clustered revealing strong relationships between those baits and confirming previous characterizations. New differential clusters of proteins were also detected, such as CD proteins with CD81, GTP-binding proteins with EWI-2, or both baits with Ras-related proteins.

Our results reveal a coherent network of EWI-2 and CD81 intraluminal interactions and provide new relevant information about the recruitment in exosomes of specific proteins that are essential for intracellular signalling pathways.