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ABSTRACT BOOOK

The role of extracellular vesicles in TDP-43 proteinopathies

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TDP-43 proteinopathies are a group of diseases characterized by an abnormal accumulation of pathological inclusions of the TAR DNA-binding protein of 43 KDa (TDP-43), in the cytosol of affected cells. Among these, there are Amyotrophic lateral scerosis (ALS), Frontotemporal lobar degeneration (FTLD), Alzheimer's disease (AD) and Parkinson's disease (PD). Disease-related inclusions contain insoluble forms of TDP-43 and of its C-terminal fragments of 35 (TDP-35) and 25 KDa (TDP-25), that are neurotoxic for cells. Interestingly, TDP-43 and its C-terminal fragments have been also found extracellularly, both secreted as free proteins or into extracellular vesicles (EVs), in particular into exosomes. These extracellular species are able to spread from one cell to another, therefore they could be toxic for the recipient cells and contribute to the disease. However, they could also represent a protective mechanism for the secreting cell, working together with the intracellular protein quality control (PQC) system, in the clearance of TDP species.
In order to better understand the role of extracellular TDP-43 in health and disease, we investigated the secretion of TDP-43 by taking into account both large and small vesicles (LVs and SVs, respectively). In particular, we carefully analysed LVs and SVs protein cargo in terms of TDP-43 species (FL vs fragments) and solubility (soluble vs insoluble), by comparing it with the intracellular TDP-43. Moreover, we analysed how and if PQC blockage might affect TDP-43 secretion.
For these purposes, we isolated EVs produced by an immortalized neuronal cell line using the differential ultracentrifugation method, and we analysed them for size and count through the Nanoparticle Tracking Analysis, for morphology through the transmission electron microscopy and for their protein content through western blot analysis.
We found that both TDP-43 and its C-terminal fragments (especially TDP-35) were physiologically secreted in EVs, in particular into LVs; interestingly, secreted TDP species were mainly insoluble, while intracellular TDPs were in a soluble form. Moreover, we discovered that LVs were immunoreactive for the lipidated form of the Microtubule-associated protein 1B-light chain 3 (MAP1BLC3-II), that has been recently proven to be a crucial component of a new secretory autophagy pathway, the LC3-dependent extracellular vesicle loading and secretion (LDELS), and they contained important other PQC components, known to be involved in TDPs clearance. Finally, we observed that a complete PQC blockage boosted EVs secretion, in particular that of MAP1BLC3-II positive LVs, further increasing the secretion of TDP C-terminal fragments and PQC components.
In conclusion, we demonstrated that, in physiological condition, EVs, in particular LVs, possibly secreted via LDELS, could represent an important mechanism for the clearance of insoluble TDPs species. This mechanism is specifically boosted when PQC is impaired. This aspect is particularly relevant, since PQC impairment is a common condition observed in TDP-43 proteinophaties.

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