Astrocyte-derived extracellular vesicles: A double-edged sword in central nervous system disorders

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ARTICLE INFO

Keywords:
Astrocyte
Extracellular vesicles
Biomarker
Neurodegeneration
Neuroinflammation
Central Nervous System
Neurological disorders

ABSTRACT

Recent studies suggest that astrocytes released a great quantity of extracellular vesicles (AEVs) to communicate with other brain cells. Under pathological conditions, AEVs are widely associated with the pathogenesis of neurobiological diseases by horizontally transferring pathogenic factors to neighboring cells or peripheral immune cells. Their beneficial role is also evident by the fact that they are involved in neuroprotection and neuroregeneration through alleviating apoptosis, maintaining neuronal function, and repairing neural injuries. The strong association of AEVswith neurological disorders makes AEVs a promising target for disease diagnosis, treatment, and prevention. The identification of disease-specific cargos in AEVs isolated from the patients’ biofluids suggests AEVs as an attractive platform for biomarker development. Furthermore, the inhibition of inflammatory/toxic AEV release and the preservation of neuroprotective AEV release have been considered as potential therapeutic strategies in CNS disorder treatment and prevention, respectively. Here, we summarize the biological roles of AEVs as pathological contributors, protective/regenerative factors, and potential diagnostic biomarkers and therapeutic targets for neurological disorders, with a focus on recent progresses and emerging concepts.

Abbreviations: Aβ, Amyloid β-peptide; ABI, Acquired brain injury; ACR, Acute cytokine response; AEVs, Astrocyte-derived extracellular vesicles; AD, Alzheimer’s disease; ALS, Amyotrophic lateral sclerosis; AUC, Area under the curve; BACE1, β-site amyloid precursor protein-cleaving enzyme 1; BBB, Blood-Brain barrier; cART, Combined antiretroviral therapy; CaSR, Calcium-sensing receptor; CNS, Central nervous system; CR1, Complement receptor type 1; CRP, C-reactive protein; CRYAB, B-Crystallin; CSF, Cerebrospinal fluid; Cx43, Connexin-43; DAF, Decay-accelerating factor; DAMPs, Damage- or danger-associated molecular patterns; EAE, Experimental autoimmune encephalomyelitis; EE, Environmental enrichment; EVs, Extracellular vesicles; FTD, Frontotemporal dementia; GJA1, Gap junction alpha 1; GLAST, Glutamine aspartate transporter; GLS, Glutaminase; HAND, HIV-associated neurological disorders; HD, Huntington’s disease; HIV, Human immunodeficiency virus; HSPB1, Heat-shock protein β1; IL-1β, Interleukin-1 beta; IL-6, Interleukin-6; IL-10, Interleukin-10; IVVs, Intraluminal vesicles; MCAO, Middle cerebral artery occlusion; MCI, Mild cognitive impairment; mTBI, Multivesicular bodies; Nef-PAEVs, HIV-1 Nef-positive primary human fetal astrocyte-derived extracellular vesicles; NF-kB, nuclear factor kappa B; NGB, Neuroglobin; NSCs, Neural stem cells; OGD, Oxygen glucose deprivation; PAPP1, Primary human fetal astrocyte-derived extracellular vesicles; PAMPs, Pathogen-associated molecular patterns; PPARα, Peroxisome proliferator-activated receptor α; PRRs, Pattern recognition receptors; p-Tau, Phospho-Tau; ROC, Receiver operating characteristic; sAPP, Soluble amyloid precursor protein; Sema3A, Semaphorin 3A; SMases, Sphingomyelinases; SOD1, Superoxide Dismutase; sTBI, Sports-related traumatic brain injury; TBI, Traumatic brain injury; TCA, Tricarboxylic acid; TCC, Terminal complement complex; TIGER, Transgenic inducible GFP extracellular-vesicle reporter; TLR4, Toll-like receptor 4; TLR-KO, TLR4-knockout; TLR7, Toll-like receptor 7; TNFα, Tumor necrosis factor alpha; WT, Wild type.

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https://doi.org/10.1016/j.neubiorev.2021.02.027
Received 1 April 2020; Received in revised form 28 December 2020; Accepted 16 February 2021
Available online 21 February 2021

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1. Introduction

Astrocytes are the largest and most prevalent type of glial cells in the central nervous system (CNS) (Sofroniew and Vinters, 2010). Originally, astrocytes were considered as space-filling support cells with single function. Growing evidence has demonstrated that astrocytes express a wide range of receptors, various channels, and second messenger systems, making them a key type of regulatory cells in the CNS (Acosta et al., 2017). Under physiological conditions, astrocytes play important roles in supporting neuronal functions, constituting the Blood-Brain Barrier (BBB), and controlling ion balance. Under pathological conditions, astrocyte reactivity is triggered by a wide variety of stimuli from diverse sources, including age-mediated intracellular alterations and damage within molecular pathways, molecules (e.g., cytokines, growth factors, pattern-associated molecular patterns (PAMPs), and damage- or danger-associated molecular patterns (DAMPs)) secreted by other brain cells and circulating inflammatory ones, microbial pathogens, or environmental toxins (Sofroniew, 2020). Reactive astrocytes contribute to neuroinflammation, acute neural injury, and neurodegenerative diseases (Yoshikawa et al., 2020). Recently, emerging evidence implicates that astrocytes may achieve their functions via secreting extracellular vesicles (EVs) (Alessandrini et al., 2018; Dickens et al., 2017; Goetzl et al., 2018; Wang et al., 2012).

EVs are small bi-layer-enclosed vesicles which are released from virtually all eukaryotic cells for intercellular communication (Cocucci and Meldolesi, 2011; Johnstone et al., 1987). Emerging evidence has implicated that EVs are widely involved in regulating various biological processes through horizontally transferring their cargos including nucleic acids, proteins, and lipids (Xia et al., 2019). As secretory cells (Verkhratsky et al., 2016), normal/resting astrocytes release a large number of EVs that can regulate neurogenesis, angiogenesis, and neuroprotection in vitro (Pascua-Maestro et al., 2018; Proia et al., 2008; Upadhya et al., 2020; You et al., 2020; Yu et al., 2018). Moreover, under stimulation in pathological or aging conditions, reactive/activated astrocytes secrete more EVs with altered protein (Datta Chaudhuri et al., 2020; You et al., 2020) and miRNA (Chaudhuri et al., 2018) contents (Wang et al., 2017a; You et al., 2020; Yu et al., 2018). In response to anti-inflammatory cytokines in the microenvironment, such as Interleukin-10 (IL-10) that are abundantly produced by anti-inflammatory microglia and macrophages (Martinez and Gordon, 2014), astrocytes release EVs containing a set of proteins that promote neuronal plasticity, neurite outgrowth, and neuronal survival (Datta Chaudhuri et al., 2020). In contrast, when astrocytes are activated by aging or inflammatory stimuli like pro-inflammatory microglia- and macrophages-secreted Interleukin-1 beta (IL-1β) and Tumor necrosis factor alpha (TNFα) (Martinez and Gordon, 2014), they secrete EVs loaded with proteins and miRNAs that are involved in inflammatory signal transmission, neurite extension and branching inhibition (Datta Chaudhuri et al., 2020; You et al., 2020), and neuronal excitability reduction (Chaudhuri et al., 2018). Thus, astrocyte-derived EVs (AEVs) not only participate in the occurrence and development of neurological diseases, but also play a protective role in a variety of CNS disorders. Besides, the distinct profiles of proteins and miRNAs in AEVs under different physiological and pathological conditions also imply the possibility to utilize AEVs and their cargos as a new diagnostic index and therapeutic targets. In this review, we provide a comprehensive summarization of AEVs’ roles as pathological mediators, neuroprotective factors, potential biomarkers, and therapeutic targets for CNS diseases due to their unique properties.

2. The biogenesis, uptake, composition, and general function of EVs

2.1. The biogenesis and uptake of EVs

EVs are phospholipid-bilayer-enclosed extracellular spherical structures, with a size ranging between 30 nm and 10 μm, which contain a wide variety of bioactive molecules (Lotvall et al., 2014; Matarredona and Pastor, 2019). EVs can be divided into many classes, and exosomes and microvesicles are the two under most extensive investigation (Fig. 1) (Xu et al., 2016). Exosomes (30–100 nm) are originated from the inward budding of multivesicular bodies (MVBs), leading to the formation of intraluminal vesicles (ILVs). ILVs that released into extracellular spaces by the fusion of MVBs with plasma membrane are referred to as exosomes (Baietti et al., 2012; Cocucci and Meldolesi, 2015; Colombo et al., 2013; Perez-Hernandez et al., 2013; Strauss et al., 2010; Tamai et al., 2010; Trajkovic et al., 2008). However, the term exosome is also used in the literature for EVs with small size (less than 200 nm in diameter) or those that can only be spun down by high-speed ultracentrifugation, without validating their endosomal origin (Kowal et al., 2016). In contrast to exosomes, microvesicles (50–1000 nm) are assembled with small membrane domains and released by direct outward budding of plasma membrane (Mause and Weber, 2016; Raposo and Stoorvogel, 2013). Besides, there are other types of EVs including apoptotic bodies (Atkin-Smith et al., 2015), blood-derived vesicles (Aaartzen et al., 2014), autophagosomes (Pallet et al., 2013), large oncosomes (Minciacchi et al., 2015), migrasomes (Ma et al., 2015), and extracellular microhondrial particles (mito-particles) (Hayakawa et al., 2016) that are not covered in this review.

After being released into extracellular space, EVs interact with recipient cells in three distinct modes that display different engagements with plasma membrane (Mulcahy et al., 2014). First, EVs can be taken up by endocytic pathways including non-specific phagocytosis/micropinocytosis and receptor-ligand interaction-mediated endocytosis, and deliver their contents to the cytosol of the target cell. Second, EVs may directly fuse with the plasma membrane and release their cargos intracellularly. Third, after receptor-ligand interaction, EVs can remain attached to the plasma membrane and manipulate downstream signaling pathways through regulating the activity of receptor.

2.2. The composition of EVs

EVs carry diverse molecules including proteins, RNA species (mRNA, miRNA, IncRNA, and other RNA species), DNAs (mtDNA, ssDNA, dsDNA), and lipids. The contents of EVs are determined by multiple factors such as the intracellular origins of EVs, the types and pathological states of parent cells. For example, due to their endosome origination, exosomes are specifically enriched with many proteins in endosomal pathways, such as MVB biogenesis-related proteins (Alix and TSG101), syntenin-1, and tetraspanins (e.g., CD81) (Kowal et al., 2016). Microvesicles’ biogenesis requires cortical actin reorganization and various contractile and enzymatic proteins including KIP23, RAGCAG, CSE1L, ARF6, EMMPRIN (Greening et al., 2017). Therefore, aforementioned proteins have been used as markers of different EV subtypes, which, provide a biological relevant approach to distinguish and separate these vesicles besides the simple size-based one (Thery et al., 2018).

The contents of EVs also vary with their parent cell types. Neuron-derived EVs have been reported to contain multiple neuronal specific membrane proteins including L1CAM (Goetzl et al., 2015). Similarly, astrocyte-derived EVs are enriched with astroglial surface protein glutamine aspartate transporter (GLAST) (Goetzl et al., 2016). By using antibodies that bind to these proteins specifically, cell type specific EVs can be isolated from plasma through immunoisolation, which, has been utilized in biomarker screening for various neurological disorders (Goetzl et al., 2015, 2016).

The pathophysiological status of parent cells that changes in a dynamic manner also significantly influences the content profiles of EVs. Our recent studies have demonstrated the miRNA profiles of EVs derived from resting microglia are significantly different from that of EVs released from activated microglia (Gao et al., 2019). Similarly, the levels of pro-inflammatory proteins are also significantly higher in EVs isolated from middle cerebral artery occlusion (MCAO) mouse brain, compared...
with sham controls (Gao et al., 2020). These observations all suggest that the contents of EVs reflect the biogenesis of EVs and the types and status of parent cells, which can be utilized in the determination and isolation of different EV population, the diagnosis and progression monitoring of neurological disorders, and many other purposes.

### 2.3. The function of EVs

EVs have been emerged as key intercellular communicators with multiple functions via transporting their contents. During CNS development, neuronal EVs regulate proliferation and neural circuit development in dentate gyrus of neonatal mouse brain (Sharma et al., 2019). In adult brain, neuronal EVs play an important role in maintaining brain vascular integrity through transferring neuron-enriched miR-132 to endothelial cells (Xu et al., 2017). Under pathological conditions, EVs are reported to regulate the pathogenesis of multiple CNS disorders including neurodegeneration, neuroinflammation, and tumorigenesis (Budnik et al., 2016; Xia et al., 2019). For instance, disease-associated molecules, such as Amyloid β-peptide (Aβ) oligomer, phospho-Tau (p-Tau), α-synuclein, cytokines, or prion, are detected in EVs isolated from disease models or patients’ cerebrospinal fluid (CSF) and plasma (Dinkins et al., 2014; Falker et al., 2016; Gao et al., 2020, 2019; Stuendl et al., 2016; Wang et al., 2017b). On one way, EVs speed up the spreading and accumulation of these molecules among cells, which contributes to disease progression (Asai et al., 2015; Gao et al., 2019; Guo et al., 2016; Stuendl et al., 2016). On the other way, EVs carry these neurotoxic molecules out of cells, exhibiting neuroprotective effects (Falker et al., 2016; Spencer et al., 2016).

Given its highly heterogeneous population, it is important to investigate the effects of EVs on physiological and pathological processes in a cell type specific manner. To date, a large number of research works have corroborated the strong association of EVs derived from neurons and microglia, the basic sensory/effect units and native immune responders in the CNS, respectively (for reviews see (Paolicelli et al., 2019; Trotta et al., 2018)). Being the largest and most prevalent type of glial cells in the CNS, astrocytes can also secret EVs with pathological or beneficial effects that regulate the pathogenesis of neurological diseases.

### 3. AEVs: aggravating factors in the pathogenesis of neurological disorders

EVs, as one of the important secretome components from astrocytes, were narrowly assigned the function of removing useless proteins from cells as firstly identified. However, recent researches show that AEVs serve as a crucial aggravating factor under neuropathological conditions (Fig. 2) (Siracusa et al., 2019).
3.1. The contribution of AEVs to aging

Aging is an important and common risk factor for almost all CNS disorders including age-related neurodegenerative diseases (Hou et al., 2019) and stroke (Donnan et al., 2008). Neural aging is a progressive loss of function of central and peripheral neural cells including neurons and NSCs (Chang and Guarente, 2013; Satoh et al., 2013). Key changes in neural aging include the decrease in brain volume and gray matter loss in the medial frontal cortical regions, which can cause impairment of neurological functions in the elderly (Bergfield et al., 2010; Courchesne et al., 2000; Peters et al., 2008; Salthouse, 2009; Sowell et al., 2004). Astrocyte reactivity has been considered as a hallmark of aging in many mammalian species including non-human and human primates (Matias et al., 2019; Nichols et al., 1993). Furthermore, astrocyte reactivity is mainly observed in brain regions that strongly associated with synaptic loss and age-related cognitive decline, such as the hippocampus and frontal cortex (Rodriguez et al., 2016). Thus, the effects of AEVs on aging have emerged as an important and interesting topic.

Growing evidence has implicated the essential roles of EVs in modulating the progress of brain aging. For instance, when compared to young animal serum-derived EVs, EVs isolated from serum of aged animals lost anti-aging effects including promoting oligodendrocyte precursor cells proliferation and differentiation, enhancing myelin production and remyelination (Pusic and Kraig, 2014). Being the most predominant type of secretory cells in the CNS, this functional deficit of EVs may be mediated, partially at least, by AEVs. This premise is supported by following studies. It is shown that normally aged astrocytes take on a reactive phenotype of neuroinflammatory A1-like reactive astrocytes (Clarke et al., 2018). A1-like astrocytes release EVs, enriched with pro-inflammatory molecules and neurotoxic effectors C3b and C5b-9 terminal C complex, that may directly induce neural damage and neurodegeneration (Goetzl et al., 2018, 2020). In addition, Willis et al. found that EVs derived from young astrocytes were able to convey support for oligodendrocyte differentiation while this support was lost in EVs released from aged astrocytes (Willis et al., 2020). Thus, AEVs play an important role in the establishment of aging microenvironment in the CNS which may contribute to multiple neurological disorders.

3.2. The pathogenic effects of AEVs on neuroinflammation

Neuroinflammation is a major pathological process in many CNS disorders (Cederberg and Siesjo, 2010; Heneka and O’Banion, 2007). Damaged or degenerating neurons in the brain release cellular debris into microenvironment, priming astrocytes and macrophage-like microglia in the CNS. These resident cells express various pattern recognition receptors (PRRs) including the family of Toll-like receptors on cell surface and/or within the cell, causing an immune response (Gorina et al., 2011; Holm et al., 2012; Karve et al., 2016). Interestingly, Ibanez et al. reported that Toll-like receptor 4 (TLR4) participates in the release of AEVs (Ibanez et al., 2019). When cultured under an ethanol-induced inflammatory condition, wild type (WT) astrocytes exhibit an elevation in EVs secretion capacity. In contrast, TLR4-knockout (TLR4-KO) astrocytes release similar amount of EVs in both ethanol-treated and untreated groups. Besides the positive effects

Fig. 2. Involvement of astrocyte-derived extracellular vesicles (AEVs) in the pathogenesis of CNS disorders.
The figure illustrates AEV-associated mechanisms for the initiation and progression of neurological diseases. Astrocytes are activated by stimuli secreted from damaged/degenerating neurons or pro-inflammatory microglia, accelerating AEVs release. AEVs transfer neurotoxic molecules (e.g., Aβ42, p-Tau, SOD1, miRNAs) into neurons to induce or exacerbate neurodegeneration, leading to various neurodegenerative diseases. AEVs also transfer pro-inflammatory molecules to activate neighboring microglia, or diffuse across blood-brain barrier to promote the transmigration of leukocytes from periphery to the brain, which, in turn, causes neuroinflammation.
on AEV release, TLR4 also regulates the sorting of inflammatory-related proteins (e.g., IL1R, NLRP3, NfκB-p65) and miRNAs (mir-146a, mir-182, and mir-200b) into AEVs, confirmed by that TLR4-KO abrogates the accumulation of pro-inflammatory proteins and miRNAs in AEVs post ethanol treatment.

Activated astrocyte-derived pro-inflammatory EVs can be directly internalized by neurons and cause neuronal damage. For example, post ethanol and pro-inflammatory cytokines (e.g., IL-1β and TNFα) stimulation, AEVs significantly reduce dendritic growth, dendritic complexity, spike rates, and burst activity (Chaudhuri et al., 2018). Bioinformatic analysis identified that miR-125a-5p and miR-16-5p are abundantly expressed in these AEVs. These miRNAs target TNRK3 and its downstream effector Bcl2, therefore repress neurotrophic signaling in neurons. The pro-inflammatory and neurotoxic effects of AEVs are compromised by TLR4-KO (Dicken et al., 2017). These observations suggest that TLR4-mediated AEVs release as a biological process that induces neuroinflammation.

Besides the direct inflammatory effects of AEVs after uptake by neurons, AEVs also play an essential messenger-role for the communication between astrocytes and microglia during neuroinflammation. After intracerebral injection of IL-1β, mouse astrocytes shed more EVs than microglia (Dicken et al., 2017). The effects of AEVs on microglial activation were examined using transgenic inducible codable GFP extracellular-vesicle reporter (TIGER) mouse (Neckles et al., 2019). The Nestin positive neural stem cells (NSCs)-derived astrocytes secrete abundant EVs that are predominantly internalized by CD11b/IBA1 positive microglia. The knockdown of Rab27a, an EVs release-required protein, in subventricular zone astrocytes reduced the proportions of immune active microglia. It is noteworthy that the miRNAs profile in AEVs considerably overlaps with NSC-derived EVs that were previously reported to control the activation of microglia, suggesting AEVs as key immunomodulators of the CNS. This finding is confirmed in the situation of human immunodeficiency virus (HIV)-associated neurological disorders (HANEs), where chronic neuroinflammation remains a common underlying feature of HIV-infected patients on combined antiretroviral therapy (cART) (Yang et al., 2018). Post exposure to HIV Tat, a cytotoxic viral protein, human astrocytoma (A172) cells release EVs enriched with mir-9, which, was taken up by microglia, resulting in the migratory phenotype transition of the latter cells. The effects of AEVs on microglia migration can be blocked by exosome release inhibitor GW4869, exosomal mir-9 knockdown, or the administration of protectors of PTEN, a mir-9 specific target. Furthermore, AEVs are also involved in the regulation of phagocytic functions of microglia. Both in vivo and in vitro studies demonstrated that EVs derived from morphine-stimulated astrocytes were taken up by microglial endosomes, leading, in turn, to the activation of Toll-like receptor 7 (TLR7) with a subsequent upregulation of lineRNA-CoX2, and ultimately resulted in the impairment of microglial phagocytosis (Hu et al., 2018). Hence, these results suggest that AEVs mediate the activation, migration, and phagocytic capacities of microglia, causing further inflammation process.

Except activating CNS immune cells, AEVs also regulate brain-to-peripheral immune communication. Under the condition of neuroinflammation, the protein and miRNA contents of AEVs promote the peripheral acute cytokine response (ACR) (Dicken et al., 2017). This process is achieved by that AEVs pass across the BBB and target to peroxisome proliferator-activated receptor α (PPARα) in peripheral organs such as liver. AEVs then raise the peripheral immune response by increasing nuclear factor kappa B (NF-kB) activity and enhancing the inflammatory cytokine expression in liver through suppressing PPARα. In vivo study further showed that AEVs promote peripheral leukocyte transmigration into the brain, which probably aggravates neuroinflammation.

3.3. The pathogenic effects of AEVs on acute neural injury

Brain injury refers to the physical injury of brain tissue caused by violence on the head. According to whether the injury is caused by non-external force or external force, it is divided into traumatic brain injury (TBI) and acquired brain injury (ABI). In the past decades, the United States government has invested a lot of money to treat veterans who suffered from brain damage caused by explosive devices and ordnance attacks. In addition, the frequent brain injury accidents in sports field also deepen people’s concern of this disease. Recent efforts show enrichment of neurotoxic molecules in AEVs isolated from the plasma of sports-related TBI (sTBI) (Goetzl et al., 2020) and TBI in military veterans (mTBI) (Winston et al., 2019b) patients. For instance, TBI activates the complement system to release more neurotoxic C-reactive protein (CRP) through AEVs after mild or moderate injury. Mild TBI (mTBI) also facilitates the release of AEVs with a significantly higher level of Aß42, compared with healthy donors, even though Aß42 in AEVs may not induce cytotoxicity. Both results suggest that AEVs partly participate in the aggravation of brain injury.

3.4. The pathogenic effects of AEVs in chronic neurodegenerative diseases

Chronic neurodegenerative diseases are caused by the loss of neurons and/or their myelin sheath, which deteriorates with time and results in dysfunction. Common neurodegenerative diseases include Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), amyotrophic lateral sclerosis (ALS), HANDs, etc.

AD is the most common neurodegenerative disease in elderly people. The etiology of AD is not clear, which is mainly related to genetic and environmental factors like p-Tau and Aß. In the process of AD, a great deal of Aß42 protofibrils accumulates in astrocytes leading to the formation of Aß. The excessive Aß induces astrocytes to express high levels of p-Tau, prostate apoptosis response 4 (PAR-4) and ceramide, and to form giant endosomes for AEV release (Sollvander et al., 2016). PAR-4, ceramide, and p-Tau are associated with AEVs, which exhibit strong neurotoxic and apoptotic influences on neural cells (Chiarini et al., 2017; Wang et al., 2012). Calciytic NPS 2143, an antagonist of Aß—the calcium-sensing receptor (CaSR) signaling, suppresses the formation of Aß to protect astrocytes and inhibit the release of p-Tau-loaded AEVs. Moreover, the high levels of complement proteins (e.g., CRP) in both plasma- and CSF-isolated AEVs are continuously detected during the transformation from mild cognitive impairment to severe dementia, which, contribute to AD-related neurotoxicity as well (Goetzl et al., 2018; Winston et al., 2019a).

PD is also a common neurodegenerative disease among the elderly. The main pathological change of PD is the degeneration of dopaminergic neurons in substantia nigra of midbrain, resulting in significant decrease of dopamine content in the striatum. During the pathological process of PD, α-synuclein, a neuronal cytoplasmic protein, displays a polymorphous and fibrillar conformation, causing neurodegeneration and cell death (Chistiakov and Chistiakov, 2017). To prevent α-synuclein accumulation in neurons, astrocytes and microglia remove extracellular α-synuclein via endocytosis (Choi et al., 2020; Loria et al., 2017). However, extensive uptake of α-synuclein by neuroglia induces inflammatory response, resulting in an elevation of EV secretion. EVs-mediated glia-glia and glia-neuron communication further promotes the spreading of inflammatory response and the aggravation of neurodegeneration (Valdinoj et al., 2017). Besides the classic causative agents, other disease-related molecules such as miRNAs are also involved in the pathogenesis of PD. In the 1-methyl-4-phenylpyridinium (MPP)-induced PD model, a subset of miRNAs are misexpressed in EVs released from MPP-stimulated astrocytes. Among these miRNAs, miR-200a-3p was the most down-regulated one (Schildknecht et al., 2017). miR-200a-3p in AEVs contributed to cell survival in SH-SY5Y cells, primary mesencephalic dopaminergic neurons, and primary hippocampal neurons by targeting mitogen-activated protein kinase kinase 4 (MKK4) and repressing the expression of MKK4 at mRNA and protein levels (Shakespeare et al., 2020). This lack of miR-200a-3p in AEVs leads to activation of caspase-3 signaling and neuronal apoptosis, deteriorating...
dopaminergic neuron degeneration.

Huntington’s disease (HD) is a particularly insidious autosomal-dominant genetic neurodegenerative disease. The main characteristics of the disease are dance-like movement with progressive dysfunction of cognition and mental situation, leading to dementia. Evidence has shown that mutant huntingtin (mHtt) accumulates to suppress the expression of α-B-crystallin (CRYAB), a heat shock protein that represses neuroglial activation and neuroinflammation, in astrocytes (Hong et al., 2017). This process inhibits EV secretion and the sorting of CRYAB into AEVs. Without the supporting function of AEVs on the survival of neighboring neurons, mHtt leads to non-cell-autonomous neurotoxicity in HD.

Amyotrophic lateral sclerosis (ALS) is also known as motor neuron disease. Most evidence supports that ALS is caused by continuous death of nerve cells. The etiology of the disease is still unknown. 20 % of the cases may be related to genetic defects, in addition to some environmental factors. Recent results show that the level of Interleukin-6 (IL-6) increases in AEVs isolated from the plasma of sporadic ALS Patients (Chen et al., 2019). Bioinformatic analysis of miRNA profiles in EVs secreted by human induced astrocytes from 3 ALS patients carrying C9orf72 mutations and 3 non-affected donors also detected the dysregulation of miRNA cargos in AEVs. Functional verification experiment further showed that the lack of miR-494-3p, a negative regulator of axonal maintenance-related gene semaphorin 3A (Sema3A), in C9orf72-mutated AEVs is a key inducer of motor neuron death (Varcianna et al., 2019). Besides, AEVs are involved in the spread of Mutant Copper-Zinc Superoxide Dismutase (SOD1) in the CNS. The uptake of mutant SOD1-enriched AEVs by microglia and motor neurons induces neuroglial activation and neuroinflammation, in astrocytes (Hong et al., 2016; Wang et al., 2011). Guittart et al. showed that PrP in astrocytes serves as a sensor for oxidative stress and mediates the release of EVs carrying PrP and other beneficial molecules (e.g., 37/67 kDa laminin receptor, apolipoprotein E, and the ribosomal proteins S3 and P0), leading to improved survival of neurons under hypoxic and ischemic conditions (Guittart et al., 2016). Synapsin I, an oligomannosae-carrying glycoprotein, is also detected in AEVs, when astrocytes were cultured in either oxygen/glucose deprivation or hydrogen peroxide-induced ischemic models (Wang et al., 2011). Application of EVs derived from wild-type or synapsin-deficient astrocytes confirmed that AEVs promote neuronal survival in aforementioned models via synapsin I. Similar to that in ischemic models, AEVs exhibit neuroprotective function in TBI models as well. Chen et al. found that AEVs contain GJA 1-20 k, a 20 kD fragment produced by internal translation of the gap junction alpha 1 (GJAI) gene transcript (Smyth and Shaw, 2013). AEVs carrying GJA 1-20 k protect the integrity of mitochondrial morphology and function in damaged neurons by reducing Connexin-43 (Cx43) phosphorylation, thus enhance the survival of damaged neurons.

AEVs also exhibit neuroprotective effects in neurodegenerative diseases including AD, multiple sclerosis (MS), and HD through their specific cargos. In AD, astrocytes released heat-shock protein 1 (HSPB1) via EVs when stimulated by a low concentration of Aβ. HSPB1 interacts with Aβ directly and exerts potential protective effects on neurodegeneration (Nafar et al., 2016). In experimental autoimmune encephalomyelitis (EAE) mouse, a disease model for studying MS in rodents, the expression levels of CRYAB were significantly increased in astrocytes. CRYAB was then packaged into AEVs and released to extracellular space. Internalized by neuroglia, AEV-loaded CRYAB down-regulates the expression of pro-inflammatory factors (e.g., IL-6, IL-1β, and TNFα) and suppresses the inflammatory response of recipient cells (Guo et al., 2019). Besides, in vivo and in vitro models of HD, mHtt inhibits EV secretion from primary astrocytes and mouse striatum, respectively, together with the reduction of CRYAB expression. The injection of AEVs into HD mouse striatum decreases the accumulation of mHtt and reduces related neuropathology. Overexpression of CRYAB in HD mouse has increased AEV secretion, which alleviates HD pathology (Hong et al., 2017). What’s more, AEVs were proven positive for neuroglobin (NGB), a protein that functions as a neuroprotectant against cell insult. AEVs could selectively target neurons and were involved in astrocyte-neuron communication of CNS. The possibility that AEVs might transfer NGB to neurons adds a general mechanism to the potential astrocytic neuroprotection (Venturini et al., 2019).
forward the EV-based cell-free therapeutic strategy in treating CNS disorders. They will also push us to expand our knowledge in related fields rapidly. Meanwhile, better systematic functional analyses of specific cargos will still far away from understanding the entire effects of AEVs and their underlying mechanisms, the development of more powerful high throughput screening methods for minuscule changes of AEVs contents and better systematic functional analyses of specific cargos will greatly simplify the difficulty and complexity in the isolation of AEVs from patients’ serum/plasma and the screening of potential biomarkers in AEVs. To date, multiple disease-related molecules have been identified in EVs, including β-site amyloid precursor protein-cleaving enzyme 1 (BACE-1), γ-secretase, soluble Aβ42, soluble amyloid precursor protein (sAPP)β, P-T181-tau, P-S396-tau, IL-6, and some complement proteins. Each or a combination of them may be utilized as potential biomarkers for AD, frontotemporal dementia (FTD), mild cognitive impairment (MCI), and ALS (Goetzl et al., 2016, 2018; Winston et al., 2019a). In 2016, Goetzl et al. isolated and enriched plasma AEVs from patients with AD and FTD and their corresponding controls, and then quantitatively detected the components in Aβ42-generating system (Goetzl et al., 2016). For example, the expression levels of BACE-1 and sAPPβ in AEVs isolated from AD patients were significantly higher than those isolated from healthy donors. According to the receiver operating characteristic (ROC) curve, BACE-1 and sAPPβ significantly distinguish AD patients from matched healthy controls. The area under the curve (AUC) of ROC values are 0.78 and 0.83 for BACE-1 and sAPPβ, respectively. Discriminant classifier analyses correctly classified 63.6 % of AD patients and 91.7 % of healthy donors. Moreover, sAPPβ also significantly distinguishes AD patients from FTD patients. The AUC value is 0.86, and discriminant classifier analysis scores are 66.7 % and 92.9 % for AD and FTD patients, respectively (Goetzl et al., 2016). In addition to the components of the Aβ42 production system described above, levels of complement proteins in EVs of AD patients also show significant differences from the cognitively normal controls and MCI patients. Normalized by exosomal marker CD81, the AEVs levels of C1q, C4b, C3d, factor B, factor D, Bb, C3b and C5b-C9 terminal complement complex (TCC) in AD patients were significantly higher than that in controls, whereas CDS9, CDA6, decay-accelerating factor (DAF) and complement receptor type 1 (CR1) were significantly lower than that in controls (Goetzl et al., 2018). The ROC evaluation shows that the sensitivity of multiple complements reaches more than 90 % for distinguishing AD patients from healthy controls, of which the sensitivity of DAF and CDS9 can reach 100 %, and that of C1q and C4b are 99.3 ± 0.009 % and 99.0 ± 0.011 %, respectively. When distinguishing between MIC and AD patients, the two complement proteins with the highest sensitivity value are C4b (100 %) and Bb (85.5 ± 0.059 %) (Winston et al., 2019a). These observations suggest that AEVs can be recruited as a promising biomarker candidate in diagnosing MCI, AD, and FTD. AEVs also have the potential to be used as an ALS biomarker since elevated levels of certain cytokines have been found in AEVs from patients with ALS (Chen et al., 2019). For example, the levels of IL-6 in AEVs of ALS patients are almost two folds higher than that of IL-6 in AEVs of healthy donors. Furthermore, the elevated levels of IL-6 in AEVs in patients with sporadic ALS are positively related to disease progression, especially in the first 12 months of ALS. However, it remains vague that whether AEVs can be applied as biomarkers for ALS or general neuroinflammation, as IL-6 levels in AEVs are also elevated in AD patients (Goetzl et al., 2016). Therefore, more studies are needed to clarify the sensitivity and specificity of AEV cytokines in the diagnosis of different inflammation-related neurological diseases.

5. AEVs: potential biomarkers in the diagnosis of neurological disorders

Due to the distinct profiles of AEVs cargos in various neurological diseases as stated above, disease-related factors in AEVs have been perceived as potential biomarkers for diagnosing CNS disorders. AEVs are able to diffuse through the BBB and into the periphery, where they can be selectively captured by antibodies that target the membrane protein glutamine aspartate transporter (GLAST) specifically presented on astrocytes and AEVs (Goetzl et al., 2018). This approach greatly simplified the difficulty and complexity in the isolation of AEVs from patients’ serum/plasma and the screening of potential biomarkers in AEVs. To date, multiple disease-related molecules have been identified in AEVs, including β-site amyloid precursor protein-cleaving enzyme 1 (BACE-1), γ-secretase, soluble Aβ42, soluble amyloid precursor protein (sAPP)β, P-T181-tau, P-S396-tau, IL-6, and some complement proteins. Each or a combination of them may be utilized as potential biomarkers for AD, frontotemporal dementia (FTD), mild cognitive impairment (MCI), and ALS (Goetzl et al., 2016, 2018; Winston et al., 2019a).
6. AEVs: potential therapeutic targets for neurological disorder treatment or prevention

6.1. AEVs as therapeutic targets for treating neurological disorders

Due to the essential roles of AEVs in exacerabating the pathological processes of neurological disorders through transferring various pro-inflammatory or neurotoxic cargos, many studies have been carried out to examine the feasibility of EVs as therapeutic targets in treating these diseases via suppressing the production of EVs. Currently, multiple proteins have been identified as key regulators of EV release, in which Sphingomyelinases (SMases) is the most widely recognized one (Ding et al., 2020; Trajkovic et al., 2008). SMases enhances budding of exosome vesicles into multivesicular endosomes via promoting ceramide formation, and GW4869, the SMase inhibitor, effectively suppresses the exosome biogenesis (Trajkovic et al., 2008). The administration of GW4869 has been proved to halt amyloid plaque accumulation (Dinkins et al., 2014) and tau propagation (Asai et al., 2015) in AD mice, accelerate clearance of pathological TDP-43 in ALS mice (Guguchi et al., 2016), and alleviate neuroinflammation and neural injury in animal models of TBI (Kumar et al., 2019) and stroke (Gao et al., 2020). More importantly, our studies have demonstrated that GW4869 significantly inhibits EV release from astrocytes, which blocks pro-inflammatory cytokine production, Zika virus propagation and release in astrocytes (Huang et al., 2018; Wang et al., 2017a). Besides SMases, our group also identified glutaminase (GLS) as another important regulator of EV release (Ding et al., 2020). GLS is the enzyme that catalyzes the hydrolysis of glutamine to produce glutamate, therefore regulating cellular metabolism through the tricarboxylic acid (TCA) cycle and excitatory neurotransmission in CNS cells (Ding et al., 2020). The treatment of GLS inhibitor CB-839 or BPTES exerts similar inhibitory effects as GW4869 on AEV release and astrocyte-mediated neuroinflammation in vitro and in vivo (Gao et al., 2020; Wang et al., 2017a). Therefore, our observations, together with others’ findings, all imply AEVs as a promising target in treating neurological disorders.

6.2. AEVs as targets for preventing neurological disorders

In physiological conditions, EVs, especially AEVs, are widely involved in the maintenance of homeostasis of CNS microenvironment (Upadhya et al., 2020; Wooff et al., 2020). For instance, astrocytes release miR-195-enriched EVs to improve BBB integrity, therefore preventing cerebrovascular diseases (Chen et al., 2020b). The removal of EVs in normal conditions may result in neurodegeneration and neuroinflammation (Wooff et al., 2020). Thus, to preserve the release of neuroprotective AEVs has been considered as a novel approach to prevent neurological disorders. One simple and safe strategy is cerebral preconditioning which increases brain resistance to subsequent neural injury (Thushara Vijayakumar et al., 2012). Preconditioned astrocytes release EVs shuttled miR-92b-3p to enhance neuronal survival under oxidative stress and ischemic conditions (Guitart et al., 2016; Xu et al., 2019). Another approach that can be used in clinical application is to provide environmental enrichment (EE) including volitionally increased activity and stimuli (Verkhratsky et al., 2014). One simple model is A1 & A2 astrocytes. It has been reported that the same population of astrocytes can show diverse phenotypes, identified via high throughput analyses, which is far faster than the phenotype transition of astrocytes. Accordingly, the functions and contents of AEVs may change dynamically during the process of astrocyte reactivity. Similarly, the perturbations in EV release and cargo via chemical or biological strategies may also increase the efficiency and specificity in modifying AEV release and contents.

7. Future perspectives

All aforementioned studies have suggested the wide association of AEVs in various stages of neurological diseases. These findings suggest the necessity to further dig out the functions of AEVs and the underlying mechanisms in the CNS, which can be summarized by the following points.

7.1. To expound the heterogeneity of AEVs

Due to the “double-edged sword” effects of AEVs on the initiation and progression of CNS disorders, to expound the heterogeneity of AEVs is a key topic in future investigation.

First, the heterogeneity of AEVs is determined by the diversity of their parent cells. Astrocytes, the most abundant cell type in human brain, exhibit significantly heterogeneous morphologies and features due to the different ontogenetic origin for astrocytes during brain development (Bribian et al., 2016). For example, multiple subtypes of astrocytes are found to co-exist in human cerebral neocortex, including the types that do not appear to present in rodent cortex (Oberheim et al., 2009). Astrocytes in different brain regions (e.g., white matter and gray matter) are also highly heterogeneous and have dissimilar roles (Lundgaard et al., 2014; Rajkowska et al., 2018). Furthermore, astrocytes can be activated into distinct sub-groups by different stimuli in neurological disorders (Habib et al., 2020). Additionally, the subpopulation of astrocytes may also be influenced by gender, a key factor that also contributes to various neurological disorders (Nebel et al., 2018; Thibaut, 2016). Thus, different subpopulations of astrocytes may display distinct physiological functions in the brain, such as blood flow regulation, synapse formation and function maintenance, energy supply, and BBB construction (Sofroniew and Vinters, 2010). The unequal population of astrocytes, that are used for AEVs collection intentionally or unintentionally, interferes the functional analyses of AEVs under physiological and pathological conditions significantly.

Second, the heterogeneity of AEVs is influenced by the phenotype of astrocytes. It has been reported that the same population of astrocytes can show diverse phenotypes based on different pathological conditions and stimuli (Verkhratsky et al., 2014). One simple model is A1 & A2 model, which, in turn, classified astrocytes into three phenotypes, resting/quiescent A0-like astrocytes, pro-inflammatory/neurotoxic A1-like astrocytes, and anti-inflammatory/protective A2-like astrocytes (Hidde et al., 2017). Distinct gene expression profiles are observed in different phenotypes, identified via high throughput analyses, which significantly influence the contents and functions of AEVs (Zamanian et al., 2012).

Third, the heterogeneity of AEVs is also due to the dynamic changes of cellular status. The astrogial exocytosis takes place in seconds to minutes after mechanical stimulation (Ramamoorthy and Whim, 2008) or stimuli (e.g., Ca2+ ionophore ionomycin) treatment (Liu et al., 2011), which is far faster than the phenotype transition of astrocytes. Accordingly, the functions and contents of AEVs may change dynamically during the process of astrocyte reactivity. Similarly, the perturbations in EV release and cargo via chemical or biological strategies may also modify AEV functions and contents in a dynamic manner. Hence, it is important to track these dynamic changes in different models of CNS.
disorders, which may clarify key mechanisms in the pathogenesis and essential therapeutic targets to treat these disorders.

Fourth, the heterogeneity of AEVs is caused by the different production processes of EVs. EVs are a mixed population that can be classified into multiple types due to their biogenesis and cell sources. It is worth-noting that, besides exosomes (Chen et al., 2020a) and microvesicles (Sollvander et al., 2016), astrocytes also can secrete mito-particles, which is triggered by a calcium-dependent mechanism involving CD38/cyclic ADP ribose signaling (Hayakawa et al., 2016). The transferring of functional mitochondria via mito-particles amplified cell survival signals in mice stroke model, suggesting a novel mechanism of neuroglial crosstalk that contributes to endogenous neuroprotection and neurorecovery post brain injury. Moreover, A1-type astrocyte induction under the neurodegenerative circumstance results in the dysfunction and fragmentation of mito-particles, diminishing the beneficial effects of functional astrocyte-derived mito-particles (Joshli et al., 2019). Hence, to further clarify all types of EVs including mito-particles that are generated by astrocytes and to unveil their functions are of great importance to fully understand the involvement of AEVs in CNS disorders.

For these reasons and more, to fully understand the physiological and pathological roles of AEVs, it is important to compare the functions of EVs derived from gender-, brain region-, subtype-, and phenotype-specific astrocytes in future studies.

7.2. To develop novel therapeutic strategies for the diagnosis, prevention, treatment of CNS diseases by targeting AEVs

The distinct expression profiles of disease-related materials in AEVs isolated from patients with various CNS disorders confer the rationality and feasibility to recruit AEVs as potential biomarkers. ROC analyses reveal high sensitivity and specificity in utilizing biological fluid-derived AEVs as early biomarkers for the diagnosis of CNS diseases, especially before the onset of irreversible neurological damages. However, the facts that only very low amounts of AEVs were isolated from limited human biofluids and that the sample sizes in aforementioned studies were relatively small severely restricted the application of AEVs as a diagnostic index. Inspiringly, newly developed methodologies for detecting minute amounts of disease-related molecules in EVs significantly reduce the threshold for utilizing AEVs as diagnostic biomarkers. By recruiting large clinical cohorts to increase sample sizes, the reliability of AEVs as biomarkers for CNS disorders will be further verified, which, removes another main barrier to the application of AEVs in clinical settings.

Besides, although AEVs may be a promising target in treating neurological disorders, great efforts are urgently needed to develop efficient strategies for specifically blocking AEV release. To achieve this goal, SMases and GLS are two main candidates. The generation of transgenic animals that are able to conditionally knockout either SMases or GLS in astrocytes will be a great approach to remove AEVs from brain microenvironment, which demonstrates the temporal effects of AEVs in the pathogenesis of CNS disorders. To translate the basic scientific research achievement from bench to bedside is another challenge. To date, multiple small chemicals have been identified as inhibitors of either genes and some of them, such as JHU-083, has been designed to improve CNS penetration for targeting neurological and psychiatric disorders (Ding et al., 2020; Trajkovic et al., 2008; Zhu et al., 2019). Additionally, EVs decorated with homing molecules have been considered as a powerful tool to deliver cargos into specific types of cells and tissues in the body (Rufino-Ramos et al., 2017). After being loaded into EVs that can target astroglia, these inhibitors may be internalized by astrocytes and block AEV release specifically, therefore reversing or at least slowing down disease progression.

8. Conclusions

In this review, we summarize the different roles of AEVs in neurological diseases. Under pathological stimulations, activated astrocytes release EVs containing pro-inflammatory factors to facilitate the accumulation and spreading of the latter in the CNS, aggravating neuroinflammation. AEVs also deliver neurotoxic proteins to neurons and induce neurodegeneration. However, AEVs also contain a lot of proteins and nuclei acids that are beneficial for neurons such as CRYAB and PrP. Internalized by neurons, AEVs release these beneficial materials to promote cell survival and neural function recovery. Furthermore, current studies have also made a great progression in screening AEV contents as diagnostic biomarkers of various neurological diseases, identifying key targets for manipulating AEV release, and clarifying different AEV subtypes and their functions.

Taken together, with information on the pathological roles of AEVs in general and AEV-contained disease-related cargos in particular, the applications of AEVs as therapeutic targets or agents in preventing and treating CNS diseases are a near possibility. In addition, the extension of our knowledge in the distinct expression signatures of disease-related molecules in AEVs will shed light on the development of highly efficient and sensitive detection strategies in the diagnosis of neurological diseases.

Ethical approval and consent to participate

Not applicable.

Consent for publication

The authors approved the final manuscript.

Availability of supporting data

Not applicable.

Funding

This work was supported in part by research grants from the National Natural Science Foundation of China (No. 91949204 and No. 81830037 to JCZ, No. 81971145 and No. 81901333 to XX), Shanghai Sailing Program (No. 19YF1451700 to XX), Shanghai Blue Cross Brain Hospital Co., Ltd., and Shanghai Tongji University Education Development Foundation (No. 000000381/2018108 to JCZ).

Authors’ contributions

JCZ XX conceived the manuscript. SZ SS XX LD XX collected references. XX SZ SS YW wrote the manuscript. SZ XX prepared illustrations.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

We thank Jie Zhu, Yanyan Zhang, Drs. Ling Ye and Xinrui Qi for proofreading the manuscript.

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