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Proteomic insights in extracellular microvesicles from multiple sclerosis patients

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To date the most important biomarkers for Multiple Sclerosis (MuS) diagnosis are the oligoclonal bands (OCBs) in CSF and Link Index. CSF is the body fluid that might better provide information about the pathological processes occurring in the CNS, because of its proximity. Anyway, it is obtained through an invasive procedure, thus tears, may represent an useful alternative source of biomarkers. Emerging evidences showed that distinct types of brain cells release high number of Extracellular Vesicles (EVs), that play important roles in the CNS, and represent a relevant source of biomarkers, relative free from confounding factors. In the present study, we analysed EVs from MuS patients obtained from tears and CSF samples. In details, 50µl of CSF or 50 µl of tears/sample were processed by a common flow cytometry no-lyse and no-wash method, in order to identify EVs. Exosomes and microvesicles (MVs) were sorted (70 µm nozzle, FACSAria III cell sorter, BD) from pooled CSF samples on the basis of their positivity to specific tetraspainins (for exosomes) or markers identifying each MV subset. Fractions were analysed by electron microscopy and Dynamic Light Scattering. Purified MV fractions undergone to FASP tryptic digestion and nanoLC-ESI-QTOF-MS/MS based shotgun proteomic approach. Identified MVs proteins were processed by Ingenuity Pathway Analysis (IPA) and PANTHER - Gene List Analysis.

Our data shows the presence of subpopulations of extracellular MVs of neuronal and microglia origins in tears , indicating a cross talk between the two compartment. We also identified 55 proteins (FDR<2.38) for the MVs fraction. To uncover the molecular events underlying these proteins profiles, we studied the Gene Ontology (GO) information in terms of biological process and molecular function by using PANTHER software and we observed that about 70% of the identified proteins resulted were involved in binding processes, while 40% of them were related to cell communication. Ingenuity Pathway Analysis (IPA) of the identified MVs proteins revealed that the top network associated to them are "Cellular Movement, Hematological Disease, Immunological Disease", well matching with MS. Among the upstream regulators, the most significant one is PRDM with a p-value of 6.68E-07. The remaining upstream regulators, including APOE (the most relevant lipid carrier protein in the brain involved in brain development and repair), well related to neurological disease. These data underlined that MVs form neuronal and microcglial origin are detectable not only in the CSF, but also in tears from MuS patients. Of note, MVs of CSF origin carry relevant targets involved in immune responses in MuS patients, therefore they might be proposed as useful tools in MuS diagnosis and characterization.

Keywords

Proteomic, Extracellular vesicles, Flow cytometry, Biomarker