NSC-34 cells as a model to study extracellular vesicles release-based cell-to-cell communication in amyotrophic lateral sclerosis

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The NSC-34 cell line is a widely used model for studying the pathogenetic mechanisms that characterize the forms of amyotrophic lateral sclerosis (ALS). The main feature of SLA is the functional damage of alpha motoneurons due to the accumulation of misfolded proteins, including SOD1, FUS, and TDP43. In the last years, *in vivo* experiments and clinical studies suggest the role of neuroinflammation in the pathophysiology of the disease, indicating the alteration of cell-to-cell communication an actual area of research in ALS. In particular, the involvement of peripheral immune cells seems to be very interesting. Among the various modalities of intercellular communication, extracellular vesicles (EVs) released by cells arouse increasing interest in the scientific community.

Here, we explore if NSC-34 cells are a suitable model for studying the EVs-mediated neuroinflammation occurrence in ALS. EVs obtained from the motoneuron-like cell line NSC-34 transfected with different SOD1 mutations (G93A, A4V, G85R, G37R) were characterized by transmission electron microscopy, cytofluorimetry, and western blot. The EVs were isolated in small and large vesicles fractions and were used to culture Raw 246.7 murine macrophages and their response in terms of polarization in the M1 or M2 phenotype was evaluated by analysis of mRNA levels of pro- and anti-inflammatory cytokines, suggesting that the EVs from mSOD1 NSC-34 induce in Raw 264.7 macrophages the switch to mixed M1 and M2 subpopulations.

Overall results suggest the suitability of mSOD1 NSC-34 as a model to study EVs-based inflammatory modulation relevant to modulating neuroinflammation in ALS patients.