P-1.46

The Extracellular Secretion of Misfolded Toxic Proteins Through Extracellular Vesicles Is Enhanced by Proteostasis Impairment

Andrea Carlotta Conti [1], Elena Casarotto [2], Cristina Cecchi [1], Fabrizio Chiti [1], Angelo Poletti [2], Valeria Crippa [2], Roberta Cascella [1]

[1] University of Florence, Florence, Italy

[2] University of Milan, Milan, Italy

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are neurodegenerative diseases linked to dysfunction of the ubiquitin-proteasome system (UPS) and autophagy-lysosome pathway (ALP), the two major catabolic systems that cooperate in the maintenance of the proteostasis network (PN). PN prevents toxic protein aggregation and promotes their degradation. We previously showed that PN impairment enhances extracellular secretion of misfolded proteins, particularly TDP-43, via extracellular vesicles (EVs), which can release their content into other cells. Here, we characterized EV structure, protein cargo composition, and evaluated their toxicity in recipient cells. EVs were isolated from NSC-34 cells, and analyzed using biophysical techniques, including far-UV circular dichroism (CD), intrinsic tryptophan fluorescence (IF), Thioflavin T (ThT) and 8-anilino-1-naphthalenesulfonic acid (ANS) fluorescence assays. PN inhibition increased secretion of hydrophobic, β-sheet-rich aggregates within EVs. STED microscopy revealed TDP-43 inside EVs and on membranes, while MTT assays confirmed EVs cytotoxicity in recipient cells. These findings highlight EVs as key players in TDP-43 proteinopathies, modulating protein homeostasis and aggregate propagation. This study was supported by MUR (PRIN 2022, CUP « B53D23018750006», project 2022KSJZF5 to R.C. and V.C.).