THE CROSSTALK BETWEEN CIRCULATING EXOSOME CARRIED miRNAs AND FERROPTOSIS RELATED GENES IN MULTIPLE SCLEROSIS

Jovana Kuveljic[1], Ivan Jovanovic[1], Maja Kosanovic[2], Natasa Macak[1], Tamara Djuric[1], Aleksandra Stankovic[1], Maja Zivkovic[1]

[1] Laboratory for Radiobiology and Molecular Genetics, "Vinca" Institute of Nuclear Sciences, National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia [2] Department for Immunology and Immunoparasitology, Institute for the application of nuclear energy, University of Belgrade, Belgrade, Serbia

Introduction

Ferroptosis is one of the processes that could drive immune-mediated neurodegeneration in multiple sclerosis (MS). Exosomes as biologically active extracellular vesicles (EVs) can carry miRNAs and are easily delivered across blood - brain barrier. The aim of FerroReg project is to provide data about the difference in network interplay of exosome carried miRNAs and PBMC mRNA between mild and progressive MS phenotypes, in context of ferroptosis process regulation.

Methods

Mild MS phenotype (12 RRMS patients) vs Progressive MS phenotype (12 SPMS patients)

PBMC

mRNA sequencing
of selected ferroptosis related genes
on Illumina iSeq100 NGS instrument

DESeq2 algorithm
was used for obtaining differentially expressed ferroptosis related genes between two MS phenotypes

miRNET platform was used for wovening the miRNA/mRNA interplay

EXOSOMES

Purification of EVs and extraction of total RNA was performed using Plasma/Serum Exosome RNA Isolation Kit (Norgen Biotek) + microRNA (cel-miR-39) Spike-in Kit

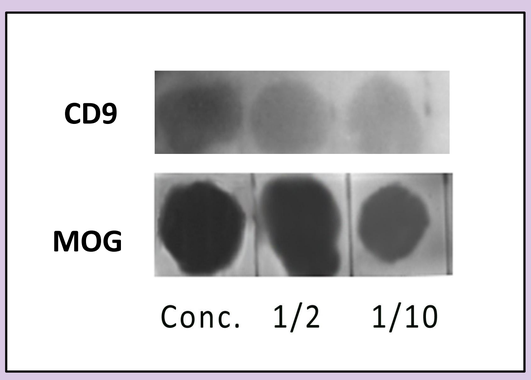
EVs were evaluated on the ZetaView instrument

Dot blot detection of exosomes with CD9, CD63 and CD81 antibodies and detecting the presence of brain derived exosome fraction with L1CAM and MOG antibodies

miRNA expression levels were detected with TaqMan™ MicroRNA Assays:
hsa-let-7b-5p, as bioinformatically selected target miRNA,
hsa-miR-16, as endogenous control
cell-miR-39, as control of RNA extraction

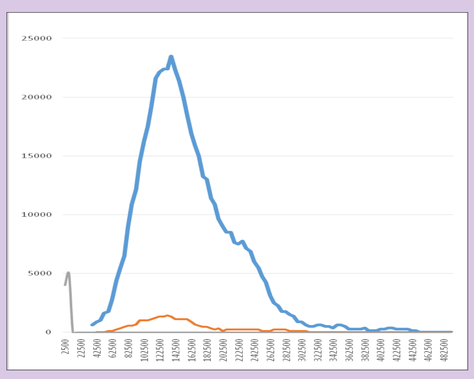
Results

CD9 and MOG presence on EVs of MS patients



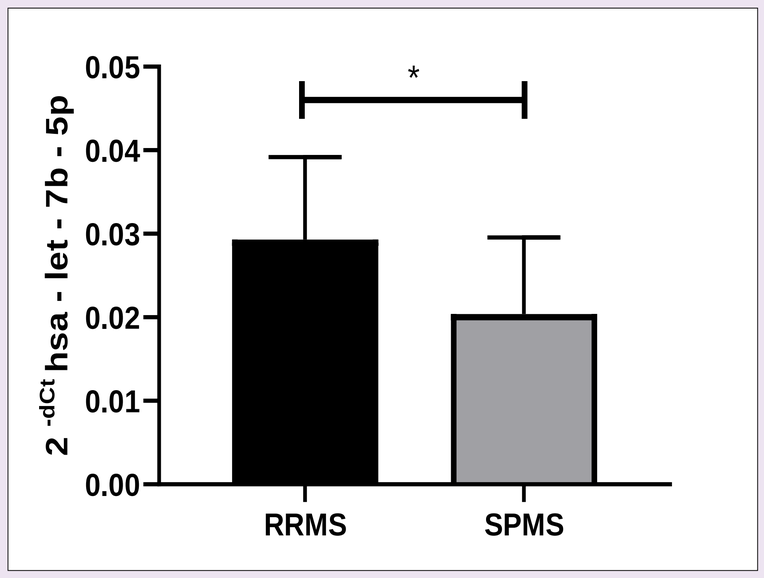
The average size of EVs was 106,2 nm and the average concetration was $2x10^{11}$ particles/ml.
There was no difference in EVs size and concentration between two MS phenothpes.

Total EVs (blue) compared to CD9-positive EVs (red)



CD9 antibody representes exosome presence in circulation of MS patients.
MOG antibody (myelin oligodendrocyte glycoprotein) representes the brain derived fraction of exosomes in circulation of MS patients.

hsa-let-7b-5p expression in EVs of MS patients



hsa-let-7b-5p was the largest hub, since it was conected with
9 out of 12 DE genes related to ferroptosis:
NFS1, SAT1, GCLC, MAP1B, SCL7A11, EGLN2,TP53, SCL11A2, CDKN1A

hsa-let-7b-5p expression levels in EVs were positively correlated with ***TP53*** (R=0,56; p=0,005), ***EGLN2*** (R=0,55; p=0,005) and ***SCL11A2*** (R=0,43; p=0,04) mRNA expression in PBMC of MS patients

hsa-let-7b-5p levels were significantly down-regulated (p=0,017) in EVs of patients with SPMS phenotype

Conclusion

Exosome cargo could serve as easily accessible biomarker for monitoring of MS course and severity. Validation of hsa-let-7b-5p in exosomes and its correlation with ferroptosis related genes TP53, EGLN2 and SCL11A2 in MS will elucidate regulatory role of ferroptosis in MS. Further detection of brain fraction exosomes in circulation will provide additional data on exosome signature and miRNA content in MS patients.

Acknowledgments:
This research was supported by the Science Fund of the Republic of Serbia, Program Ideje, FerroReg Project, Grant No. 7753406 and by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia. Grant No. 451-03-47/2023-01/200017.

Small New World 2.0 4.-5. September 2023. Graz, Austria