miRNAs shuttled by exosomes derived from immunomodulatory mesenchymal stem cells modulate microglia activation

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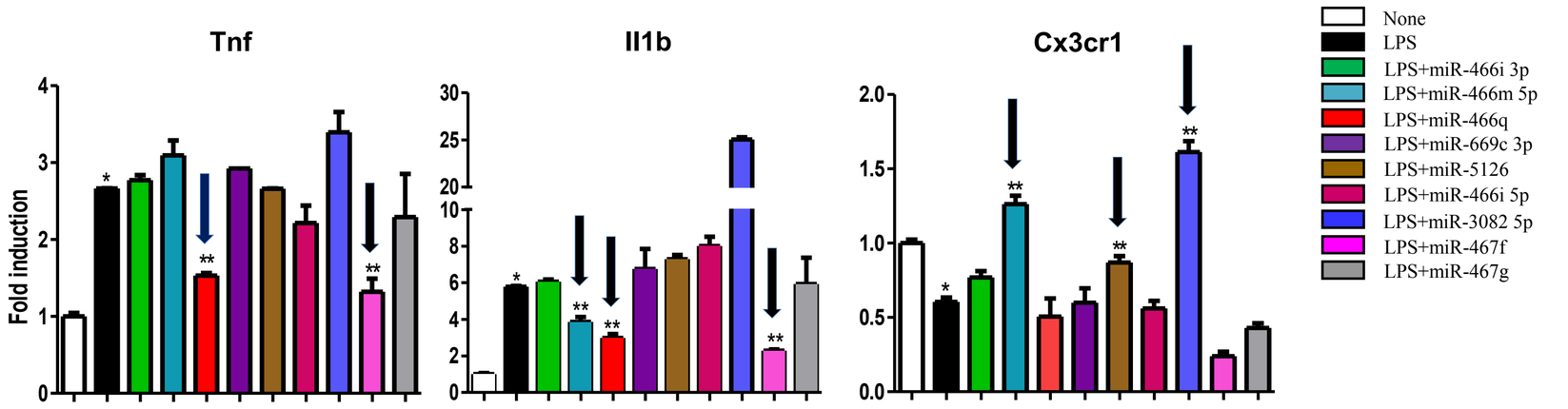
Motor neuron degeneration in amyotrophic lateral sclerosis (ALS) is associated with neuroinflammation, in which activated microglia play a prominent role. Accordingly, the neuroinflammatory reaction is being considered as potential therapeutic target for ALS. Treatment of murine ALS with mesenchymal stem cells (MSCs) that have shown neuroprotective, modulatory, and possible neuroregenerative potential, results in clear disease amelioration at clinical and pathological levels. While a number of secreted factors have been considered in the paracrine mode of action of MSCs, we have postulated that MSCs can ameliorate clinical murine ALS (G93A- SOD1 transgenic mice, or mSOD1 mice) in part via modulation of microglial phenotype through exosome-mediated transfer of specific miRNA that affect microglial gene expression.

RESULTS

Specific miRNAs are implicated in modulating microglia activation

miRNAs identified by microarray as upregulated in IFN-γ-primed MSCs and validated by RT-PCR

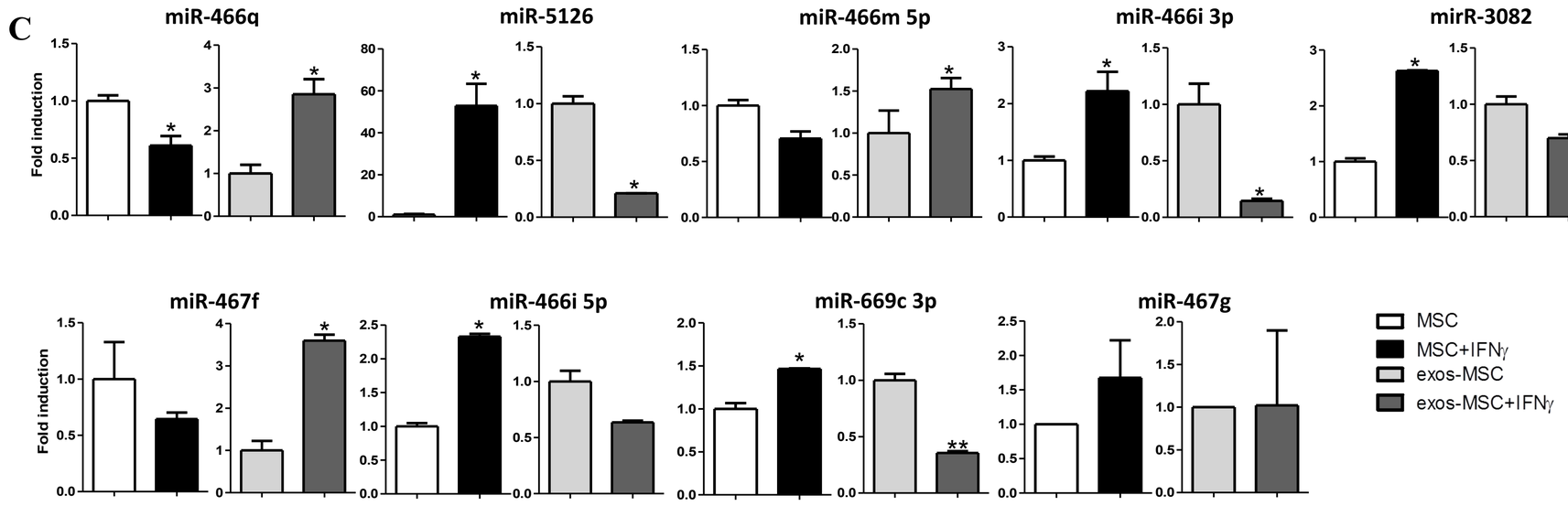
miR-	*P* value
466q	$P<0.05$
467g	NS
466m 5p	$P<0.05$
466i 3p	$P<0.05$
467f	$P<0.05$
466i 5p	$P<0.05$
3082 5p	$P<0.05$
669c 3p	$P<0.05$
5126	$P<0.05$



LPS-activated N9 microglia were transfected with synthetic mimics of the nine miRNAs upregulated in modulatory MSC. RT-PCR analysis shows that miR-466q and miR-467f significantly reduce the expression of TNF and IL1b (pro-inflammatory genes, markers of M1-like phenotype), while miR-466m5p, -5126 and -3082 5p significantly upregulated the mRNA expression of Cx3cr1 (marker of M2-like phenotype). $*P < 0.05$; $**P < 0.01$

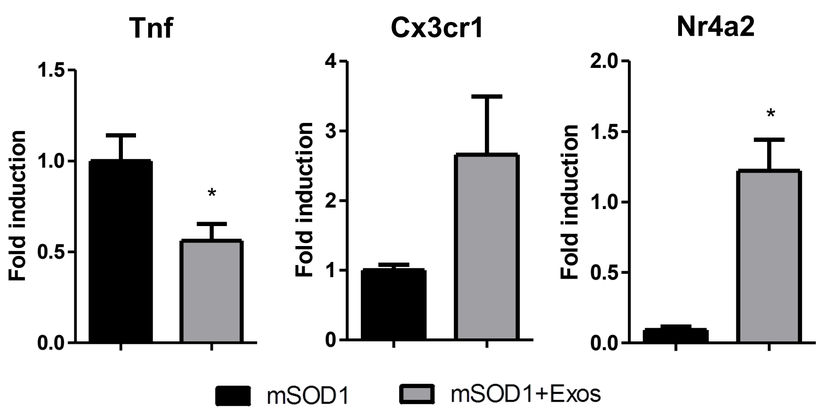
Exosomes derived from IFN-γ-primed modulatory MSC contain modulatory miRNAs

A: Western blotting of exosomes isolated from supernatant of IFN-γ-stimulated MSC with ALIX and CD9 as exosomal markers.
B: Electron microscopy image of MSC-derived exosomes (arrow heads).



C: RT-PCR analysis of miRNAs expression in unstimulated and IFNγ-primed modulatory MSC, and in their derived exosomes, respectively, shows differential sorting with increased levels of the modulatory miRNAs in exosomes derived from IFNγ-primed MSCs. $*P < 0.05$; $**P < 0.01$

Exposure to exosomes derived from IFNγ-primed MSCs modulate the molecular phenotype of mSOD1-microglia



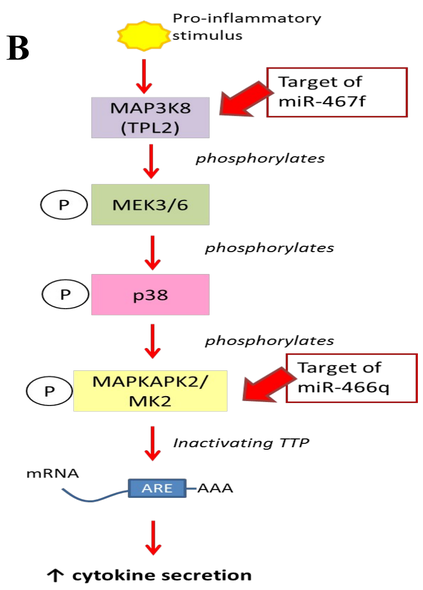
Exposure to exosomes derived from modulatory MSCs results in downregulation of the pro-inflammatory gene (Tnf) and upregulation of genes associated with anti-inflammatory/neuroprotective phenotype (Cx3cr1 and Nr4a2) in microglia isolated from end-stage mSOD1 mice. $*P < 0.05$

Prediction and preliminary validation of possible gene targets for the "modulatory" miRNAs

A

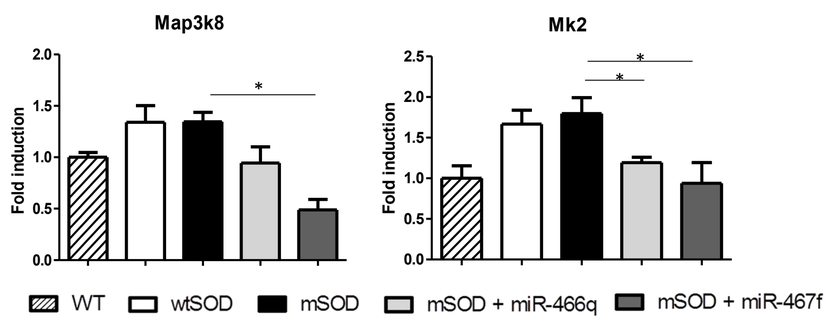
miR-466q	miR-467f	
Pathways in cancer	Pathways in cancer	Dorso ventral axis formation
Neurotrophin signaling pathway	TGF beta signaling pathway	Adipocytokine signaling pathway
Colorectal cancer	Endometrial cancer	Vascular smooth muscle contraction
Endometrial cancer	Acute myeloid leukemia	Wnt signaling pathway
Amyotrophic lateral sclerosis ALS	Chronic myeloid leukemia	Regulation of actin cytoskeleton
MAPK signaling pathway	Colorectal cancer	Ubiquitin mediated proteolysis
Thyroid cancer	MAPK signaling pathway	Melanoma
VEGF signaling pathway	B cell receptor signaling pathway	Glioma
Fc epsilon RI signaling pathway	ErbB signaling pathway	Gap junction
	Prostate cancer	Basal cell carcinoma
	Renal cell carcinoma	Fc epsilon RI signaling pathway
	Chemokine signaling pathway	Jak STAT signaling pathway
	T cell receptor signaling pathway	GnRH signaling pathway
	Sphingolipid metabolism	

B



A: MirWalk database analysis of the predicted pathways for miR-466q and miR-467f indicates that MAPK pathway is likely affected by both miRNAs. **B:** Scheme of p38 MAPK signaling pathway. The red arrows indicate the target genes of miR-466q and -467f in this pathway

C



C: Preliminary RT-PCR results suggest that miR-466q and miR-467f could act in mSOD-1-microglia in part through blocking the p38 MAPK signaling pathway. $*P < 0.05$

CONCLUSIONS

These data suggest that specific miRNAs shuttled by exosomes derived from modulatory MSCs alter microglial gene expression, inducing a switch from neurotoxic to neuroprotective phenotype. As exosomes can cross the blood-brain barrier, such mode of action could be exploited towards a therapeutic approach that circumvent the use of MSCs themselves.

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