Differentially expressed microRNAs in extracellular vesicles of ALS and AD patients

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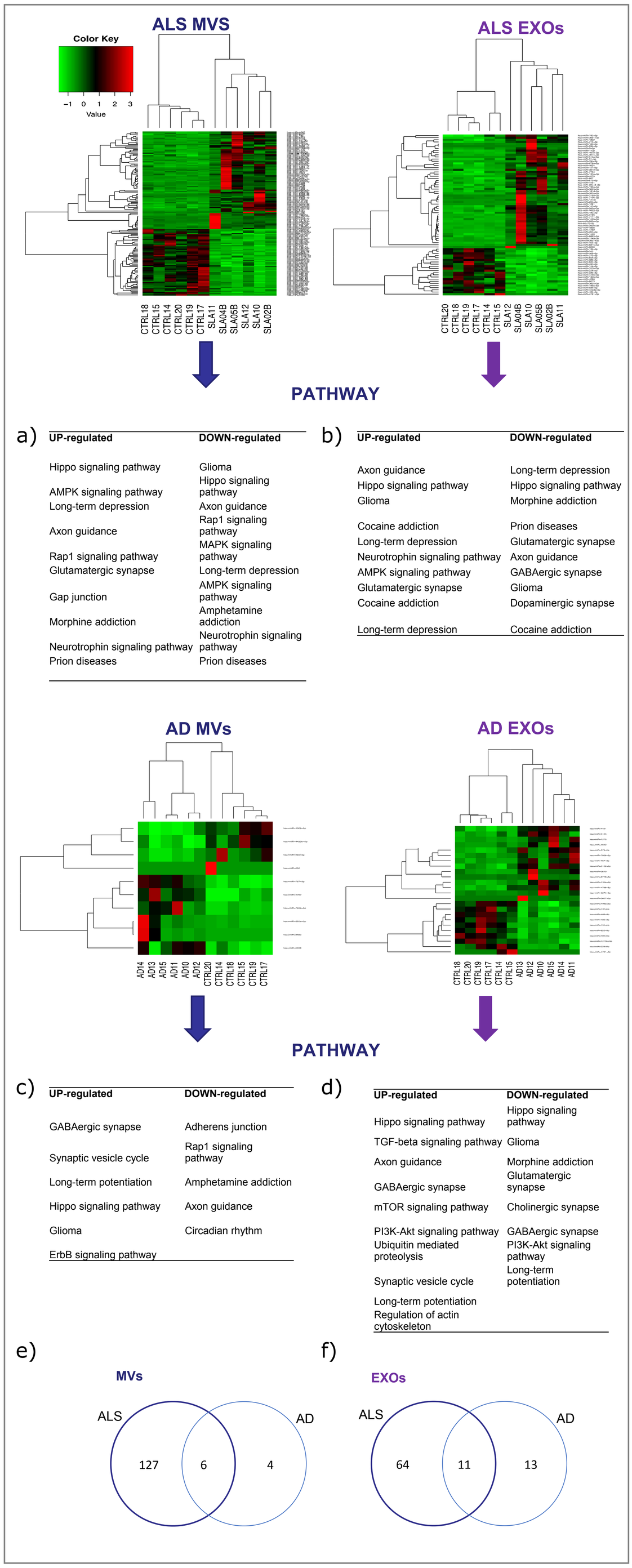
Objectives. Exploring robust biomarkers is essential for early diagnosis of neurodegenerative diseases. Blood contains microvesicles (MVs) and exosomes (EXOs), extracellular vesicles of different sizes and biological functions, which transfer mRNA, miRNAs, or proteins among different cell types. Aim of our study was to investigate mRNA/miRNA signature in plasma derived MVs and EXOs of Amyotrophic Lateral Sclerosis (ALS) and Alzheimer's Disease (AD) patients.

Methods. MVs and EXOs were isolated from plasma of 6 sALS, 6 AD patients and 6 healthy volunteers (CTRLs) by ultracentrifugation and whole RNA was extracted. mRNAlibraries were prepared by TruSeq Stranded Total RNA kit (Illumina) (60 million reads). miRNA libraries were prepared by TruSeq Small RNA Library kit (Illumina) (5-8 millionreads). Data were analyzed with ad hoc Bioconductor packages and pathways were predicted using DIANA-miRPath v3 (Kegg 2016). Statistically significant enriched pathways related to neurological diseases were shown.

Results. In ALS group 133 miRNA were DE in MVs while 75 miRNA DE in EXOs as shown in the heatmaps. In AD group 10 miRNA were DE in MVs while 24 miRNA DE in EXOs. Pathways of DE miRNA of ALS compared to CTRLs were determined and a number of these pathways related to neurodegenerative diseases were distinguished between MVs and EXOs. These included figure a) for ALS MVs - hippo signaling pathway, axon guidance, glioma; b) for ALS EXOs - axon guidance, hippo signaling pathway, long term depression; c) for AD MVs - GABAergic synapses, synaptic vesicle cycle, adherens junction, Rap1 signaling pathway; b) for AD EXOs - hippo signaling, TGF-beta signaling pathway, morphine addiction. Only 6 miRNAs (e) for MVs and 11 miRNAs (f) for EXOs are common between ALS and AD. Remaining miRNAs are specific for each disease, providing a signature for MVs and EXOs in the two diseases.

Conclusions. Our results show a great number of deregulated miRNA in plasma derived EVs from ALS patients compared to control and only a small number for AD patients. miRNA cargo is greatly deregulated in EVs from Amyotrophic Lateral Sclerosis (ALS) compared to Alzheimer's Disease (AD) patients. These results make hypothesize a major impact of RNA metabolism dysfunction in ALS pathogenesis, not observed in AD disease.



a)

UP-regulated	DOWN-regulated
Hippo signaling pathway	Glioma
AMPK signaling pathway	Hippo signaling pathway
Long-term depression	Axon guidance
Axon guidance	Rap1 signaling pathway
Rap1 signaling pathway	MAPK signaling pathway
Glutamatergic synapse	Long-term depression
Gap junction	AMPK signaling pathway
Morphine addiction	Amphetamine addiction
Neurotrophin signaling pathway	Neurotrophin signaling pathway
Prion diseases	Prion diseases

b)

UP-regulated	DOWN-regulated
Axon guidance	Long-term depression
Hippo signaling pathway	Hippo signaling pathway
Glioma	Morphine addiction
Cocaine addiction	Prion diseases
Long-term depression	Glutamatergic synapse
Neurotrophin signaling pathway	Axon guidance
AMPK signaling pathway	GABAergic synapse
Glutamatergic synapse	Glioma
Cocaine addiction	Dopaminergic synapse
Long-term depression	Cocaine addiction

c)

UP-regulated	DOWN-regulated
GABAergic synapse	Adherens junction
Synaptic vesicle cycle	Rap1 signaling pathway
Long-term potentiation	Amphetamine addiction
Hippo signaling pathway	Axon guidance
Glioma	Circadian rhythm
ErbB signaling pathway	

d)

UP-regulated	DOWN-regulated
Hippo signaling pathway	Hippo signaling pathway
TGF-beta signaling pathway	Glioma
Axon guidance	Morphine addiction
GABAergic synapse	Glutamatergic synapse
mTOR signaling pathway	Cholinergic synapse
PI3K-Akt signaling pathway	GABAergic synapse
Ubiquitin mediated proteolysis	PI3K-Akt signaling pathway
Synaptic vesicle cycle	Long-term potentiation
Long-term potentiation	
Regulation of actin cytoskeleton	

e)

f)

References

1)Cocucci E et al., J. Trends Cel Biol 2015 S0962-8924.