MODULATION OF THE ADULT SOD1^{G93A} ASTROCYTE PHENOTYPE BY TREATMENT WITH EXOSOMES AND miRNAs DERIVED FROM MESENCHYMAL STEM CELLS

[1]Francesca Provenzano, [1,2]Marco Milanese , [3]Debora Giunti, [1]Carola Torazza , [3]Chiara Marini, [4]Cesare Usai, [3]Nicole Kerlero de Rosbo, [2,3]Antonio Uccelli, [1,2]Giambattista Bonanno

[1]Department of Pharmacy, Unit of Pharmacology and Toxicology, University of Genoa, Italy. [2]Centre of Excellence for Biomedical Research, University of Genoa, Italy. [3]Department of Neurosciences Ophthalmology and Genetics, University of Genoa, Italy. [4]Institute of Biophysics, National Research Council (CNR), Genoa, Italy.

BACKGROUND. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects upper and lower motor neurons (MNs) leading to muscle atrophy and paralysis. Within 3-5 years from diagnosis, patients succumb due to respiratory failure (1). ALS is a non-cell autonomous disease, in which microglia and astrocytes play a central role in the clinical progression secreting neurotoxic and pro-inflammatory factors (2). In a previous work we investigated the effect of intravenous administration of mesenchymal stem cells (MSCs) in SOD1^{G93A} mice (3). A single injection of MSCs determined increase of survival probability, improvement of motor skills and reduction of gliosis and inflammation in spinal cord. These beneficial effects were determined by paracrine mechanisms, rather than MSC differentiation. We have speculated that MSCs exert their action at least in part through the transfer to target cells of miRNA shuttled by their released exosomes.

AIM. The objective of this study was to verify whether exosomes derived from IFNγ-primed MSCs and their shuttled miRNAs modulate the phenotype of cultured astrocytes prepared from the spinal cord of late symptomatic SOD1^{G93A} mice and reduce the inflammatory environment surrounding MNs. We analyzed astrocyte activation state, cytokine expression and release, the impact of astrocytes on MN viability in astrocyte/MN co-cultures.

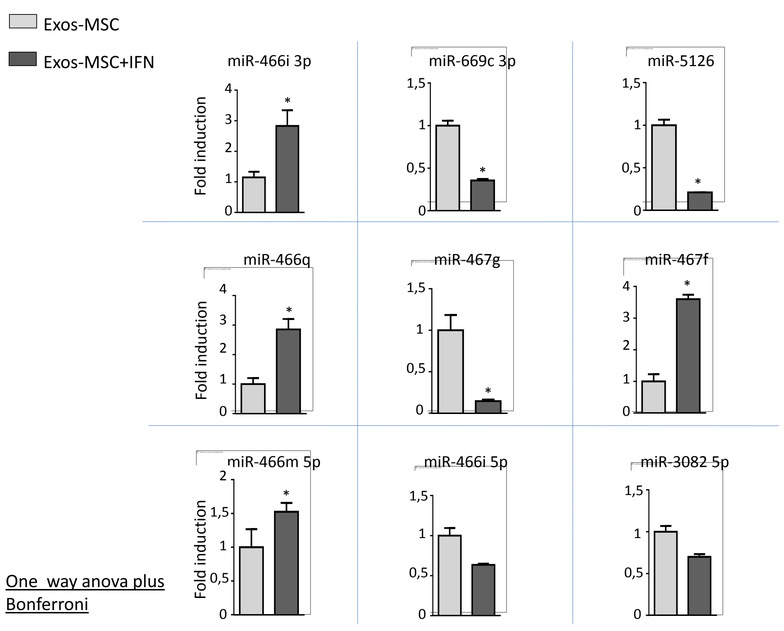
HIGHLIGHTS

- Exosome treatment reduced the SOD1^{G93A} astrocyte activated phenotype.
- The expression of pro-inflammatory cytokines, increased in SOD1^{G93A} astrocytes, was reduced in exosome-treated SOD1^{G93A} astrocytes.
- Synthetic miRNAs mimicked the beneficial effects of exosomes in SOD1^{G93A} astrocytes.
- Treating astrocytes with exosomes had a positive impact on motor neuron viability .

RESULTS

1. MSCs AND EXOSOME CHARACTERIZATION

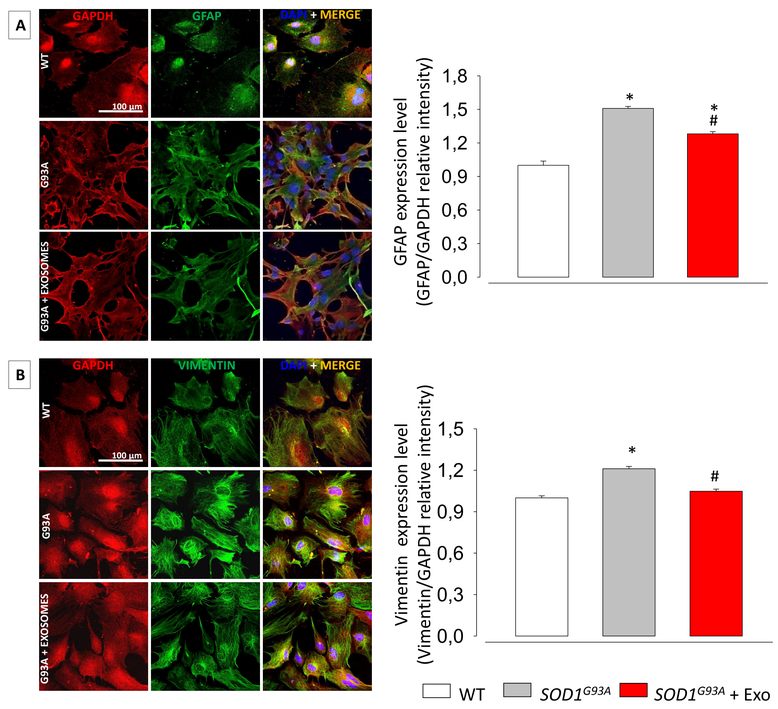
Nine miRNAs are up-regulated in MSCs activated by IFN-γ (not shown). These miRNAs were also expressed in exosomes .



*p<0.05 vs exos-MSC

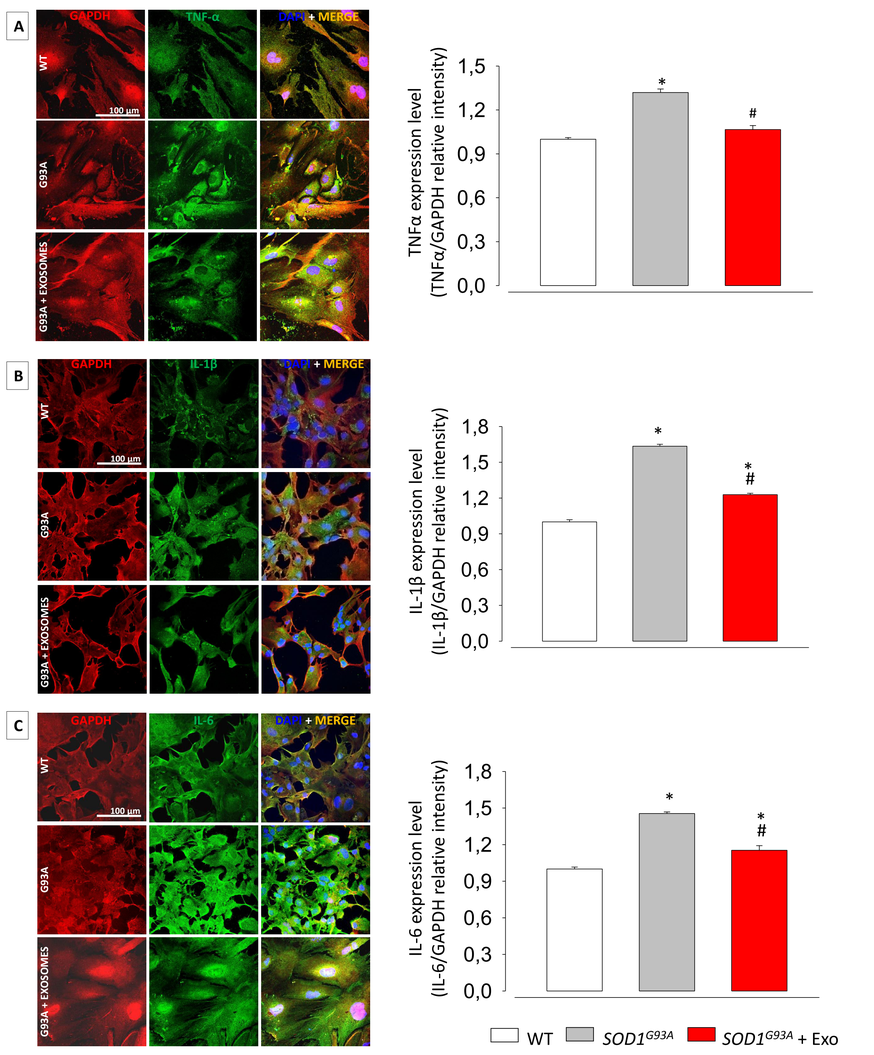
2. ASTROGLIOSIS

The expression of astrocyte activation markers GFAP (A) and Vimentin (B) was significantly increased in spinal cord astrocytes from adult SOD1^{G93A} mice. Exosome reversed GFAP and Vimentin over-expression.



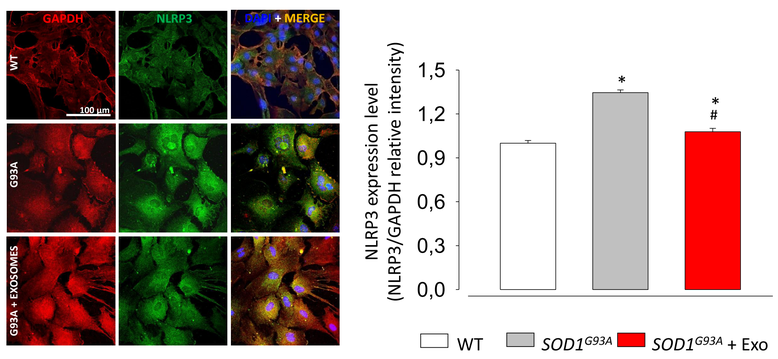
3. PRO-INFLAMMATORY MARKERS

The expression of TNF-α (A), IL-1β (B) and IL-6 (C) was significantly increased in spinal cord astrocytes from adult SOD1^{G93A} mice. Reduction of the three pro-inflammatory marker over-expression was detected after exosome treatment.



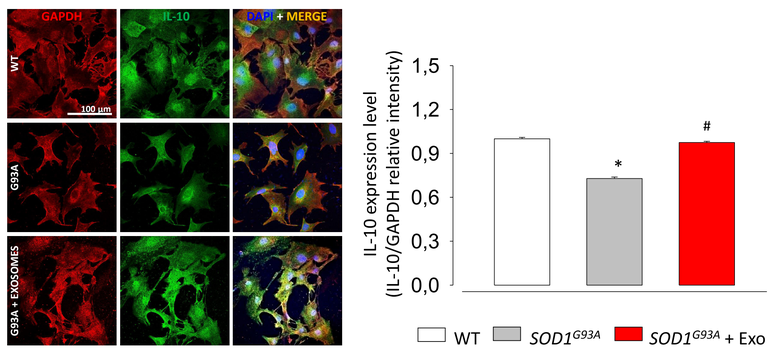
4. NLRP3

The expression of the astrocyte-mediated inflammation marker NLRP3 was significantly increased in spinal cord astrocytes from adult SOD1^{G93A} spinal mice. NLRP3 overexpression was reduced after exosome treatment.



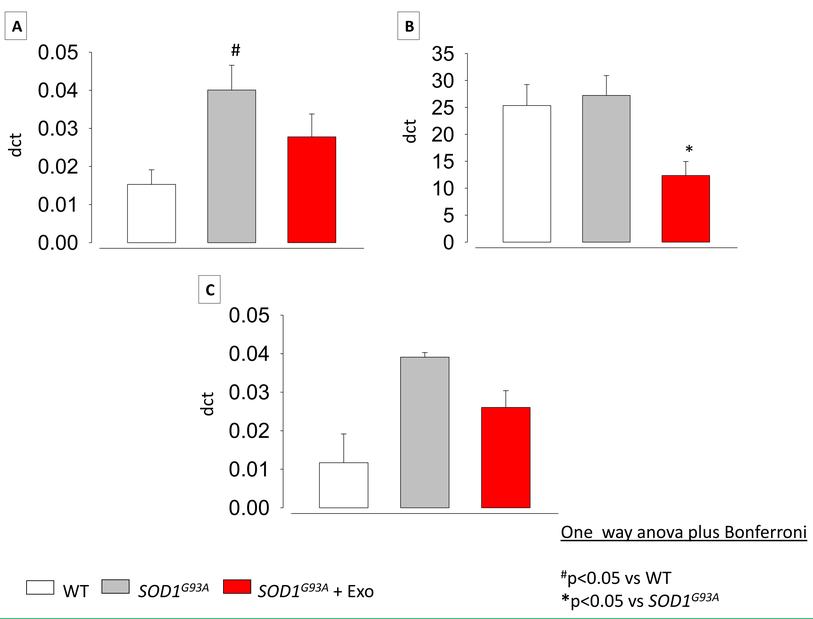
5. IL-10

The expression of the anti-inflammatory cytokine IL-10 was significantly decreased in spinal cord astrocytes from adult SOD1^{G93A} mice. IL-10 down-regulation was normalized after exosome treatment.



6. GENE EXPRESSION

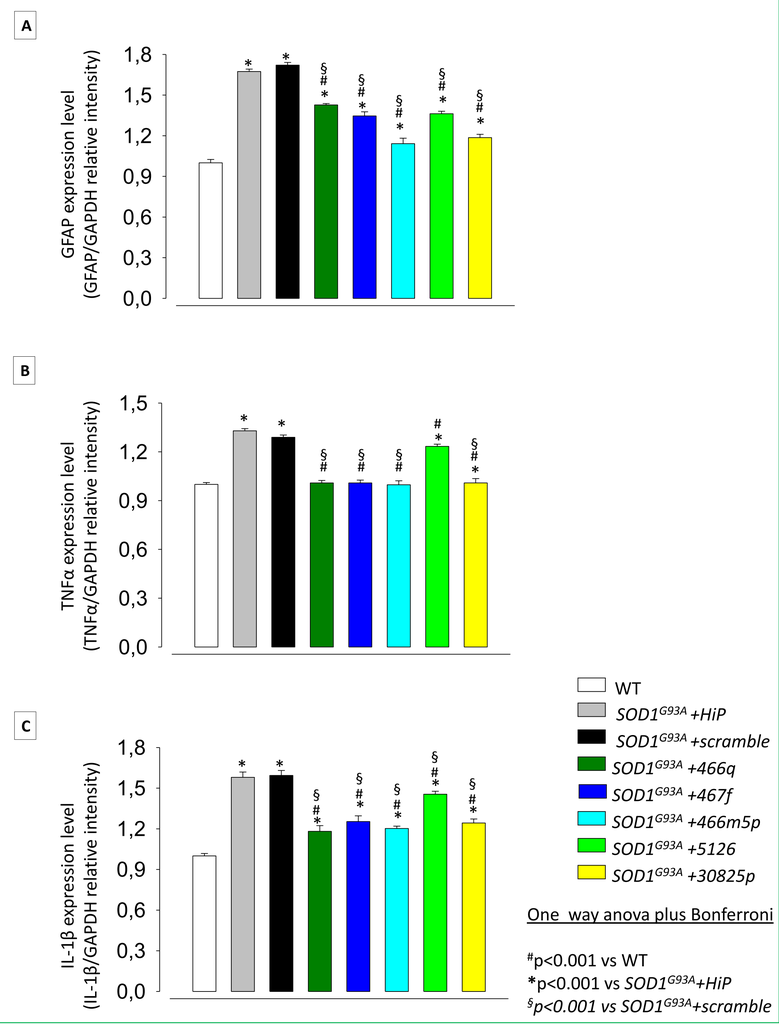
Expression of mRNA for GFAP (A), Vimentin (B) and Serping1 (C) was reduced in exosome-treated astrocytes.



#p<0.05 vs WT
*p<0.05 vs SOD1^{G93A}

7. SYNTHETIC miRNAs

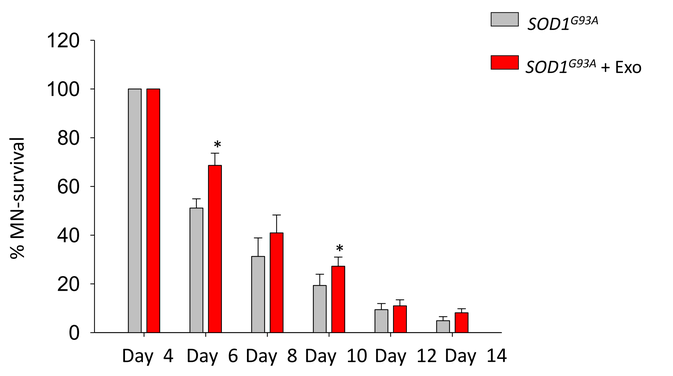
The expression of GFAP (A), TNF-α (B) and IL-1β (C) was quantified by immunofluorescence. in spinal cord astrocytes from adult SOD1^{G93A} mice. The 466q, 467f, 466m5p, 5126 or 30825p synthetic miRNA transfection reduced the over-expression of GFAP, TNF-α and IL-1β.



#p<0.001 vs WT
*p<0.001 vs SOD1^{G93A}+HiP
§p<0.001 vs SOD1^{G93A}+scramble

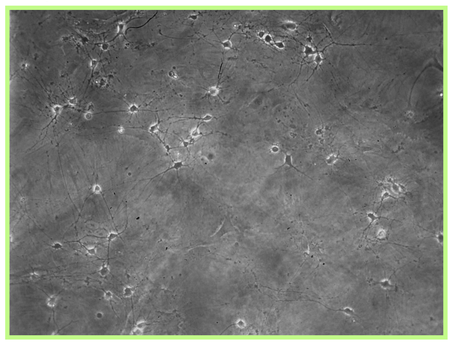
8. MOTOR NEURON SURVIVAL

MN viability was analyzed in presence of spinal cord astrocytes from adult SOD1^{G93A} mice, that were treated or not with exosomes for 24 hours. Preliminary data showed an improvement of survival of MNs cultured in the presence of astrocytes treated with exosomes, when compared to co-culture with untreated astrocytes.

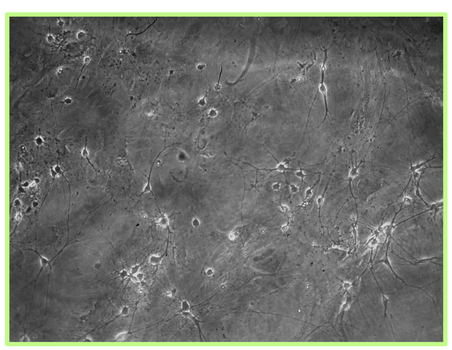


*p<0.1 vs SOD1^{G93A}

Day 6



SOD1^{G93A} astrocytes and SOD1^{G93A} MNs



SOD1^{G93A} astrocytes + exosomes and SOD1^{G93A} MNs

METHODS

Adult astrocytes culture prearation Primary astrocyte cell cultures were prepared from 120 day-old spinal cord WT and SOD1^{G93A} mice. Tissue was mechanically dissociated in DMEM high Glucose medium + Glutamine (1%), Pen-Strep (1%) and Fetal Bovine serum (10%). Aliquots were cultured at 37°C and 5% CO_2 for 20 DIV before experiments.

Exosomes isolation and astrocyte treatment. Exosomes were isolated by Total Exosome Isolation Kit (Invitrogen) from the supernatant of MSCs stimulated with IFN-γ for 24h. Astrocytes were exposed to exosomes (6x10^5 MSC-derived exosomes / 1x10^5 astrocytes) for 24h.

Astrocyte transfection. Astrocytes were transfected with a mix of HiPerFect Transfection Reagent (Qiagen) and synthetic mimics of exosome-contained miRNAs for 48h in DMEM serum-free.

Protein determination Spinal cord astrocytes were cultured onto pre-coated (poly-ornitine) glass coverslips, fixed with paraformaldehyde 4%, permeabilized with methanol at -20°C and incubated with appropriate primary antibodies (o.n., at 4°C) and fluorochrome-conjugated secondary antibodies (45 min, at r.t.). Protein content was determined by confocal microscopy and quantitative immunofluorescence.

mRNA determination RNA was isolated by phenol-chloroform extraction, using QIAzol Lysis Reagent. Purity was evaluated by spectrophotometric analysis. Quantification of gene expression was measured by qPCR uinsg a LightCycler 480 (Roche) in a final volume of 20 µl containing 50 ng cDNA, 1 µl of each pair of forward or reverse primers (20 µM) and 10 µl of FastStart Essential DNA Green Master. GAPDH was adopted as housekeeping gene.

Co-colture preparation and MN count MNs were prepared from the spinal cord of SOD1^{G93A} E13,5 embryos and isolated by Optiprep gradient. MNs were seeded on exosome-treated or untreated astrocytes at confluence. MNs were counted for 20 days starting at day 4 after seeding.

REFERENCES

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(2) Lee J. et al., 2016 Exp Neurobiol. 25(5):233-240
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E-MAIL ADDRESS: provenzano.phd@difar.unige.it

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