Proteomics characterization of FACS-sorted Extracellular Vesicles sub-types as liquid biopsy: new challenges in biomarkers discovery

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Extracellular Vesicles (EVs) *in vivo* are emerged to have pivotal roles in pathophysiological processes. Their molecular outfit reflects the origin cell by conveying information towards the recipient cells/tissues, thus they can be considered an excellent source of biomarkers. In this scenario, EVs proteomics remain a challenge due to the lack of a well-established purification step. EVs *in vivo* are extremely heterogeneous in terms of size and, above all, in terms of phenotype. Ideally, for proteomics purpose, EVs should be free of soluble contaminants and collected separately based on their cell of origin. We undertook a pioneering proteomics approach of EVs purified from body fluids through Fluorescent-Activated Cell Sorting, combining a lipophilic dye and a specific panel of antibodies to gather EVs subtypes. As a proof of concept, we applied the SORT-omics workflow to the study of leukocyte derived EVs (Leuko-EVs) from blood and tears. Besides, we analyzed the proteome of Leuko-EVs from tears of Multiple Sclerosis patients. Functional proteomics revealed as Leuko-EVs carry a specific protein cargo able to trigger events related to "leukocyte mediated immunity". An up-regulation of "migration of endothelial cells" via TGFB1 regulator was highlighted in Multiple Sclerosis, demonstrating how EVs act as shuttles in the Immune System Machinery in response to neuroinflammation and neurodegeneration. Our data point out as proteomics of EV subtypes from biological fluids could be considered a "liquid biopsy" in the assessment of EVs clinical significance.