Microglia extracellular vesicles: focus on molecular composition and biological function

Lorenzo Ceccarelli[1#], Chiara Giacomelli[1#], Laura Marchetti[1*], Claudia Martini[1*]

[1] Department of Pharmacy, University of Pisa, Via Bonanno 6, 56126 Pisa, Italy;

# These authors contributed equally to the work

*Correspondence: Claudia Martini (claudia.martini@unipi.it); Laura Marchetti (laura.marchetti@unipi.it)

Keywords

Extracellular vesicle; microglia; protein; miRNA; lipid; EV function

Abstract

Extracellular Vesicles (EVs) are a heterogeneous family of cell-derived lipid bounded vesicles comprising exosomes and microvesicles. They are potentially produced by all types of cells and are used as a cell-to-cell communication method that allows protein, lipid, and genetic material exchange. Microglia cells produce a large number of EVs both in resting and activated conditions, in the latter case changing their production and related biological effects. Several actions of microglia in the central nervous system are ascribed to EVs, but the molecular mechanisms by which each effect occurs are still largely unknown. Conflicting functions have been ascribed to microglia-derived EVs starting from the neuronal support and ending with the propagation of inflammation and neurodegeneration, confirming the crucial role of these organelles in tuning brain homeostasis. Despite the increasing number of studies reported on microglia EVs, there is also a lot of fragmentation in the knowledge on the mechanism at the basis of their production and modification of their cargo. In this review, a collection of literature data about the surface and cargo proteins and lipids as well as the miRNA content of EVs produced by microglial cells has been reported. A special highlight was given to the works in which the EV molecular composition is linked to a precise biological function.

Introduction

The term ‘extracellular vesicles’ defines a heterogeneous family of cell-derived membrane vesicles originating from membrane shedding, which constitutes a major component in cell-to-cell communication. The number of biological functions and cargos, either on the membrane and in the lumen, ascribed to EVs has witnessed an enormous increase in the last 20 years [1–3].

According to their size and biogenesis [4,5], EVs can be divided into Microvesicles (MVs) and exosomes [6–11]. MVs refer to vesicles released by either healthy or apoptotic cells originating from the outward budding of the plasma membrane [4], and typically have dimensions spanning from 150 to 800 nm in diameter. Exosomes have peculiar biogenesis, starting with the formation of a Multivesicular Body (MVB) by endocytosis and ending with exocytosis [6]. Exosomes are characterized by dimensions spanning from 30 to 250 nm in diameter. Exosome and MV isolation from a biological sample is tricky due to their partial overlap in size and biomarker composition. This is one of the reasons because now EVs are classified as small EVs with a size of 30–250 nm and large EVs with a size of 150–800 nm [5,7,11].

The International Society Of Extracellular Vesicles (ISEV) proposed the Minimal Information For Studies Of Extracellular Vesicles (MISEV) guidelines for the field in 2014 [12], which was later updated in 2018 [11], in which the minimal requirements to validate an EV sample are extensively described. Briefly, there is the need to test/confirm the presence of: i) at least one protein associated with the plasma membrane or endosomes, ii) one cytosolic protein associated with EVs, iii) one component of non-EVs co-isolated structures (e.g. lipoprotein), and iv) one component associated to the specific EV sub-type of interest [11]. Several studies name EVs as exosomes without clear evidence of the EV nature; however, different authors have recently begun to align to the aforementioned guidelines and provide concrete evidence of the exosome isolation [7,13–15]. To avoid confusion, we decided to adopt the MVs and exosome nomenclature.

The first study relative to EVs in the Central Nervous System (CNS) was performed on Drosophila about 20 years ago [16]. From there on, the work on EV role in the brain has dramatically increased, and EVs have been directly implicated in the modulation of neuronal development [17], as well as in the exchange of membrane proteins within the brain cells [18]. EVs have been proposed as markers for pathologies such as Traumatic Brain Injuries (TBI) and strokes, Alzheimer’s disease, and generally for some types of cancers and CNS disorders [19–21]. Microglia cells, fundamental cells ensuring the immune response

in the CNS [22], have revealed to be both producers and recipient cells for EVs. While the role of microglia as recipient cells for EVs has been reviewed elsewhere [6], here we focus on microglia cells as producers of EVs, and in detail describe the correlation between their molecular composition and exerted biological functions. The state-of-the-art of the biogenesis mechanism of both small and large EVs from microglia has been recently exhaustively described by Aires et al. [23,24]. Notably, some processes in microglia EVs biogenesis are still unclear, but the use of methods for interfering with EVs secretion, either pharmacologically or genetically, will possibly allow to fill this gap [25]. An example of this approach is provided by Bianco et al., who modulated acid sphingomyelinase activity to block or trigger MVs release from microglia [26].

The molecular composition of microglia EVs

EVs secretion from microglia cells can occur in unstimulated conditions or can be enhanced by different stimuli (**Table 1**), which can be pro-inflammatory or pro-regenerative, but also not related to the M1 and M2 states (**Fig. 1**). The cellular models used to derive microglia-EVs are discussed elsewhere [22]. Microglia phenotype is highly affected by the microenvironment; accordingly, the EV molecular composition produced by resting or activated microglia is widely variable. The proteins, lipid, and miRNA present in the membrane or as cargo (**Fig. 2**) can vary in composition or abundance based on the state of donor cells [27,28] causing a wide spectrum of effects in recipient cells (**Table 2**).

Protein Composition

The content of resting and stimulated microglia-derived EVs was investigated by Potolicchio et al., who reported an extensive proteomic analysis of the content of EV originated from the N9 murine cell line [29]. These vesicles include metabolic enzymes (e.g. glyceraldehyde 3-phosphate dehydrogenase, GAPDH, pyruvate kinase), chaperones, tetraspanins, and membrane receptors, very similar to the profile of exosomes originated by both B-cells and dendritic cells, together with specific microglia markers CD13 and MCT-1 [29]. Similar results were obtained using primary microglia extracted from SJL/J mice [29]. Moreover the identification of the lactate transporter on exosomes reveals their ability to deliver energy substrates to match the energy needs during increased neuronal activity, strengthening a model in which lactate flux may serve as support to neurons during synaptic activation [30].

ATP is a typical stimulus used to increase the secretion of EVs by cultured microglia [6] both in N9 cells and primary rat and mouse microglia [31,32]. The purinergic P2X7 receptor has been reported as the

major mechanism underlying the ATP-mediated EV shedding and secretion in N9 cells. It has been hypothesized that EV loaded with functional P2X7 can be a way for the cells to downregulate their plasma membrane pool, thereby reducing P2X7-mediated apoptosis [28]. This observation also suggests that EVs may serve as a carrier to transfer functional receptors out of the cell membrane modifying the responsiveness of cells to specific extracellular stimuli.

ATP stimulation is capable of modifying the content of EVs released from rat primary microglia, enriching them in proteins implicated in cell adhesion/extracellular matrix organization, metabolism, and autophagolysosomal pathway (**Table 1**) [33]. The increase of neuronal and microglial autophagy has been correlated with synaptic pruning [34], underling a possible correlation with the ATP-mediated release of EVs. Interestingly, in EVs released upon ATP stimulation, Drago et al. reveal the presence of the complement protein C1q involved in the pruning process [35], suggesting the role of microglia-derived EVs as carriers to tag aberrant synapses. ATP stimulus also increases metabolism-related proteins and enzymes, which are not present in constitutive EVs. Among these, there are enzymes of glycolysis, lactate production, pentose phosphate pathway, glutamine metabolism, and fatty acid synthesis (**Table 1**) [33]. All these proteins reflect the metabolic changes occurring after ATP stimulation that promote the generation of the fatty acid pool needed for microglia scanning process and phagocytic activity.

Capsaicin has been reported as another activation stimulus by Marrone et al. for microglia-derived shedding [36]. They found that the activation of the Transient Receptor Potential Vanilloid Type 1 (TRPV1) receptor by capsaicin on C57BL/6-derived murine microglial cells increases the production of MVs similarly to ATP. These MVs bear the capability of inducing miniature excitatory postsynaptic currents (mEPSCs) in neurons, confirming their role in the modulation of the synaptic activity of neurons [37].

Notably, the inflammatory status of microglia cells can affect the responsiveness to extracellular ATP. Takenouchi et al. describe how ATP may induce the release of EV enriched with GAPDH from MG6 mouse cells only under inflammatory conditions [32]. Extracellular GAPDH is involved in the regulation of neuritogenesis, and these results further support the role that microglia-EVs can play in the modulation of neuronal development.

Inflammation mimicked by lipopolysaccharides (LPS) treatment has demonstrated the importance of EVs in the CNS as evidenced by Asai et al. [38]. They have demonstrated that the ATP stimulation of primary cultured murine microglia, but not neurons or astrocytes, led to secretion of Tau in the exosome

fraction, albeit not phosphorylated. However, Tau is then transmitted from exosomes to cultured neurons where it is phosphorylated. As phosphorylated Tau is a pathological hallmark of different neurodegenerative pathologies [39], this work confirms the importance of microglia exosomes in the propagation of Tau pathology.

Using α-synuclein as a stimulus in EV production from BV2 cells, Chang et al. have observed an increased secretion of EVs bearing a high level of mTNF-α (EVs-T) and a higher surface amount of MCH-II receptor, with respect to EVs obtained by untreated microglia. The EVs-T are able to increase apoptosis in rat cortical neurons [40] and this effect can be counteracted by mTNF-α neutralizing antibodies supporting the active role of EVs-T in this process. Interestingly, only EVs can produce this specific effect on recipient cells, in fact, a concentration of soluble TNF-α equal to that contained in the α-synuclein-induced EVs does not show the same apoptotic effects on cortical neurons.

Recently, it has also been reported that α-synuclein can not only be a microglia stimulus to produce EV, but also a cargo [41]. Specifically, plasma exosomes derived by PD patients are internalized by BV2 cells where they caused cell autophagy dysregulation, leading to an increased accumulation of intracellular α-synuclein and its accelerated secretion into the extracellular medium, as exosome cargo. These data strengthen the role played by microglia in neuroinflammatory conditions through the release of EVs, carrying pathological proteins and inflammatory mediators.

Neuroinflammation is the primary response of microglia not only in case of infection but also upon TBI in which it can mediate neuronal damage [42]. Kumar et al. analyzed microparticles (MPs) extracted from the blood of C57BL/6 mice after surgical TBI, and showed that TBI increases the release of microglia-derived MPs. TBI-derived MPs can upregulate pro-inflammatory signaling in recipient cells. The same authors also evaluate the effects of LPS in the modulation of microglia-derived EV production. They show how the presence of IL-1β and miR-155 is highly increased in LPS-derived EVs and sufficient to activate resting microglia and propagate the pro-inflammatory response [42–44]. In fact, the treatment of primary and immortalized microglia (BV2) cells with these MPs increases the expression of IL-1β, TNF-α, CCL2, IL-6, NOS2, and miR-155. MPs extracted both from BV2 cells upon LPS treatment and from mice primary microglia isolated *ex vivo*, following TBI, can initiate neuroinflammation upon intracortical injection in naïve animals, confirming the pro-inflammatory capability of TBI-derived MPs [6,42,43].

The ischemic injury induced in rat primary microglia culture (Oxygen-Glucose Deprivation, OGD model) is also able to promote the release of exosomes carrying Nicotinamide Phosphoribosyltransferase

(NAMPT), a key enzyme of the nicotinamide adenine dinucleotide synthesis pathway which acts as a pro-inflammatory agent in the CNS [45]. The release of exosomes seems to be mediated by the increase of extracellular ATP in response to the ischemic injury.

It has been recently demonstrated that the strength of the inflammatory response mediated by microglia depends on the tissue microenvironment. Murgoci et al. report an extensive proteomic analysis on exosomes extracted from neonatal rat microglial cells derived from two different CNS locations, the Spinal Cord Microglia (SpC-M) and Cortex Microglia (Cx-M), in resting condition and upon stimulation with LPS. The results demonstrate that microglia functions and their inflammatory response depend on their origin and these characteristics are preserved if microglia cells are maintained *in vitro* [46]. Cx-M and SpC-M derived exosomes displayed some of the typical EVs proteins (including tetraspanin CD81, transmembrane protein Anxa2, cytosolic protein S100, C1q) and proteins related to development, (such as axonal growth promoters, neuroprotective factors, promoters of neurites outgrowth), and have been implicated in neurodevelopment (**Table 1**). By contrast, only Cx-M derived exosomes revealed an enrichment in neurite outgrowth, nerve regeneration and axonogenesis, whereas only SpC-M-derived exosomes showed an enrichment of inflammation and injury categories. Specifically, in control and LPS-induced SpC-M exosomes, several proteins involved in inflammatory processes have been identified. LPS-treated Cx-M exosomes present proteins involved in chemotaxis, NFκB pathway, and metabolic processes. Finally, exosomes purified from LPS-treated microglia revealed the presence of pro-inflammatory chemokines, as well as the presence of IL-6 and TNF-α [46]. Interestingly, both sources of microglia exosomes enhance the neurite outgrowth of Dorsal Root Ganglion Cells (DRGs). Conversely, only SpC-derived exosomes under LPS stimulation significantly attenuated glioma proliferation supporting the idea that the origin of microglia can ensure different biological functions [46].

Glebov et al. have provided first evidence of neurotransmitter-induced communication between neurons and microglia via released exosomes [47]. Serotonin (5-HT) can induce exosome release from BV2 microglial cells, by stimulation of 5-HTRs. The stimulation of 5-HTRs causes an increase of intracellular Ca^{2+} via PLC phosphorylation that seems to favor the fusion of MVBs with the plasma membrane and consequently increases the secretion of EVs. The vesicles have shown an increased concentration of Insulin Degrading Enzyme (IDE), flotillin-1, and actin. Glebov et al. also suggest an active communication of serotoninergic neurons with microglia mediated by the EVs; however, this suggestion is not supported by a direct evaluation.

Another peculiar stimulus promoting the EV release is the Wnt3a protein. Wnt3a is a morphogenic protein of the WNT family, which is emerging to play diverse roles in the adult CNS [48,49]. Hooper et al. study the effects of recombinant Wnt3a administration to primary rat microglial cells on EVs production and composition [50]. Wnt3a does not increase EVs secretion; however, a proteomic analysis reveals that Wnt3-induced EVs are enriched in proteins associated with cellular metabolism, architecture, and protein synthesis [50]. Wnt3a-induced EVs also contain Wnt3a, ubiquitin, and proteasome components such as proteasome subunit beta type-7 and alpha type-2 (**Table 1**), suggesting a possible role in the extracellular space clearance.

The leech is also a model to study microglia [22], and proteomic analysis of its microglia-derived EVs have been found to contain components involved in neuronal development, neurite growth, axon guidance, filopodium assembly, and dendrite development, all effects confirmed by *in vitro* administration to recipient neurons [51,52].

Lipid Composition

Hundreds of lipids populate the plasma membrane [53]. The lipid environment in the membrane is related to its protein content [54], and lipids can regulate receptor-dependent cell signaling [55,56]. Accordingly, a functional role for lipids is expected also for EVs. Indeed, the interest in lipid research on EVs is growing very rapidly [57]. Most of the lipidomic studies agree in identifying Cholesterol (CHOL), Sphingomyelin (SM), glycosphingolipids, and Phosphatidylserine (PS) as the most recurrent lipids in EVs [53]. The lipid composition is reported to be highly variable among different vesicles, with the highest content of CHOL and SM observed in exosomes rather than MVs [58]. It has to be noted though that most of the studies on EV lipid composition do not include microglia-derived EVs [59]. However, Osteikoetxea et al. described the protein/lipid ratio of exosomes released from resting BV2 cells [60], suggesting a similarity between microglia derived-EVs and EVs derived from other types of cells.

The relatively few studies performed on microglia-derived EVs stand out for providing compelling evidence of the functional role of EVs lipids. A striking example is the recent observation that the lipid fraction of microglia-derived EVs can drive oligodendrocytes precursor cell (OPC) maturation [61], almost to the same extent as the same EVs enriched with miR-219, a crucial component of pro-myelinating exosomes [61,62]. The authors have characterized the lipid content of EVs derived from primary rat microglia stimulated with ATP. They have used non-polarized microglia (NS), or microglia polarized by MSC co-culture (MSC-EVs), a Th1 cocktail of inflammatory cytokines (i-EVs), or IL-4, a

pro-regenerative cytokine (IL-4-EVs). They detect the presence of sphingosine-1-phosphate (S1P) that crucially acts as an attractive guidance cue for OPC migration to the myelin lesion site *in vivo* due to S1P receptor expression on the OPC plasma membrane [61]. However, while MSC-EVs and IL-4-EVs can also promote OPC maturation and initiate remyelination, i-EVs stop OPC maturation [61]. Co-culture experiments revealed that the negative effect of i-EVs is mediated by the detrimental action of protein and/or RNA cargoes onto astrocytes, which are converted into the A1 harmful phenotype [63].

In another work, Prada et al. show that PS of MVs derived from microglia treated with ATP is involved in the recognition and contact with different recipient cells [64]. This is in line with the PS role previously reported for the targeting of monocyte/macrophage-derived MVs onto platelets [65]. However, they also found that MVs contact microglia and astrocytes in very different ways. MVs on microglia display a net drifted movement on the plasma membrane, possibly driven by cortical actin flow, before internalization. Instead, on astrocytes, they are static and almost do not internalize. Finally, they show that cloaking of MVs-related PS by annexin-V reduces MVs adhesion only in part, suggesting that other surface molecules likely contribute to their interaction with glial cells.

Microglia-derived EVs can also crucially influence the excitation/inhibition balance in the adult CNS. The endocannabinoid N-Arachidonoylethanolamine (AEA), a lipid crucially involved in the modulation of synaptic transmission, is enriched in EVs derived from ATP-stimulated N9 cells and primary rat microglia [66]. AEA is mainly present in the MVs sample, which, administrated to cultured hippocampal neurons, can modulate GABAergic transmission, due to the activation of presynaptic CB1. The same group has also shown that microglia-derived EVs positively affect spontaneous excitatory neurotransmission *in vitro* and *in vivo* by promoting neuronal production of ceramide and sphingosine [37]. These data may suggest that EVs are good vehicles of bioactive hydrophobic compounds like endocannabinoids, whose transport across the CNS would be otherwise difficult to explain [66].

miRNA Composition

miRNAs are small non-coding RNAs, which play a crucial role in the post-transcriptional regulation of gene expression [67]. Their packaging into EVs has been widely recognized as a way to protect them from degradation and potentiate their response [68]. The observation that the highest expression of tissue-specific miRNAs is found in the brain [69] has motivated several studies about the role of EVs-associated miRNA in brain pathologies [70], as well as an in-depth characterization of the miRNA content of microglia-derived EVs [71,72].

The miRNA content of microglia-derived EVs was analyzed by RNA-seq by Lemaire et al. in resting leech primary microglia, finding an enrichment of 6 miRNAs: miR-1860, miR-1705, miR-2284y-6, miR-146a, miR-858, miR-7718. Most of the selected miRNA show at least 2-fold increased expression in microglia parental cells than in recipient neurons, qualifying them also as a possible signature for microglia-derived EVs [51]. Noteworthy, some changes caused by the administration of these EVs to recipient neurons can be ascribed to these miRNAs.

This work has partially confirmed Prada et al. study in which an extensive miRNA profiling of MVs, exosomes, and parental cells from ATP-stimulated primary rat microglia was performed [71]. 21 miRNAs have been found differentially abundant in M1-EVs versus M2-EVs. miR-146a-5p, miR-181a, and miR-223, all displaying conserved synaptic targets, are overexpressed in EVs released from M1 microglia, as well as in the Cerebro Spinal Fluid (CSF) of patients with multiple sclerosis. miR-146a-5p carried by microglia-derived EVs downregulated synaptic Syt1 and Nlg1 protein levels in recipient neurons, leading to loss of dendritic spines and synaptic density *in vitro* and *in vivo* [71]. By this mechanism, inflamed microglia can selectively transfer miRNA to neurons via MVs, directly affecting synaptic transmission. The miRNA signature of microglia-derived EVs has also been measured in N9 cells polarized with LPS and then stimulated with ATP. miR-146a was confirmed to be part of M1-EVs, together with miR-155 and, in line with their increase in the parental cells after the M0-M1 transition. EVs from M1-N9 cells also showed a reduction of miR-124, another characteristic typical of this transition [73], further confirming that the composition and biological effect of EVs strictly depends on the condition of the parental cell. Tian et al. analyzed miRNAs in EVs derived from BV2 cells polarized with LPS to induce the M1 shift and with IL-4 to induce the M2 one. They identified miR-26a as a distinguishing miRNA upregulated in M2 microglia-derived exosomes. As miR-26a was previously shown to promote angiogenesis in glioma [74], and miR-26a loaded EVs can promote angiogenesis *in vitro* and *in vivo*, they claim that exosomes released by IL-4-polarized microglia may have therapeutic potential in the treatment of stroke [75].

Interestingly, EVs secreted by LPS-treated primary rat microglia can have anticancer effects, on rat glioma. The molecular mechanism by which this occurs is unknown but it has been proposed to derive from the miRNA cargo of EVs, in particular the family of miR-16 [76].

Fernandes et al. used SH-SY5Y cells transfected with the Swedish mutant of APP695 (SH_{Swe}) as a source of APP- and Aβ-enriched exosomes and they administrated them to human microglia HMC3 cells [77]. While SH_{Swe} exosomes are enriched in miR-155, miR-146a, miR-124, miR-21, and miR-125b, exosomes

in turn released by HMC3 cells contain just one upregulated miRNA, miR-21, one of the most common regulators of the inflammatory response. This work thus suggests an important role for miR-21 in neuron-microglia communication during AD pathogenesis [78].

There is a wide interest in understanding the role of EVs-associated miRNAs in several pathologies of the brain such as TBI [79], neuroinflammation and depression [80], and neurodegeneration [70,81]. Understanding whether these miRNAs represent a pathology biomarker or also a target for therapeutic intervention will certainly constitute an exciting avenue for future investigations.

Conclusion

The composition of EVs can vary greatly depending on both the stimulus received from the parental cells and the cell model used making the overall picture stimulating but also puzzling. It is worth noticing that, even though there are a lot of studies regarding the biological effect of EVs in the CNS, including their interaction with tumors and neurodegenerative diseases, the majority of them still consider microglial cells as recipient cells for EVs produced by other components of the CNS [82–84]. Considering the role of microglia and its nature of restless immune cells, it will be important to identify all the possible effects of these cells on neighboring cells mediated by EVs.

Recently, EVs have been extensively manipulated and engineered by different approaches such as passive or active drug loading, active targeting, and pH-responsive EVs. These can be focused on modifying both the secreting cells (endogenous engineering) and the EVs after isolation (exogenous engineering). EVs can be produced with artificial features aimed to improve their translational potential. However, the lack of knowledge on the mechanisms of action and the specific biological effects of microglia-derived EVs in different pathological conditions has so far hindered their development and use in this field. Understanding deeper the functional roles of these structures would pave the way for the development of therapies that include microglia-EVs, as already done and consolidated for mesenchymal stem cells [85–87].

Perspective

1. EVs are a pivotal method of cell-to-cell communication in both physiological and pathological conditions. Although accumulating evidence demonstrates the crucial role of microglia-derived

EVs in the CNS, the molecular composition and the mechanisms underlying their release have not been fully understood yet.

2. Thanks to omics approaches, this field is accumulating knowledge about the EVs composition, but there is a lack of a unifying vision allowing the correlation of a precise EV component with one or more biological effects.
3. Most of the data in the field mostly derive from animal models, and the interspecies differences call for the need to look for reliable human models. Further studies will be necessary to deepen the composition-function relationship of human EVs and understand if they can be exploited for therapeutic applications.

Abbreviations

5-HT	Serotonin
5-HTRs	5-HT Receptors
AEA	N-Arachidonoylethanolamine
CHOL	Cholesterol
CNS	Central Nervous System
CSF	Cerebro Spinal Fluid
Cx-M	Cortex Microglia
DRGs	Dorsal Root Ganglion Cells
EVs	Extracellular Vesicles
IDE	Insulin Degrading Enzyme
ISEV	International Society of Extracellular Vesicles
LPS	Lipopolysaccharides
MCHII	Major Histocompatibility Complex Class II
mEPSCs	Miniature Excitatory Postsynaptic Currents
miRNA	MicroRNA
MISEV	Minimal Information for Studies of Extracellular Vesicles
MPs	Microparticles
MVB	Multivesicular Body
MVs	Microvesicles
NAMPT	Nicotinamide Phosphoribosyltransferase
OGDNAMPT	Oxygen-Glucose Deprivation Nicotinamide phosphoribosyltransferase
OPCOGD	Oligodendrocytes Precursor CellOxygen-Glucose Deprivation
PDOPC	Parkinson's DiseaseOligodendrocytes Precursor Cell
PSPD	PhosphatidylserineParkinson's Disease
S1PPS	Sphingosine-1-PhosphatePhosphatidylserine
SMS1P	SphingomyelinSphingosine-1-Phosphate
SpC-MSM	Spinal Cord MicrogliaSphingomyelin
TBISpC-M	Traumatic Brain InjuriesSpinal Cord Microglia
TRPV1TBI	Transient Receptor Potential Vanilloid Type 1Traumatic Brain Injuries
TRPV1	Transient Receptor Potential Vanilloid Type 1

Funding

None

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Acknowledgements

The authors acknowledge Prof. Paolo Bergese and Miriam Romano for useful discussions and apologize to the authors whose work could not be cited due to space limitations.

Author Contribution

L.C. and C.G. conceived the area of focus for this review. L.C., C.G. and L.M. wrote the main text. C.M. and L.M. edited.

References

[1] Doyle L, Wang M. Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. Cells 2019;8:727. https://doi.org/10.3390/cells8070727.

[2] O'Brien K, Breyne K, Ughetto S, Laurent LC, Breakefield XO. RNA delivery by extracellular vesicles in mammalian cells and its applications. Nat Rev Mol Cell Biol 2020;21:585–606. https://doi.org/10.1038/s41580-020-0251-y.

[3] Minciacchi VR, Freeman MR, Di Vizio D. Extracellular Vesicles in Cancer: Exosomes, Microvesicles and the Emerging Role of Large Oncosomes. Semin Cell Dev Biol 2015;40:41–51. https://doi.org/10.1016/j.semcdb.2015.02.010.

[4] Raposo G, Stoorvogel W. Extracellular vesicles: Exosomes, microvesicles, and friends. J Cell Biol 2013;200:373–83. https://doi.org/10.1083/jcb.201211138.

[5] Busatto S, Zendrini A, Radeghieri A, Paolini L, Romano M, Presta M, et al. The nanostructured secretome. Biomater Sci 2020;8:39–63. https://doi.org/10.1039/c9bm01007f.

[6] Paolicelli RC, Bergamini G, Rajendran L. Cell-to-cell Communication by Extracellular Vesicles: Focus on Microglia. Neuroscience 2019;405:148–57. https://doi.org/10.1016/j.neuroscience.2018.04.003.

[7] Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol 2014;30:255–89. https://doi.org/10.1146/annurev-cellbio-101512-122326.

[8] Cocucci E, Racchetti G, Meldolesi J. Shedding microvesicles: artefacts no more. Trends Cell Biol 2009;19:43–51. https://doi.org/10.1016/j.tcb.2008.11.003.

[9] Théry C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. Nat Rev Immunol 2009;9:581–93. https://doi.org/10.1038/nri2567.

[10] György B, Szabó TG, Pásztói M, Pál Z, Misják P, Aradi B, et al. Membrane vesicles, current state-of-the-art: Emerging role of extracellular vesicles. Cell Mol Life Sci 2011;68:2667–88. https://doi.org/10.1007/s00018-011-0689-3.

[11] Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal

information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 2018;7. https://doi.org/10.1080/20013078.2018.1535750.

[12] Lötvall J, Hill AF, Hochberg F, Buzás EI, Vizio D Di, Gardiner C, et al. Minimal experimental requirements for definition of extracellular vesicles and their functions: A position statement from the International Society for Extracellular Vesicles. J Extracell Vesicles 2014;3. https://doi.org/10.3402/jev.v3.26913.

[13] Trams EG, Lauter CJ, Norman Salem J, Heine U. Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. BBA - Biomembr 1981;645:63–70. https://doi.org/10.1016/0005-2736(81)90512-5.

[14] Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem 1987;262:9412–20. https://doi.org/10.1016/s0021-9258(18)48095-7.

[15] Raposo G, Nijman HW, Stoorvogel W, Leijendekker R, Harding C V., Melief CJM, et al. B lymphocytes secrete antigen-presenting vesicles. J Exp Med 1996;183:1161–72. https://doi.org/10.1084/jem.183.3.1161.

[16] Greco V, Hannus M, Eaton S. Argosomes: A potential vehicle for the spread of morphogens through epithelia. Cell 2001;106:633–45. https://doi.org/10.1016/S0092-8674(01)00484-6.

[17] Marzesco AM, Janich P, Wilsch-Bräuninger M, Dubreuil V, Langenfeld K, Corbeil D, et al. Release of extracellular membrane particles carrying the stem cell marker prominin-1 (CD133) from neural progenitors and other epithelial cells. J Cell Sci 2005;118:2849–58. https://doi.org/10.1242/jcs.02439.

[18] Fauré J, Lachenal G, Court M, Hirrlinger J, Chatellard-Causse C, Blot B, et al. Exosomes are released by cultured cortical neurones. Mol Cell Neurosci 2006;31:642–8. https://doi.org/10.1016/j.mcn.2005.12.003.

[19] Ramirez SH, Andrews AM, Paul D, Pachter JS. Extracellular vesicles: Mediators and biomarkers of pathology along CNS barriers. Fluids Barriers CNS 2018;15. https://doi.org/10.1186/s12987-018-0104-7.

[20] Yuana Y, Sturk A, Nieuwland R. Extracellular vesicles in physiological and pathological conditions. Blood Rev 2013;27:31–9. https://doi.org/10.1016/j.blre.2012.12.002.

[21] Koniusz S, Andrzejewska A, Muraca M, Srivastava AK, Janowski M, Lukomska B. Extracellular

vesicles in physiology, pathology, and therapy of the immune and central nervous system, with focus on extracellular vesicles derived from mesenchymal stem cells as therapeutic tools. Front Cell Neurosci 2016;10. https://doi.org/10.3389/fncel.2016.00109.

[22] Ceccarelli L, Giacomelli C, Marchetti L, Martini C. Advances in Microglia Cellular Models: Focus on Extracellular Vesicle production [Submitted to Biochem Soc Trans.]. Biochem Soc Trans 2021. https://doi.org/10.1042/BST20210203

[23] Aires ID, Ribeiro-Rodrigues T, Boia R, Ferreira-Rodrigues M, Girão H, Ambrósio AF, et al. Microglial extracellular vesicles as vehicles for neurodegeneration spreading. Biomolecules 2021;11. https://doi.org/10.3390/biom11060770.

[24] Tricarico C, Clancy J, D'Souza-Schorey C. Biology and biogenesis of shed microvesicles. Small GTPases 2017;8:220–32. https://doi.org/10.1080/21541248.2016.1215283.

[25] Catalano M, O'Driscoll L. Inhibiting extracellular vesicles formation and release: a review of EV inhibitors. J Extracell Vesicles 2020;9. https://doi.org/10.1080/20013078.2019.1703244.

[26] Bianco F, Perrotta C, Novellino L, Francolini M, Riganti L, Menna E, et al. Acid sphingomyelinase activity triggers microparticle release from glial cells. EMBO J 2009;28:1043–54. https://doi.org/10.1038/emboj.2009.45.

[27] Bernimoulin M, Waters EK, Foy M, Steele BM, Sullivan M, Falet H, et al. Differential stimulation of monocytic cells results in distinct populations of microparticles. J Thromb Haemost 2009;7:1019–28. https://doi.org/10.1111/j.1538-7836.2009.03434.x.

[28] Turola E, Furlan R, Bianco F, Matteoli M, Verderio C. Microglial microvesicle secretion and intercellular signaling. Front Physiol 2012;3 MAY:149. https://doi.org/10.3389/fphys.2012.00149.

[29] Potolicchio I, Carven GJ, Xu X, Stipp C, Riese RJ, Stern LJ, et al. Proteomic Analysis of Microglia-Derived Exosomes: Metabolic Role of the Aminopeptidase CD13 in Neuropeptide Catabolism. J Immunol 2005;175:2237–43. https://doi.org/10.4049/jimmunol.175.4.2237.

[30] Bröer S, Rahman B, Pellegri G, Pellerin L, Martin JL, Verleysdonk S, et al. Comparison of lactate transport in astroglial cells and monocarboxylate transporter 1 (MCT 1) expressing Xenopus laevis oocytes. Expression of two different monocarboxylate transporters in astroglial cells and neurons. J Biol Chem 1997;272:30096–102. https://doi.org/10.1074/jbc.272.48.30096.

[31] Bianco F, Pravettoni E, Colombo A, Schenk U, Möller T, Matteoli M, et al. Astrocyte-Derived ATP Induces Vesicle Shedding and IL-1β Release from Microglia. J Immunol 2005;174:7268–

77. https://doi.org/10.4049/jimmunol.174.11.7268.

[32] Takenouchi T, Tsukimoto M, Iwamaru Y, Sugama S, Sekiyama K, Sato M, et al. Extracellular ATP induces unconventional release of glyceraldehyde-3-phosphate dehydrogenase from microglial cells. Immunol Lett 2015;167:116–24. https://doi.org/10.1016/j.imlet.2015.08.002.

[33] Drago F, Lombardi M, Prada I, Gabrielli M, Joshi P, Cojoc D, et al. ATP modifies the proteome of extracellular vesicles released by microglia and influences their action on astrocytes. Front Pharmacol 2017;8:910. https://doi.org/10.3389/fphar.2017.00910.

[34] Lieberman OJ, McGuirt AF, Tang G, Sulzer D. Roles for neuronal and glial autophagy in synaptic pruning during development. Neurobiol Dis 2019;122:49–63. https://doi.org/10.1016/j.nbd.2018.04.017.

[35] Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, et al. The Classical Complement Cascade Mediates CNS Synapse Elimination. Cell 2007;131:1164–78. https://doi.org/10.1016/j.cell.2007.10.036.

[36] Marrone MC, Morabito A, Giustizieri M, Chiurchiù V, Leuti A, Mattioli M, et al. TRPV1 channels are critical brain inflammation detectors and neuropathic pain biomarkers in mice. Nat Commun 2017;8. https://doi.org/10.1038/ncomms15292.

[37] Antonucci F, Turola E, Riganti L, Caleo M, Gabrielli M, Perrotta C, et al. Microvesicles released from microglia stimulate synaptic activity via enhanced sphingolipid metabolism. EMBO J 2012;31:1231–40. https://doi.org/10.1038/emboj.2011.489.

[38] Asai H, Ikezu S, Tsunoda S, Medalla M, Luebke J, Haydar T, et al. Depletion of microglia and inhibition of exosome synthesis halt tau propagation. Nat Neurosci 2015;18:1584–93. https://doi.org/10.1038/nn.4132.

[39] Daniele S, Giacomelli C, Martini C. Brain ageing and neurodegenerative disease: The role of cellular waste management. Biochem Pharmacol 2018;158:207–16. https://doi.org/10.1016/j.bcp.2018.10.030.

[40] Chang C, Lang H, Geng N, Wang J, Li N, Wang X. Exosomes of BV-2 cells induced by alpha-synuclein: Important mediator of neurodegeneration in PD. Neurosci Lett 2013;548:190–5. https://doi.org/10.1016/j.neulet.2013.06.009.

[41] Xia Y, Zhang G, Han C, Ma K, Guo X, Wan F, et al. Microglia as modulators of exosomal alpha-synuclein transmission. Cell Death Dis 2019;10:1–15. https://doi.org/10.1038/s41419-019-1404-9.

[42] Kumar AA, Stoica BA, Loane DJ, Yang M, Abulwerdi G, Khan N, et al. Microglial-derived microparticles mediate neuroinflammation after traumatic brain injury. J Neuroinflammation 2017;14:47. https://doi.org/10.1186/s12974-017-0819-4.

[43] Jablonski KA, Gaudet AD, Amici SA, Popovich PG, Guerau-de-Arellano M. Control of the inflammatory macrophage transcriptional signature by miR-155. PLoS One 2016;11:e0159724. https://doi.org/10.1371/journal.pone.0159724.

[44] Verderio C, Muzio L, Turola E, Bergami A, Novellino L, Ruffini F, et al. Myeloid microvesicles are a marker and therapeutic target for neuroinflammation. Ann Neurol 2012;72:610–24. https://doi.org/10.1002/ana.23627.

[45] Lu YB, Chen CX, Huang J, Tian YX, Xie X, Yang P, et al. Nicotinamide phosphoribosyltransferase secreted from microglia via exosome during ischemic injury. J Neurochem 2019;150:723–37. https://doi.org/10.1111/jnc.14811.

[46] Murgoci AN, Duhamel M, Raffo-Romero A, Mallah K, Aboulouard S, Lefebvre C, et al. Location of neonatal microglia drives small extracellular vesicles content and biological functions in vitro. J Extracell Vesicles 2020;9. https://doi.org/10.1080/20013078.2020.1727637.

[47] Glebov K, Löchner M, Jabs R, Lau T, Merkel O, Schloss P, et al. Serotonin stimulates secretion of exosomes from microglia cells. Glia 2015;63:626–34. https://doi.org/10.1002/glia.22772.

[48] Miller JR. The Wnts. Genome Biol 2002;3. https://doi.org/10.1186/gb-2001-3-1-reviews3001.

[49] Inestrosa NC, Arenas E. Emerging roles of Wnts in the adult nervous system. Nat Rev Neurosci 2010;11:77–86. https://doi.org/10.1038/nrn2755.

[50] Hooper C, Sainz-Fuertes R, Lynham S, Hye A, Killick R, Warley A, et al. Wnt3a induces exosome secretion from primary cultured rat microglia. BMC Neurosci 2012;13:144. https://doi.org/10.1186/1471-2202-13-144.

[51] Lemaire Q, Raffo-Romero A, Arab T, Van Camp C, Drago F, Forte S, et al. Isolation of microglia-derived extracellular vesicles: Towards miRNA signatures and neuroprotection. J Nanobiotechnology 2019;17:1–21. https://doi.org/10.1186/s12951-019-0551-6.

[52] Arab T, Raffo-Romero A, Van Camp C, Lemaire Q, Le Marrec-Croq F, Drago F, et al. Proteomic characterisation of leech microglia extracellular vesicles (EVs): comparison between differential ultracentrifugation and Optiprep™ density gradient isolation. J Extracell Vesicles 2019;8. https://doi.org/10.1080/20013078.2019.1603048.

[53] Skotland T, Sagini K, Sandvig K, Llorente A. An emerging focus on lipids in extracellular

vesicles. Adv Drug Deliv Rev 2020;159:308–21. https://doi.org/10.1016/j.addr.2020.03.002.

[54] Corradi V, Mendez-Villuendas E, Ingólfsson HI, Gu RX, Siuda I, Melo MN, et al. Lipid-Protein Interactions Are Unique Fingerprints for Membrane Proteins. ACS Cent Sci 2018;4:709–17. https://doi.org/10.1021/acscentsci.8b00143.

[55] Arkhipov A, Shan Y, Das R, Endres NF, Eastwood MP, Wemmer DE, et al. Architecture and membrane interactions of the EGF receptor. Cell 2013;152:557–69. https://doi.org/10.1016/j.cell.2012.12.030.

[56] Marchetti L, Bonsignore F, Gobbo F, Amodeo R, Calvello M, Jacob A, et al. Fast-diffusing p75NTR monomers support apoptosis and growth cone collapse by neurotrophin ligands. Proc Natl Acad Sci U S A 2019;116:21563–72. https://doi.org/10.1073/pnas.1902790116.

[57] Skotland T, Sandvig K, Llorente A. Lipids in exosomes: Current knowledge and the way forward. Prog Lipid Res 2017;66:30–41. https://doi.org/10.1016/j.plipres.2017.03.001.

[58] Pienimaeki-Roemer A, Kuhlmann K, Böttcher A, Konovalova T, Black A, Orsõ E, et al. Lipidomic and proteomic characterization of platelet extracellular vesicle subfractions from senescent platelets. Transfusion 2015;55:507–21. https://doi.org/10.1111/trf.12874.

[59] Basso M, Bonetto V. Extracellular vesicles and a novel form of communication in the brain. Front Neurosci 2016;10. https://doi.org/10.3389/fnins.2016.00127.

[60] Osteikoetxea X, Balogh A, Szabó-Taylor K, Németh A, Szabó TG, Pálóczi K, et al. Improved characterization of EV preparations based on protein to lipid ratio and lipid properties. PLoS One 2015;10. https://doi.org/10.1371/journal.pone.0121184.

[61] Lombardi M, Parolisi R, Scaroni F, Bonfanti E, Gualerzi A, Gabrielli M, et al. Detrimental and protective action of microglial extracellular vesicles on myelin lesions: astrocyte involvement in remyelination failure. Acta Neuropathol 2019;138:987–1012. https://doi.org/10.1007/s00401-019-02049-1.

[62] Pusic AD, Kraig RP. Youth and environmental enrichment generate serum exosomes containing miR-219 that promote CNS myelination. Glia 2014;62:284–99. https://doi.org/10.1002/glia.22606.

[63] Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. Nature 2017;541:481–7. https://doi.org/10.1038/nature21029.

[64] Prada I, Amin L, Furlan R, Legname G, Verderio C, Cojoc D. A new approach to follow a single

extracellular vesicle–cell interaction using optical tweezers. Biotechniques 2016;60:35–41. https://doi.org/10.2144/000114371.

[65] Del Conde I, Shrimpton CN, Thiagarajan P, López JA. Tissue-factor-bearing microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. Blood 2005;106:1604–11. https://doi.org/10.1182/blood-2004-03-1095.

[66] Gabrielli M, Battista N, Riganti L, Prada I, Antonucci F, Cantone L, et al. Active endocannabinoids are secreted on extracellular membrane vesicles. EMBO Rep 2015;16:213–20. https://doi.org/10.15252/embr.201439668.

[67] Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: Are the answers in sight? Nat Rev Genet 2008;9:102–14. https://doi.org/10.1038/nrg2290.

[68] Patton JG, Franklin JL, Weaver AM, Vickers K, Zhang B, Coffey RJ, et al. Biogenesis, delivery, and function of extracellular RNA. J Extracell Vesicles 2015;4. https://doi.org/10.3402/jev.v4.27494.

[69] Sempere LF, Freemantle S, Pitha-Rowe I, Moss E, Dmitrovsky E, Ambros V. Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. Genome Biol 2004;5. https://doi.org/10.1186/gb-2004-5-3-r13.

[70] Hill AF. Extracellular Vesicles and Neurodegenerative Diseases. J Neurosci 2019;39:9269–73. https://doi.org/10.1523/JNEUROSCI.0147-18.2019.

[71] Prada I, Gabrielli M, Turola E, Iorio A, D'Arrigo G, Parolisi R, et al. Glia-to-neuron transfer of miRNAs via extracellular vesicles: a new mechanism underlying inflammation-induced synaptic alterations. Acta Neuropathol 2018;135:529–50. https://doi.org/10.1007/s00401-017-1803-x.

[72] Raffo-Romero A, Arab T, Al-Amri IS, Marrec-Croq F Le, Van Camp C, Lemaire Q, et al. Medicinal leech CNS as a model for exosome studies in the crosstalk between microglia and neurons. Int J Mol Sci 2018;19. https://doi.org/10.3390/ijms19124124.

[73] Cunha C, Gomes C, Vaz AR, Brites D. Exploring New Inflammatory Biomarkers and Pathways during LPS-Induced M1 Polarization. Mediators Inflamm 2016;2016. https://doi.org/10.1155/2016/6986175.

[74] Qian X, Zhao P, Li W, Shi ZM, Wang L, Xu Q, et al. MicroRNA-26a promotes tumor growth and angiogenesis in glioma by directly targeting prohibitin. CNS Neurosci Ther 2013;19:804–12.

https://doi.org/10.1111/cns.12149.

[75] Tian Y, Zhu P, Liu S, Jin Z, Li D, Zhao H, et al. IL-4-polarized BV2 microglia cells promote angiogenesis by secreting exosomes. Adv Clin Exp Med 2019;28:421–9. https://doi.org/10.17219/acem/91826.

[76] Murgoci AN, Cizkova D, Majerova P, Petrovova E, Medvecky L, Fournier I, et al. Brain-Cortex Microglia-Derived Exosomes: Nanoparticles for Glioma Therapy. ChemPhysChem 2018;19:1205–14. https://doi.org/10.1002/cphc.201701198.

[77] Fernandes A, Ribeiro AR, Monteiro M, Garcia G, Vaz AR, Brites D. Secretome from SH-SY5Y APPSwe cells trigger time-dependent CHME3 microglia activation phenotypes, ultimately leading to miR-21 exosome shuttling. Biochimie 2018;155:67–82. https://doi.org/10.1016/j.biochi.2018.05.015.

[78] Sheedy FJ. Turning 21: Induction of miR-21 as a key switch in the inflammatory response. Front Immunol 2015;6. https://doi.org/10.3389/fimmu.2015.00019.

[79] Panaro MA, Benameur T, Porro C. Extracellular vesicles miRNA cargo for microglia polarization in traumatic brain injury. Biomolecules 2020;10:1–22. https://doi.org/10.3390/biom10060901.

[80] Brites D, Fernandes A. Neuroinflammation and depression: Microglia activation, extracellular microvesicles and microRNA dysregulation. Front Cell Neurosci 2015;9:1–20. https://doi.org/10.3389/fncel.2015.00476.

[81] Christoforidou E, Joilin G, Hafezparast M. Potential of activated microglia as a source of dysregulated extracellular microRNAs contributing to neurodegeneration in amyotrophic lateral sclerosis. J Neuroinflammation 2020;17. https://doi.org/10.1186/s12974-020-01822-4.

[82] Abels ER, Maas SLN, Nieland L, Wei Z, Cheah PS, Tai E, et al. Glioblastoma-Associated Microglia Reprogramming Is Mediated by Functional Transfer of Extracellular miR-21. Cell Rep 2019;28:3105-3119.e7. https://doi.org/10.1016/j.celrep.2019.08.036.

[83] Van Der Vos KE, Abels ER, Zhang X, Lai C, Carrizosa E, Oakley D, et al. Directly visualized glioblastoma-derived extracellular vesicles transfer RNA to microglia/macrophages in the brain. Neuro Oncol 2016;18:58–69. https://doi.org/10.1093/neuonc/nov244.

[84] Matarredona ER, Pastor AM. Extracellular Vesicle-Mediated Communication between the Glioblastoma and Its Microenvironment. Cells 2019;9. https://doi.org/10.3390/cells9010096.

[85] Tsiapalis D, O'Driscoll L. Mesenchymal Stem Cell Derived Extracellular Vesicles for Tissue Engineering and Regenerative Medicine Applications. Cells 2020;9.

https://doi.org/10.3390/cells9040991.

[86] Cai J, Wu J, Wang J, Li Y, Hu X, Luo S, et al. Extracellular vesicles derived from different sources of mesenchymal stem cells: Therapeutic effects and translational potential. Cell Biosci 2020;10. https://doi.org/10.1186/s13578-020-00427-x.

[87] Baek G, Choi H, Kim Y, Lee HC, Choi C. Mesenchymal Stem Cell-Derived Extracellular Vesicles as Therapeutics and as a Drug Delivery Platform. Stem Cells Transl Med 2019;8:880–6. https://doi.org/10.1002/sctm.18-0226.

[88] Song Y, Li Z, He T, Qu M, Jiang L, Li W, et al. M2 microglia-derived exosomes protect the mouse brain from ischemia-reperfusion injury via exosomal miR-124. Theranostics 2019;9:2910–23. https://doi.org/10.7150/thno.30879.

[89] Gabrielli M, Battista N, Riganti L, Prada I, Antonucci F, Cantone L, et al. Active endocannabinoids are secreted on the surface of microglial microvesicles. Springerplus 2015;4:1–32. https://doi.org/10.1186/2193-1801-4-S1-L29.

[90] Casella G, Colombo F, Finardi A, Descamps H, Ill-Raga G, Spinelli A, et al. Extracellular Vesicles Containing IL-4 Modulate Neuroinflammation in a Mouse Model of Multiple Sclerosis. Mol Ther 2018;26:2107–18. https://doi.org/10.1016/j.ymthe.2018.06.024.

[91] Raffaele S, Gelosa P, Bonfanti E, Lombardi M, Castiglioni L, Cimino M, et al. Microglial vesicles improve post-stroke recovery by preventing immune cell senescence and favoring oligodendrogenesis. Mol Ther 2021;29:1439–58. https://doi.org/10.1016/j.ymthe.2020.12.009.

Tables and Figures

Table 1 *Main components of microglia-derived EVs, divided by cell model and production stimulus.*

Model	Stimulus	EVs Component
BV2	α-Syn [40]	MHCII, TNF-α [40]
	LPS [42,50]	IL1-β [42] TNF-α, IL1-β [50]
	Serotonin [47]	IDE, Flotilin-1, Actin [47]
	Wnt3 [50]	Actin, peroxidasin homolog, 40S ribosomal, ubiquitin, proteasome sub-units [50]
	IL-4 [75,88]	CD63, CD9, TSG101, miR-124 [88] miR-466, miR-6360, miR-26a, miR-1940 [75]
N9	ATP [31,89]	AEA, TSG101, flotilin-1 [89] IL1-β, P2X7 [31]
MG6	ATP [32]	GAPDH, IL1-β [32]
	LPS [32]	GAPDH [32]
Primary Rat Microglia	ATP [31,33]	GAPDH, IL1-β [31] Glucose-6-phosphate isomerase, Gpi, Lactate dehydrogenase A, LDHA, Malate dehydrogenase 2, Mdh2, Tranketolase, Glucose-6-phosphate 1-dehydrogenase, G6PD, Glutamate dehydrogenase 1, Acetyl-CoA carboxylase-β, Acacβ [33]
	LPS [46]	Cfh, mrc2, C4a, Lgal3bp, Lgals1, C1S, Cfhr1 MCT-1, CD63, CD13, GAPDH, pyruvate kinase, CXCL1, CXCL2, CXCL3, CCl2, CCL7, IL-6, TNF-α [46]
	TBI [42,61]	G6PD, ACACβ [61] P2Y12, CD45 [42]
	Th1 Cytokines cocktail [61,71]	C1q, TNF-α [61] miR-181a, miR-223, miR-146a [71]
	Resting [61]	Spp1, Spon1, Sned1, Srpx, Hspg2, Fstl1, Olfml3 Sphingomyelin, lactosylceramide, ceramide, GM3, glucosylceramide, S1P [61]
Primary Mouse Microglia	ATP [42]	miR-155, miR-146, P2Y12, CD45 [42]
	LPS+ATP [38]	TSG101, MHCII, hTau [38]
	TBI [32]	GAPDH [32]
Primary Leech Microglia	Resting [51]	miR-1860, miR-1705, miR-2284y-6, miR-146a, miR-858, miR-7718 [51]

Table 2 *Effects of EVs on recipient cells. The parental cell model, the production stimulus, the effect raised and the recipient cells are reported.*

Parental Cells	**Stimulus**	**Effect on parental cells**	**Pathways influenced**	**Recipient Cells**
BV2	α-Syn	Inflammation [40]	Neurodegeneration, Neurotoxicity [40]	Rat cortical neurons [40]
	LPS	Inflammation [42]	Neuroinflammation [42] Propagation of inflammation Neurodegeneration [50]	Primary mouse and immortalized rat microglia [42,50]
	Serotonin	Increased EVs production [47]	n/d	n/d
	Wnt3	n/d	Cellular metabolism, maintaining of cellular architecture, protein synthesis and degradation, neuroprotection and extracellular space clearance [50]	n/d
	IL-4	M2-Shift [88]	Modulation of neuroprotection, upregulation of miR-124 [88]	Rat neurons [88]
BV2 expressing IL-4	Resting	M2-Shift [90]	Anti-inflammatory pathways [90]	*in vitro* mouse microglia, *in vivo* mouse phagocytes [90]
N9	ATP	Activation [31]	Neuroinflammation [31]	n/d
MG6	LPS	Inflammation [32]	n/d	n/d
Primary Rat microglia	ATP	Activation [31,33]	Cell adhesion, extracellular matrix organization, autophagolysosomal pathways, cellular metabolism [33] Neuroinflammation [31]	Primary rat astrocytes and microglia [33]
	Resting	n/d	Neurogenesis, proliferation and growth [46] mEPSC induction, Sphingolipid metabolism [37] CB1 activation, mIPSC inhibition [66]	Rat neurons [37,66]
	LPS	n/d	Inflammation and immune response [46]	n/d
	Polarization by MSC co-culture and by IL-4	Pro-regenerative shift [61]	OPC maturation, migration and remyelination [61]	OPC and DRG neurons [61]
Primary Mouse Microglia	TBI	Inflammatory response [42]	Inflammatory signaling [42]	Primary mouse and immortalized rat microglia [42]

	Capsaicin	Increased MVs production [36]	Induction of mEPSC in neurons [36]	Mouse Neurons [36]
	IL4/ATP	M2-Shift [91]	Inhibition of cell senescence [91]	*in vivo* mouse myeloid cells [91]
Primary Leech microglia	Resting	n/d	Neuroprotection and neurite development [51]	n/d

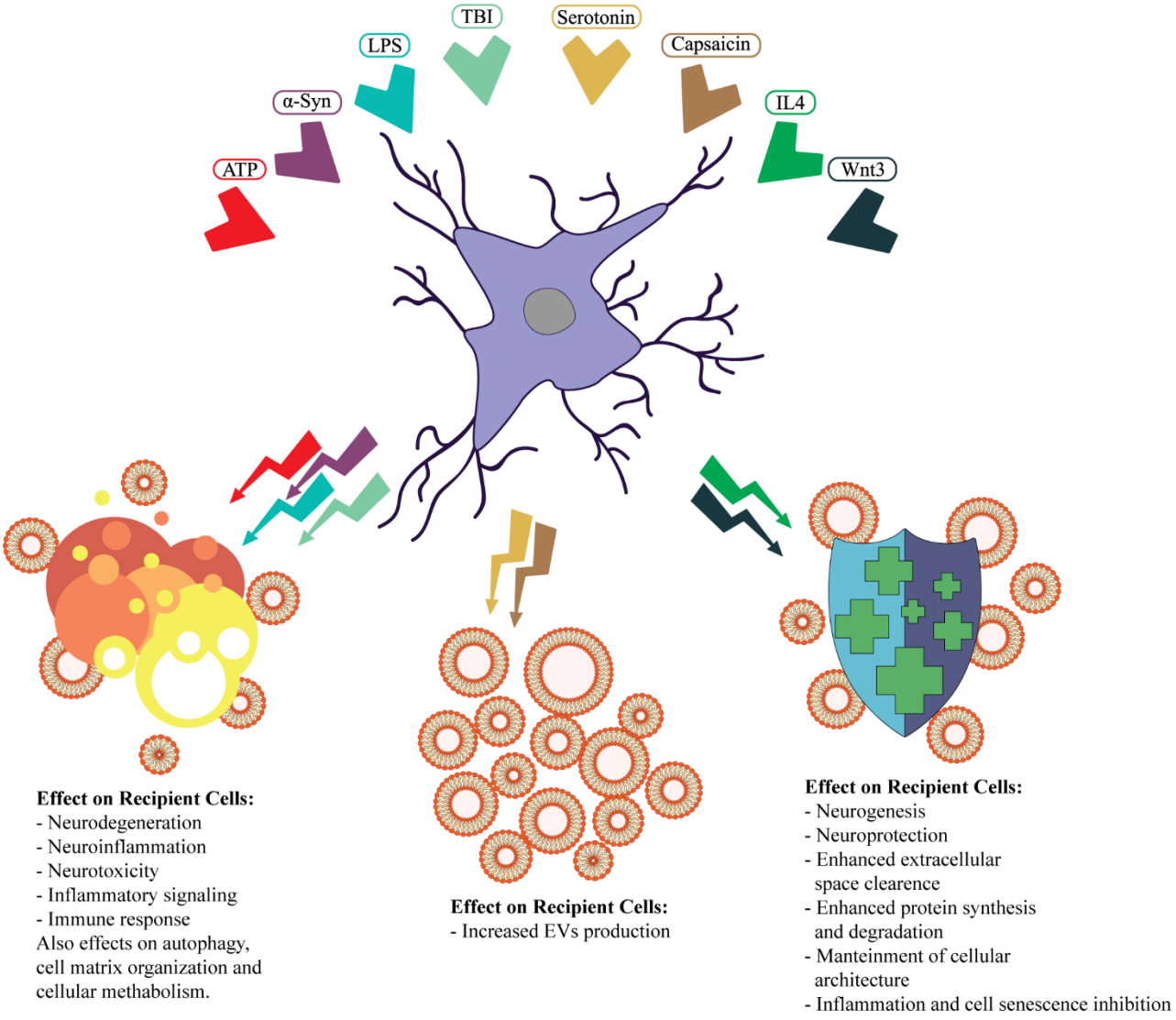

Figure 1 *Schematic representation of the stimuli used to promote EV production by microglial cells. Stimuli are color-coded; arrows at the bottom in the same color-code indicate the main biological effect raised by each stimulated EVs in the CNS.*

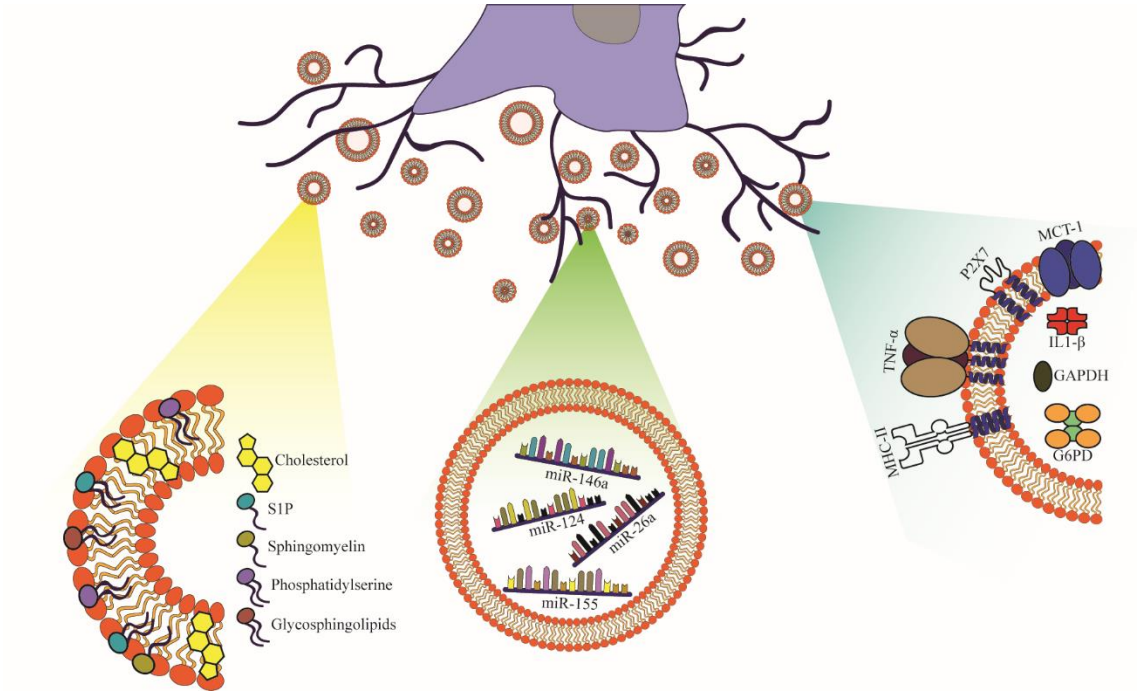

Figure 2 *Main components of microglia-derived EVs. Molecules are grouped in Lipids (left), miRNA (center) and proteins (right). The most representative member of each category has been reported.*