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BIOLOGICAL AND FUNCTIONAL ANALISYS OF INTERACTIONS AMONG TETRASPANIN-ASSOCIATED PROTEINS IN HUMAN T LIMPHOCYTES BY HIGH-THROUGHPUT METHODS USING SECOND GENERATION PROTEOMICS TECHNIQUES

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The tetraspanin superfamily of transmembrane proteins are clustered in compact structural groups forming specialized membrane microdomains (Tetraspanin Enriched Microdomains, TEM or TERM). Through heterolog and homolog interactions, tetraspanins regulate signalling processes mediated by cellular adhesion molecules, growth factor receptors and costimulatory proteins, and are also implicated in antigen presentation and viral growth in infected cells.

In spite of the growing interest for these proteins, the cytosolic interactions by which tetraspanins are involved in various receptor activation pathways, their cytoskeleton anchorage and in general the protein ligands that interact with these proteins are poorly known. In this work we made a systematic analysis of interacting partners of different proteins that are presented in TERMs, including ICAM-1, CD81, and EWI-2 by "pull-down" techniques and high-throughput MS/MS protein identification. Synthetic biotinylated peptides spanning the C-terminal cytoplasmic end of these proteins were incubated with extracts from different cell models, including HeLa cells and lymphocytes, and then captured using Streptavidin-sepharose microbeads. Proteins interacting with the peptides were subjected to digestion and automated shotgun identification from the MS/MS spectra by using the pRatio software developed by our group at a 5% error rate. In total more than 1.000 interacting proteins were identified, from which a reduced subset were only found associated to one peptide, indicating highly specific interactions.

Proteins that were selectively identified by more than 4 different peptides in each case were analyzed as a whole using Systems Biology tools (Ingenuity Pathways Analysis). The proteins associated to ICAM-1, CD81 and EWI-2 peptides showed a marked functional interrelation in each group. These results suggest that proteins linking the citoplasmic domains of tetraspanins form a coherent network of interactions playing an important role in the processes of diapedesis between T lymphocytes and vascular endothelium.