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MicroRNAs shuttled by extracellular vesicles derived from mesenchymal stem cells rescue glial activation in in-vitro models of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a progressive, fatal, neurodegenerative disease characterized by the selective death of upper and lower motor neurons (MNs). The mechanism of MN damage and death has been ascribed to several cellular and molecular alterations, including neuro inflammation. ALS is also a non-cell-autonomous disease, due to the contribution of glial cells, such as astrocytes and microglia that secrete neurotoxic factors and promote a noxious environment for MN. At present there is no effective cure for ALS. We previously demonstrated that intravenous administration of bone marrow mesenchymal stem cells (MSCs), prolonged survival probability, improved motor functions and ameliorated pathological features, including gliosis and neuro inflammation, in the spinal cord of the SOD1G93A mouse model of ALS. There is a consensus supporting that the beneficial outcomes of MSCs are unlikely due to trans-differentiation, but possibly to paracrine effects, thus we postulated that one of these mechanisms could be the transfer to target cells of microRNAs (miRNAs) shuttled by extracellular vesicles (EVs) isolated from the secretome of MSCs. To this aim we studied the activity of MSCs-derived EVs both on astrocytes isolated from the spinal cord of symptomatic SOD1G93A mice and human astrocytes (iAstrocytes) differentiated from inducible neural progenitor cells (iNPCs) of ALS patients.

Mouse or human-derived ALS astrocytes were exposed to MSCs-derived EVs or transfected with single miRNA mimics. We determined protein expression by western blot, confocal microscopy and qPCR, while cytokine release was measured by ELISA assay. Co-cultures of spinal MNs with ALS astrocytes exposed or not to EVs, were studied to assess MN viability.

The astrocyte activation markers vimentin, GFAP, and S100β were overexpressed in SOD1G93A mice, and this over-expression was reduced by exposure to EVs. The same was true for the pro-inflammatory cytokines TNF-α, IL-1β, IL-6 and CCL2 that were aberrantly secreted from adult mouse SOD1G93A astrocytes, and which secretion was reduced in astrocytes treated with EVs. In human iA strocytes, exposure to EVs increased the expression of the Nrf2 anti-oxidant factor and resulted in reduced accumulation of reactive oxygen species. Most importantly, the viability of MNs was significantly increased when co-cultured with mouse or human astrocytes previously exposed to MSCs-derived EVs. Aiming to identify possible factors that could be responsible for the neuroprotective effect of the MSC-derived EVs, we focused on microRNAs (miRNAs), which we found to be elevated in IFN-γ-primed MSCs. Of note, the transfection with specific mimics of miRNAs reverted the reactive phenotype of ALS astrocytes cultured from SOD1G93A mice. Similarly, in human iAstrocytes, transfection with the miR-29b-3p miRNA significantly upregulated the Nrf2 antioxidant pathway and rescued the viability of co-cultured MNs. Interestingly, MSCs-derived EVs also ameliorated the

neuroinflammatory phenotype of microglia cells isolated from symptomatic SOD1G93A mice.

Overall, our data suggest that EVs derived from MSCs represent a promising therapeutic strategy in ALS by releasing EV-shuttled anti-inflammatory and anti-oxidant miRNAs able to decrease glial cell toxicity towards motor neurons. In-vivo pre-clinical studies in SOD1G93A mice are on-going aimed at gathering a crucial proof-of-concept to translate our in-vitro results into effective clinical trials.

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Biography

Marco Milanese is Associate Professor at the Department of Pharmacy of the University of Genoa. Since 2005 Marco Milanese is member of the Pharmacology and Toxicology research Unit of the Department of Pharmacy, University of Genoa, working in the field of neuropharmacology and neurosciences. Marco Milanese holds a long-lasting experience in training undergraduate students and PhD students other than managing a biological research laboratory with a broad panel of expertise and facilities. The scientific research of Marco Milanese started focusing on various aspects of neurotransmission and relative molecular mechanisms underlying neurological disorders and neurodegenerative diseases: release of neuro- and glio-transmitters and modulation systems, pre-synaptic proteins, pharmacological characterizations of pre- and post-synaptic glutamatergic receptors. In this context the research activity of M. Milanese was mainly focused on the study of glutamatergic neurotransmission linked to excitotoxicity in neurodegenerative diseases with particular interest on motor neuron diseases (MND) and amyotrophic lateral sclerosis (ALS). In the last ten years the research activities of M. Milanese were characterized by in-vivo behavioural analysis and ex-vivo histological studies to assess the clinical progression and the molecular dysfunction in mouse model of ALS and/or other neurological disorders. The areas of investigation, in the context of ALS, have then expanded towards complementary in-vitro functional and biochemical studies on motoneuron, astrocyte, microglia and oligodendrocyte primary cell cultures acutely isolated from ALS mouse models. M. Milanese holds active scientific collaborations with National and International research groups, as intelligible from the publication track record.

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