The purinergic system: a new promising target for human diseases.

Proteomic analysis of ectosomes and exosomes provides new clues on microglia response to ATP

M. Lombardi, IRCCS Humanitas, Rozzano, Milan Italy
F. Drago, Laboratoire de Spectromà©trie de Masse Biologique Fondamentale et Appliquà©e Universitè© Lille Nord de France, Villeneuve d'Ascq France
I. Prada, CNR Institute of Neuroscience, Milan Italy
M.. Gabrielli, CNR Institute of Neuroscience, Milan Italy
J. Vizioli, Laboratoire de Spectromà©trie de Masse Biologique Fondamentale et Appliquà©e Universitè© Lille Nord de France, Villeneuve d'Ascq France
C. Verderio, CNR Institute of Neuroscience, Milan Italy

Extracellular ATP is among molecules promoting microglia activation and inducing the release of extracellular vesicles (EVs), which are potent mediators of intercellular communication between microglia and the microenvironment. We previously showed that EVs released from microglia under ATP stimulation (ATP-EVs) induce a robust inflammatory reaction in cultured glial cells and propagate microglia activation in mice with subclinical neuroinflammation (Verderio et al., 2012). However, the proteomic profile of ATP-EVs has not yet been elucidated.

In this study, we applied a label free proteomic approach to analyse the protein composition of the two main populations of EVs released constitutively or upon ATP stimulation from rat primary microglia, i.e. quite large ectosomes shed from the plasma membrane and exosomes originating from the endosomal compartment. To separate ectosomes from exosomes we used a classical differential ultracentrifugation protocol (Gabrielli et al., 2015). To avoid cell damage, microglia were exposed to ATP for only 1h and EVs were isolated from medium conditioned by microglia for this time period. Due to the shortness of the protocol and the limited expansion of primary microglia, small EV batches could be generated, finally limiting detection of low abundant EV proteins and quantitative analysis of EV proteome.

We found that exosomes and ectosomes have a set of specific proteins but also share a substantial fraction of proteins. Proteome overlap may derive, at least in part, from the isolation procedure, which does not allow net separation of exosomes from ectosomes. Analysis of biological processes of constitutive EVs by Gene Ontology (GO) term database revealed “response to molecules” (~32%), “response to environmental changes” (~20%), “cytoskeletal dynamics” (17%), “innate immune response” (~10%), as predominant categories. The most significant pathways identified using the Kyoto Encyclopedia of Genes and Genome (KEGG) analysis were “phagosome”, “antigen processing and presentation”, “lysosomes” and “complement and coagulation”. These categories and pathways are consistent with the surveying function of unstimulated microglia and their role in antigen presentation and degradative activity following phagocytosis. Besides immune molecules (C1q, galectin-3, CD14, Lysozyme C), constitutive EVs also contained anti-inflammatory mediators, such as the leaderless proteins Annexin A1and A2, which may balance the pro-inflammatory action of immune factors.

About ~60% of proteins of ATP-EVs were dependent on ATP, not being present in constitutive EVs. They included a set of proteins implicated in cell adhesion, extracellular matrix organization, in autophagy-lysosomal pathway and antigen processing. The functional properties of ATP-specific proteins may reflect enhancement of degradative pathways to meet increased synaptic pruning and phagocytosis in response to ATP and possible enhancement of EV movement and interaction with target cells. However, the most significant change in the proteome of ATP-EVs was related to cell metabolism. ATP-specific proteins included several enzymes involved in glycolysis, the oxidative branch of the pentose phosphate pathway, pyruvate metabolism, glutamine metabolism, and fatty acid synthesis. Panther GO pathway classification showed an increase in glycolysis, in pentose phosphate pathway, in fatty acid synthesis rather than of beta-oxidation and in glutamine metabolism, which may serve to replenish levels of TCA cycle metabolite. Changes of metabolic proteins in ATP-EVs suggest a metabolic shift of microglia from oxidative phosphorylation to anaerobic glycolysis. This metabolic alteration may accommodate the increased energy requirements to enhance routine ATP-dependent cellular behaviour of microglia such as process scanning and phagocytic activity (Grabert et al., 2016). Of note, the augmented sorting of metabolic enzymes in EVs also suggest that EVs secreted from ATP-activated microglia may influence metabolism in receiving cells.

1. Verderio C, Muzio L, Turola E et al. Myeloid microvesicles are a marker and therapeutic target for neuroinflammation. Annals of neurology. 2012; 72(4):610-24.

2. Gabrielli M, Battista N, Riganti L, et al.. Active endocannabinoids are secreted on extracellular membrane vesicles. EMBO reports. 2015; 16(2):213-20.

3. Grabert K, Michoel T, Karavolos MH, et al. Microglial brain region-dependent diversity and selective regional sensitivities to aging. Nat Neurosci. 2016 Mar;19(3):504-16.

Sponsored by European Research Projects on Neuroinflammation Era-net neuron- MicroMet to CV.