Dysregulation of miRNA expression in ALS induced Pluripotent Stem Cells-derived motor neurons

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Background and Aims

Amyotrophic lateral sclerosis (ALS) is a fatal disorder characterized by progressive degeneration of motor neurons (MNs). The pathomechanisms underlying the disease and the specific proteins involved are almost unknown, even if the role of alterations in RNA metabolism has been increasingly recognized. In particular TDP-43 and FUS have been implicated in several steps of RNA metabolism, including microRNA (miRNA) processing. MiRNA are tissue-specific small molecules that can individually regulate several hundred targets by RNA-dependent mechanism. They may play important roles in the aetiology or progression of neurodegenerative disorders. Interestingly, a deregulation of miRNAs expression has been showed in human and in murine models of ALS. The aim of this work is to investigate alterations in miRNA expression in sporadic (sALS) and familial (fALS) patients, as compared to healthy controls.

Methods

Patients fibroblasts were reprogrammed using a non-integrating vector, which carries the four pivotal transcription factors (Oct3/4, Sox2, Klf4 and c-Myc) to obtain iPSCs. Then we promoted their differentiation in spinal MNs through a multistep protocol, and we performed immunocytochemical analysis for lineage-specific MNs markers such as HB9, SMI-32 and βIII tubulin. Moreover, since a small population of miRNAs was detected to circulate packed into molecular complexes named exosomes, we isolated these vesicles from both IPSCs and MNs by ultracentrifugation. RNA sequencing of miRNA isolated from iPSCs, MNs, and exosomes from both iPSCs and MNs cultures was performed through Human Array MicroRNA Cards analysis. We screened 754 miRNAs, including 3 endogenous controls and one negative control.

Results

Comparisons between miRNAs expression profile in ALS versus control groups were performed in iPSCs, iPSC-secreted exosomes, iPSC-derived MNs and MNs-secreted exosomes. A bioinformatic analysis (miRTarBase and Mirgate database) on miRNAs identified by cards is ongoing in order to find validated and predicted target genes and related pathways associated with miRNAs deregulation in ALS. Gene Ontology Enrichment analysis and Reactome Enrichment analysis to identify molecular pathways involved will be performed. The target proteins/pathways, regulated by the selected miRNAs identified in vitro, will be further investigated into the ALS mouse model SOD1G93A.

Conclusions and future perspectives

Our study can shed new light on ALS mechanisms and on the development of effective therapies and biomarkers for ALS that can be transferred to the clinic. As a matter of fact, single miRNA can modulate the expression of multiple target genes, and changing miRNAs expression can modulate an entire gene network and thereby modify complex disease pathologies, such as ALS. In particular, our plans include modulation of selected miRNA in vivo through Adeno-Associated Virus (AAV)9-mediated over-expression or Morpholino-mediated silencing.

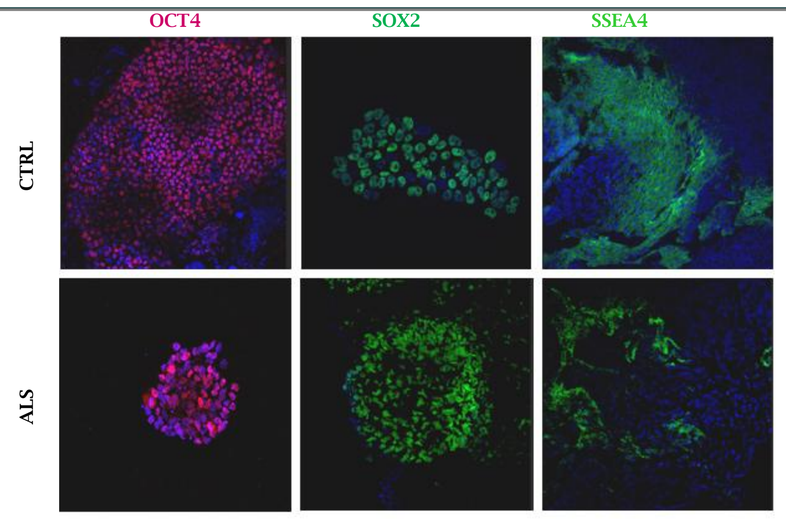


Figure 1. Immunocytochemistry performed on iPSCs lines. Both controls and fALS-iPSCs express the pluripotency markers OCT4, SOX2 and SSEA4 (nuclei are stained with DAPI, blue).

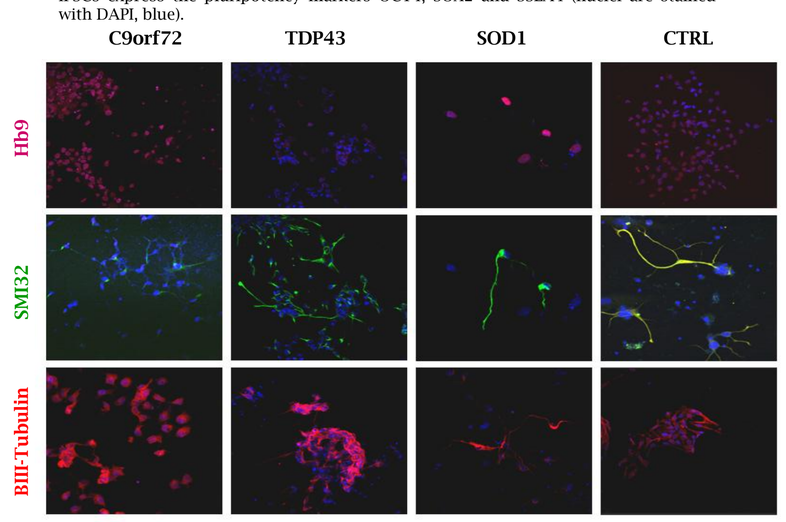
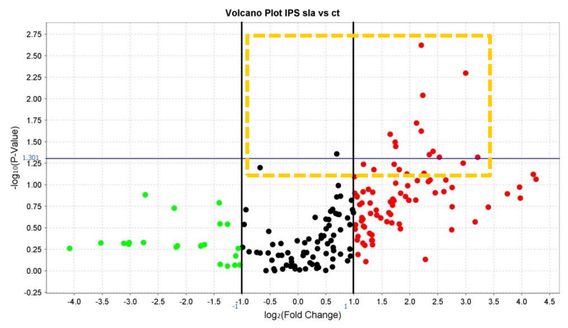


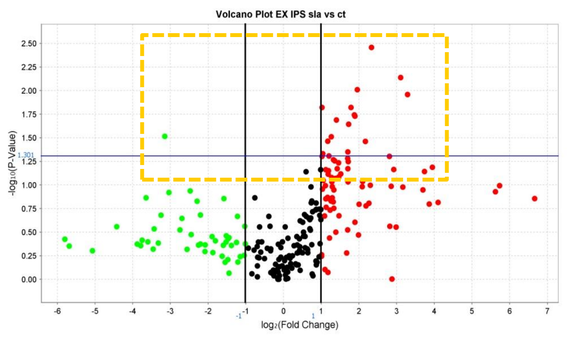
Figure 2. MNs immunocitochemistry analysis. iPSC-derived MNs from ALS and controls express typical MN markers such as Hb9, SMI32 and B-III Tubulin. SSEA4 (nuclei are stained with DAPI, blue).

a



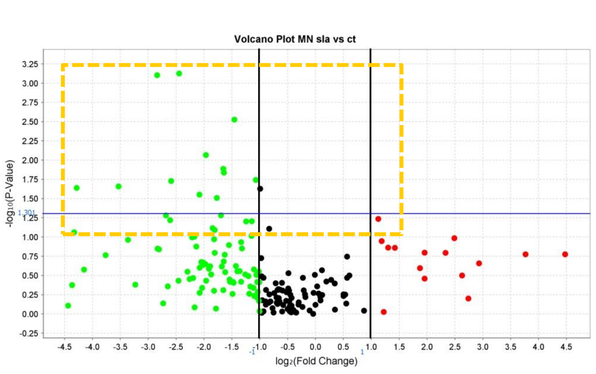
ALS IPSCs vs CTRL IPSCs		
Target Name	RQ	P-Value
hsa-miR-345-002186	4,608	0,002
hsa-miR-628-5p-002433	7,991	0,005
hsa-miR-532-3p-002355	4,702	0,009
hsa-miR-454-002323	4,340	0,019
hsa-miR-484-001821	4,604	0,024
hsa-miR-425#-002302	3,137	0,026
hsa-miR-1180-002847	3,335	0,032
hsa-miR-30b-000602	3,354	0,036
hsa-miR-340-002258	5,336	0,041
rno-miR-7#-001338	1,616	0,044
hsa-miR-103-000439	5,095	0,045
hsa-miR-128a-002216	9,268	0,048
hsa-miR-331-000545	5,771	0,048

b



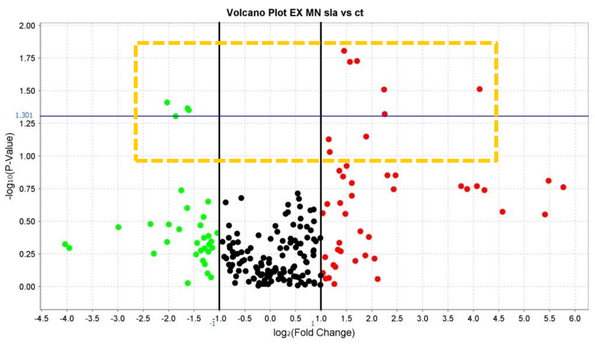
ALS IPSCs EX vs CTRL IPSCs EX		
Target Name	RQ	P-Value
hsa-miR-331-000545	5,039	0,003
hsa-miR-183-002269	8,631	0,007
hsa-miR-671-3p-002322	3,892	0,010
hsa-miR-628-5p-002433	9,799	0,011
hsa-miR-532-3p-002355	2,030	0,015
hsa-miR-103-000439	3,680	0,018
hsa-miR-30c-000419	3,727	0,019
hsa-miR-361-000554	2,650	0,021
hsa-miR-362-3p-002117	3,312	0,023
hsa-miR-29b-000413	2,408	0,031
hsa-miR-1179-002776	0,113	0,031
hsa-miR-345-002186	2,256	0,034
hsa-miR-19b-1#-002425	4,503	0,035
hsa-miR-101-002253	3,264	0,045
hsa-miR-125a-5p-002198	2,062	0,047
hsa-miR-744-002324	2,308	0,049
hsa-miR-19a-000395	2,031	0,050
rno-miR-7#-001338	7,012	0,050

c



ALS MNs vs CTRL MNs		
Target Name	RQ	P-Value
hsa-miR-34a-000426	0,184	0,001
hsa-miR-21-000397	0,140	0,001
hsa-miR-345-002186	0,365	0,003
hsa-miR-27b-000409	0,256	0,009
hsa-miR-34a#-002316	0,318	0,013
hsa-miR-24-000402	0,320	0,015
hsa-miR-636-002088	0,476	0,018
hsa-miR-152-000475	0,166	0,019
hsa-miR-335-000546	0,086	0,022
hsa-miR-10a-000387	0,051	0,023
hsa-miR-335#-002185	0,503	0,024
hsa-miR-744-002324	0,236	0,028
hsa-miR-26a-000405	0,293	0,031

d



ALS MNs EX vs CTRL MNs EX		
Target Name	RQ	P-Value
mmu-miR-491-001630	2,746	0,016
hsa-miR-192-000491	2,970	0,019
hsa-miR-194-000493	3,272	0,019
hsa-miR-375-000564	17,461	0,031
hsa-miR-502-3p-002083	4,729	0,031
hsa-miR-30a-5p-000417	0,246	0,039
hsa-miR-34a#-002316	0,325	0,043
hsa-miR-99a#-002141	0,330	0,045
hsa-miR-576-3p-002351	4,772	0,048
hsa-miR-601-001558	0,276	0,050

Figure 3. Vulcano plots of dysregulated miRNAs in cells and related exosomes. Comparisons between miRNAs expression profile in ALS versus control groups were performed in iPSCs (**a**), iPSC-secreted exosomes (EX) (**b**), iPSC-derived MNs (**c**) and MNs-secreted exosomes (EX) (**d**). The yellow boxes enclose the significant dysregulated miRNAs (green as downregulated and red as upregulated), which are listed in the specific table.

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