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The role of tetraspanins in extracellular vesicle-mediated clearance of insoluble TDP-43

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A pathological feature of amyotrophic lateral sclerosis (ALS) is the aberrant cytoplasmic aggregation of TAR DNA-binding protein 43 (TDP-43) and its C-terminal derivatives, TDP-35 and TDP-25, within affected cells. These aggregates perturb cellular homeostasis, ultimately contributing to neurodegeneration. In response, cells initiate mechanisms aimed at their clearance, including intracellular degradation and extracellular release, with the latter pathway becoming more prominent when proteolytic systems are compromised. Indeed, both our group and others have demonstrated that under impaired proteasomal and autophagic activity, insoluble TDP-43 and its C-terminal fragments are predominantly secreted via extracellular vesicles (EVs), including both large (LEVs) and small (SEVs) subtypes.
To gain further insight into the EV-mediated export of TDP-43 species, we investigated the 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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