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Tetraspanins and their role in the removal of TDP-43 insoluble species via extracellular vesicles

Annalisa Brivio[1], Elena Casarotto[1], Andrea Conti[2], Irene D'Arsiè[3], Marta Chierichetti[1], Marta Cozzi[1], Veronica Ferrari[1], Barbara Tedesco[1], Riccardo Cristofani[1], Mariarita Galbiati[1], Paola Rusmini[1], Riccardo Filadi[3,4], Roberta Cascella[2], Angelo Poletti[1], Valeria Crippa[1]

1) Department of Pharmacological and Biomolecular Sciences "Rodolfo Paoletti", University of Milan, Milan, Italy. 2) Department of Experimental and Clinical Biomedical Sciences, Section of Biochemistry, University of Florence, Florence, Italy. 3) Department of Biomedical Sciences, University of Padua, Padua, Italy. 4) Institute of Neuroscience, National Research Council (CNR), Padua, Italy.

Several data indicate that TDP-43 protein and its truncated, C-terminal, hyperphosphorylated, and ubiquitinated forms are present in the extracellular space. Our group and others have demonstrated that neurons physiologically secrete these species via extracellular vesicles (EVs), both large (LEVs) and small (SEVs). This secretion is further increased in TDP-43 proteinopathies and upon inhibition of degradative pathways such as the proteasome and autophagy, suggesting that the secretory mechanism plays a significant role in maintaining intracellular proteostasis.Despite clear evidence of TDP-43 secretion, the molecular mechanisms regulating this process remain largely unknown. To gain further insight into the EV-mediated export of TDP-43 species, we investigated the possible involvement of tetraspanins, membrane-associated proteins enriched in EVs and widely used as EV markers. For this analysis, immortalized murine motor neuron-like NSC34 cells were transiently transfected with plasmids encoding fluorescently labeled TDP-43, TDP-35, or TDP-25, either individually or in combination with tagged tetraspanins such as CD63 (GFP or Tomato) or lantern-tagged CD9 and CD81. Our data indicate that co-expression of CD63, CD9, or CD81 with TDP-43 constructs consistently led to a reduction in intracellular levels of insoluble TDP-43 species, without altering levels of their soluble forms. Importantly, CD63 overexpression significantly enhanced the EV-associated secretion of insoluble TDP-43 species, particularly within the LEVs fraction, whereas CD63 silencing increased the accumulation of insoluble TDP-43 species within the cells. These findings point to a previously unrecognized role for tetraspanins in promoting the selective packaging of misfolded TDP-43 species into EVs.
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