Identification of a novel putative interaction partner of dipeptidyl peptidase 3, SH2 domain-containing protein 3C

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Dipeptidyl peptidase 3 (DPP3) is a zinc metallopeptidase that sequentially cleaves off dipeptides from the unsubstituted amino-termini of 3 to 10 residues long peptides in vitro, showing broad specificity for 4 to 8 residues substrates. It is involved in the regulation of Nrf2/KEAP1 signalling pathway through its interaction with KEAP1 protein, which is so far the only confirmed protein interaction partner of DPP3. We have conducted SILAC-MS study on the whole proteome of HEK293T cells and identified novel putative interaction partner of DPP3, SH2 domain-containing protein 3C (SH2D3C). SH2D3C is one of the three members of the family of proteins which contain both SH2 domain and a domain similar to guanine nucleotide exchange factor domains for Ras family GTPases (Ras GEF-like domain). Several different length isoforms of SH2D3C protein are expressed in different cell types, where they have different functions, including acting as an adapter protein involved in the regulation of cell adhesion and migration, tissue organization, and the regulation of the immune response. The interaction of DPP3 with the isoforms 2 and 3 of SH2D3C was confirmed by Co-immunoprecipitation of overexpressed proteins in HEK293T cells and by GST-pulldown with both wild type DPP3 and catalytically inactive DPP3 variant, E451A. The colocalization of EGFP-DPP3 and SH2D3C-mCherry was analysed by confocal microscopy in NIH 3T3 cells and detected in cytosol and on the membrane, with weak staining signal in the nucleus. Preliminary Bimolecular fluorescence complementation (BiFC) investigation displays the interaction in the cytosol and in the membrane ruffles. Present knowledge about the DPP3 and SH2D3C proteins indicate that their interaction might represent a link between Nrf2/KEAP1 mediated oxidative stress response and the regulation of cell migration, and further investigations that will elucidate the potential physiological implications of this interaction are in progress.