Extracellular vesicles in the diagnosis and treatment of central nervous system diseases

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Abstract

Extracellular vesicles, exosomes and microvesicles, play a fundamental role in the activity of the nervous system, participating in signal transmission between neurons and providing the interaction of central nervous system (CNS) with all body systems. In various pathological processes in CNS extracellular vesicles can help eliminate toxic agents from cells, but at the same time they provide the distribution of these agents into healthy cells. Thus, extracellular vesicle cargo varies on the functional state of the CNS, the analysis of EV molecular content contributes to the development of non-invasive methods for the diagnosis of many CNS diseases. Extracellular vesicles of neuronal origin can be isolated from various biological fluids due to their ability to cross the blood-brain barrier (BBB). Today, the diagnostic potential of almost all toxic proteins involved in nervous system disease pathogenesis, specifically α-synuclein, tau protein, SOD1, FUS, LRRK2 as well as some synaptic proteins, has been proven. Special attention is paid to extracellular RNAs mostly associated with EVs, which are important in the onset and development of many neurodegenerative diseases. Depending on parental cell type, extracellular vesicles may have different therapeutic properties, including neuroprotective, regenerative, anti-inflammatory, etc. Also, modern modification approaches allow loading EVs with specific molecules and changing their surface molecules to achieve targeting of various cells, including neurons. Due to nano size, biosafety, ability to cross the BBB, possibility of targeted delivery and the lack of an immune response, extracellular vesicles are a promising vehicle for the delivery of therapeutic substances for the treatment of neurodegenerative diseases and drug delivery to the brain. This review describes modern approaches of diagnosis and treatment of CNS diseases using extracellular vesicles.

Keywords: exosomes, microvesicles, extracellular vesicles, neurodegenerative diseases, cell-mediated therapy, diagnosis, biomarkers, micro RNAs, extracellular RNAs, CNS diseases

Introduction

Extracellular vesicles (EVs) are membrane particles of cellular origin involved in the regulation of many physiological and pathological processes in the body (Chulpanova et al., 2018a; Hessvik and Llorente, 2018; Galieva et al., 2019). EVs are produced by almost all the cells of body and provide intercellular communication, transport of signal and other biologically active molecules toward target cells. (Hartmann and Burg, 1989; Merchant et al., 2017). EVs play an important role in the functioning of nervous system, providing not only the communication of nerve cells between themselves and glial cells, but also the interconnection of the central nervous system (CNS) with all body systems (Chivet et al., 2012). In CNS pathologies EVs play a dual role. On the one hand, they help maintain cellular homeostasis, cleaning the nervous system of protein aggregates and other pathogenic agents, however, they can also transfer toxic substances to healthy cells, mediating their spread throughout the body and burdening diseases. Pathogenic role of EVs is shown in neurodegeneration, neuroinflammation, cancers and disorders that affect CNS, for example, lysosomal storage disorders (Caruso Bavisotto et al., 2019).

In various pathological processes, EVs undergo significant changes in composition, quantity and size. Knowledge of these changes makes it possible to identify new biomarkers of various diseases for sensitive and specific diagnosis (Wong and Chen, 2019). Today, special attention is paid to the diagnostic potential of pathogenic proteins, synaptic proteins, and extracellular RNAs inside EVs.

EVs largely replicate cell therapy success. It is common to find works that investigate the therapeutic potential of native exosomes isolated from dendritic cells (Pitt et al., 2016; Sousa et al., 2017), macrophages (Choo et al., 2018), hematopoietic stem cells (Radosinska and Bartekova, 2017), endothelial cells (Xiao et al., 2017) and mesenchymal stem cells (MSCs) (Lopez-Verrilli et al., 2016; Mathew et al., 2019). MSC-derived exosomes contain cytokines, trophic growth factors, signal lipids, mRNAs and regulatory miRNAs, which makes them an attractive therapeutic agent for use in cell-free regenerative medicine (Phinney and Pittenger, 2017).

When developing new strategies for CNS disease treatment, special attention is paid to EVs due to their ability to cross the blood-brain barrier (BBB) (Matsumoto et al., 2017a). The mechanisms for EV passing through the BBB remain controversial and require further investigation. Malignant neoplasms, inflammatory processes and other pathological conditions of CNS can lead to dysfunction of the BBB and the substance flow of through it, which is partly due tight junction disruption (Garcia-Romero et al., 2017). It is assumed that normal transport through the BBB can occur through vesicular transcytosis, which is divided into receptor-mediated transcytosis (RMT) and adsorptive-mediated transcytosis (AMT). RMT process occurs via a binding with specific cellular receptors, but AMT is mediated by adsorption of cationic particles to the anionic components of the plasma membrane. In case of AMT there is less affinity and higher throughput. It is shown that EVs can cross the BBB through the mechanism of adsorptive-mediated transcytosis (Matsumoto et al., 2017b). Although RMT is more commonly used for therapeutic drug deliver to the brain due to the modification of surface ligands (Preston et al., 2014).

There are various methods of EV modification that allow it to be used as vehicle for therapeutic agent delivery to target cells. Drug targeted delivery is achieved by modifying of EV surface molecules. To ensure nerve cell targeting, rabies virus glycoprotein is most often used whereas it binds to an acetylcholine receptor of nerve cells (Alvarez-Erviti et al., 2011; Huey et al., 2017; Phoolcharoen et al., 2017).

1 Cells of Nervous System – potential source and targets of EVs

Nervous tissue is composed of neurons and neuroglia. Neurons are highly polarized cells, most of them consist of a body and two functionally and morphologically different processes: dendrites and axon (Giordano-Santini et al., 2016), that provide information flow through the nervous system (Takano et al., 2015). Neuroglia are involved in the metabolism and maintenance of brain homeostasis, neuron survival, development and modulation of synaptic transmission, distribution of

nerve impulses, determination of CNS structure and many other physiological processes. The role of neuroglia in the many pathologies of the nervous system, including some mental illness, epilepsy and neurodegenerative diseases, is also defined. Neuroglia include astrocytes, oligodendrocytes, Schwann cells, NG2-glia and microglia (Giordano-Santini et al., 2016). Astrocytes interact with neurons, blood vessels and many structures of the nervous system and are involved in synaptic transmission. The complex astrocyte-neurons-blood vessel is generally known as a neurovascular unit (NVU) of the blood-brain barrier. Oligodendrocytes in the CNS and Schwann cells in the peripheral nervous system produce myelin, which provides transmission speed along axons. In addition, these cells provide trophic support and affect the structure of axons (von Bernhardi et al., 2016; Mukhamedshina et al., 2019). At the periphery, Schwann cells provide the regeneration of axons and neuromuscular junctions. NG2-glia cells are precursors of oligodendrocytes and astrocytes, they provide remyelination in case of some neurodegenerative diseases and can also modulate neuron properties and activity. Microglia are CNS immune cells, which provide neuron protection against various pathogenic factors (von Bernhardi et al., 2016; Akhmetzyanova et al., 2018).

2 Classification of extracellular vesicles

There are three types of EVs that differ in the mechanism of release into the intercellular space, in size and cargo: exosomes, microvesicles and apoptotic bodies (Hessvik and Llorente, 2018). Exosomes are the smallest EVs, of 30 to 100 nm in diameter, which are formed inside endosomal organelles called multivesicular bodies (MVBs). MVBs were initially regarded as prelysosomal structures participating in protein degradation. However, new studies showed that MVBs are involved in the intra- and intercellular turnover of molecules (Kawikova and Askenase, 2015). The release of exosomes occurs in several stages: formation of intraluminal vesicles within MVBs, transport of MVBs to the plasma membrane and fusion of MVBs with the plasma membrane (Hessvik and Llorente, 2018). MVBs can also fuse with lysosomes to degrade the content (Raposo and Stoorvogel, 2013).
Microvesicles (MVs) are released directly from the plasma membrane and are 100 to 1000 nm in diameter (Raposo and Stoorvogel, 2013). MV formation occurs as a result of the aminophospholipid translocase-mediated dynamic redistribution of phospholipids and following constriction of the actin cytoskeleton due to the actin—myosin interaction (Akers et al., 2013). Apoptotic bodies are 50 nm to 5 μm in diameter (Rufino-Ramos et al., 2017) and are released only in advanced stages of apoptosis by caspase-mediated cleavage (Todorova et al., 2017).
The main mechanism to transfer EV cargo into recipient cells is endocytosis. There are many ways of endocytosis, including micropynocytosis, phagocytosis, caveolin-mediated, lipid-raft mediated and clathrin-dependent endocytosis. Proteins and glycoproteins presented on the surface of both EVs and target cells can affect the uptake mechanism (Rufino-Ramos et al., 2017). In this review, we will consider exosomes and microvesicles, their potential in the diagnosis and treatment of CNS diseases.

3 Exosome and microvesicle cargo

Depending on the type of parental cell, exosomes and MVs may have different content including bioactive molecules, membrane receptors, proteins, lipids, and genetic material that can be transported into target cells (Merchant et al., 2017; Rufino-Ramos et al., 2017; Todorova et al., 2017). Modern proteomic methods allowed analyzing the protein profile of exosomes and MVs. It has been found that exosomes and MVs contain both constitutive proteins found in cells of different origin and unique proteins, the presence of which depends on cell type and the microenvironment conditions. Unique proteins serve as potential biomarkers for diagnosis of various diseases (Haraszti et al., 2016). The proteomic profile of MVs and their parental cell has significant homology, in contrast to exosome proteomic profile, which has significant differences from the parental cell. Exosomes contain extracellular matrix proteins, heparin-binding proteins, receptors,

immune response and cell adhesion proteins, while MVs are enriched in proteasomes, endoplasmic reticulum proteins and mitochondria (Haraszti et al., 2016). Most proteins of exosomes and MVs are involved in their biogenesis, for example, tetraspanins, Rab proteins and endosomal sorting complex required for transport (ESCRT), which is the main engine of exosome biogenesis (Kalluri and LeBleu, 2016; Rufino-Ramos et al., 2017).
Exosomes and MVs also differ in lipid content. Glycolipids and free fatty acids predominate in exosomes, and the lipid composition of MVs is rich in ceramides and sphingomyelins (Haraszti et al., 2016). The lipid composition of exosomes and MVs, in contrast to their parental cells, is distinguished by a high content of phosphatidylserine, which is a determinant for vesicle entry into target cells (Record et al., 2018). The entry capacity is also determined by surface receptors and ligands of exosomes and MVs (Rufino-Ramos et al., 2017). Exosomes and MVs can contain a wide range of genetic material: chromosomal DNA, mitochondrial DNA, single-stranded and double-stranded DNA encoding messenger RNAs (mRNAs) and non-coding RNAs (long noncoding RNAs, microRNAs, and circular RNAs) (Kalluri and LeBleu, 2016; Xu et al., 2016; Kim et al., 2017). Exosome and MV nucleic acids are potential biomarkers for the diagnosis of many diseases (Kinoshita et al., 2017; Szabo and Momen-Heravi, 2017).

4 Exosomes and microvesicles in normal physiology and CNS diseases

It is known that exosomes and MVs mediate the interaction of nervous system cells between themselves and glial cells, as well as the communication of peripheral organs with the CNS (Batiz et al., 2015; Kumar et al., 2018). Neurons, astrocytes, oligodendrocytes and microglial cells release EVs and exchange signal molecules through them (Bakhti et al., 2011; Goetzl et al., 2016a; Sun et al., 2017; Vinuesa et al., 2018). It is assumed that neural exosome cargo modulates local synaptic transmission. For example, it was found out that neural exosomes released after activation of glutamatergic synapses merge only with neurons, providing interneuronal communication. Thus, exosome and MV cargo can affect the interneuronal communication and synapse activity (Chivet et al., 2014; Lu and Xu, 2016).
In case of CNS diseases, such as neurodegenerative, neuroinflammatory diseases and brain tumors, exosomes and MVs, on the one hand, can remove toxic proteins and aggregates from the affected cells, and on the other, distribute pathogenic agents to healthy cells (Rufino-Ramos et al., 2017; Sardar Sinha et al., 2018).
Exosomes carrying a prion protein (PrPC) on the surface play a protective role in beta-amyloid (Aβ)-mediated neurodegeneration. The prion protein on the surface of neuronal cells acts as a Aβ receptor which activates neurotoxic signaling. However, as part of exosome cargo, PrPC binds to the neurotoxic Aβ oligomer and contributes to its fibrillation and detoxification (Falker et al., 2016). Exosomes and MVs also contribute to angiogenesis, coagulopathy, and metastasis, in particular in CNS cancer (Kumar et al., 2018). The ability of exosomes and MVs to overcome BBB allows them to spread in body fluids and reach distant tissues, aggravating nervous system disease pathogenesis (Selmaj et al., 2017). Exosomes and MVs were shown to contribute to the proliferation of superoxide dismutase 1 (SOD1) and RNA-binding protein FUS in amyotrophic lateral sclerosis (ALS) (Sproviero et al., 2018), as well as TAR DNA-binding protein 43 (TDP-43) in frontotemporal lobar degeneration (FTLD) and ALS (Feneberg et al., 2014; Iguchi et al., 2016). It was found that TDP-43 exhibits higher toxicity as exosome cargo than in free form (Feiler et al., 2015). Exosomes and MVs also contribute to the spread of huntingtin expansion in Huntington's disease (Jeon et al., 2016), α-synuclein in Parkinson's disease (Ngolab et al., 2017), tau protein in Alzheimer's disease and some other neurodegenerative diseases (Shi et al., 2016; Wang et al., 2017). The identification of these proteins in EVs isolated from patient's body fluids helps diagnose CNS diseases.

5 The use of exosomes and microvesicles in the diagnosis of CNS diseases

The effectiveness of CNS disease treatment largely depends on the early diagnosis which can be achieved by the development of new molecular methods, including EV analysis methods (Hirshman et al., 2016). An important feature for diagnosis is that various inflammatory and signaling molecules, including RNA and pathogenic proteins, are selectively packaged in exosomes (Harischandra et al., 2018). The EV-based diagnostic approach is particularly relevant in those CNS diseases, for which direct access to the affected tissues for subsequent molecular analysis is difficult. However, to obtain brain cell-released exosomes from various biological fluids is possible due to their ability to pass through the BBB (Manek et al., 2018). For diagnostic use, EVs can be isolated from many body fluids, such as plasma, urine, cerebrospinal fluid, saliva, amniotic fluid and bile (Ko et al., 2016; Manek et al., 2018). However, EV small size and labor-intensive sample preparation limit the widespread use of EVs in diagnosis.

As noted, EV cargo may differ in normal physiological condition and in pathologies. A study of cerebrospinal fluid (CSF) in patients with Alzheimer's disease (AD) showed that exosome trafficking is different in patients with AD compared to control group (Riancho et al., 2017). In addition, it is shown that in traumatic brain injury, the size of secreted exosomes and MVs differs. If in the control CSF samples EVs were 99–104 nm in size, then after a traumatic injury their size decreased to 74–98 nm. The total amount of EVs also increased and the proportion of some proteins changed (Manek et al., 2018).

Involved in neurodegenerative disease pathogenesis proteins are identified in exosomes and MVs isolated from body fluids (most of all blood plasma and CSF). So, α-synuclein (Shi et al., 2014; Stuendl et al., 2016) and Leucine-rich repeat kinase 2 (LRRK2) (Fraser et al., 2013) in Parkinson's disease (PD), tau protein in asthma and PD spontaneous manifestation (Shi et al., 2016) are proposed to use as biomarkers. In chronic traumatic encephalopathy, which occurs as a result of repeated blows to the head and is more common in athletes, elevated levels of tau protein in plasma exosomes are observed (Stern et al., 2016). In patients with AD and frontotemporal dementia (FTD), synaptic dysfunction, which occurs in the early stages as a result of functional synaptic protein level decrease, is observed. Analysis of plasma neuron-derived exosomes showed that the level of synaptophysin, synaptopodin, synaptotagmin-2 and neurogranin proteins in patients is significantly less than in control group. Moreover, its level correlates with the patient's cognitive functioning, so it can be used as indicators of disease progression (Goetzl et al., 2016b). Similar results were observed in the study of presynaptic proteins neuronal pentraxin 2 (NPTX2), neurexin 2a (NRXN2a) and corresponding them postsynaptic proteins GluA4-containing glutamate (AMPA4) receptor and neuroligin 1 (NLGN1), which enhance excitatory synaptic activity (Goetzl et al., 2018).

Tumor cell-released EVs, called oncosomes, which contain oncogenic signals (proteins or transcripts with oncogenic functions) and tumor antigens are also described. The number of oncosomes in the patient's biological fluids increases as the disease progresses, so they can be used in the diagnosis of CNS tumors (Minciacchi et al., 2015a; Minciacchi et al., 2015b).

Extracellular RNAs are most frequently mentioned as a potential biomarker of diseases. Today, extracellular RNAs contained in neuronal cell-derived exosomes and MVs are used as biomarkers to diagnose CNS tumors neurological, neurodegenerative and mental diseases. (Rao et al., 2013). Circular RNA (circRNAs), microRNA (miRNA), piwi-interacting RNA (piRNA) and long non-coding RNAs (lncRNAs) are essential for maintaining cellular homeostasis. RNA regulation disruption and various pathological conditions are interrelated, then changes in RNA expression levels may indicate various diseases. For example, circulating U2 fragments of small nuclear RNAs have been proposed as a biomarker for primary CNS lymphoma, the expression levels of lncRNAs RP11-462G22.1, PCA3 and Sox2OT are associated with PD and AD (Gui et al., 2015), NEAT1 – with Huntington's Disease (Lu and Xu, 2016).

Micro RNAs play a special role in the contraction and progression of many neurodegenerative diseases. Today a significant number of investigations aimed at finding potential biomarkers

describes microRNAs and its diagnostic potential. MiR-195, miR-24 and miR-19b isolated from plasma exosomes and MVs are closely related to neuronal apoptosis, regeneration and neurodegenerative processes in PD. In patients with PD, decreased miR-19b level and increased miR-195 and miR-24 levels were observed (Cao et al., 2017). The results of another study describe changes in the expression of other miRNAs, and it is shown that miR-1 and miR-19b-3p levels in patients with PD are less than normal, miR-153, miR-409-3p, miR-10a-5p and let-7g-3p are higher than normal (Gui et al., 2015).
The investigation of CSF miRNA profile of patients with AD showed that miR-16-5p, miR-125b-5p, miR-451a and miR-605-5p expression in patients with early disease onset differs from the control group. In late-onset cohort, similar results are observed, however, the expression level of miR-16-5p is not different from the control group, the authors suggest this may be due to age-related changes in miRNA expression (McKeever et al., 2018). For mesial temporal lobe epilepsy with hippocampal sclerosis exosomal miR-3613-5p, miR-4668-5p, miR-8071, miR-197-5p, miR-4322 and miR-6781-5p diagnosis were also been proposed. Moreover, miR-8071 which correlates with seizure severity has the best diagnostic value (Yan et al., 2017).

6 The use of exosomes and microvesicles in CNS disease treatment

CNS damage can be caused by various factors, including vascular disease, injures, infectious and hereditary diseases. For example, in case of ischemia, inadequate tissue oxygenation leads to prolonged hypoxia, depletion of energy reserves in neurons and glial cells. This causes an energy-dependent membrane ion-pump function decreases, membrane potential loss and, as a result, cell damage and cell death (Pratt and McPherson, 1997). And, as a consequence of this, ischemic stroke can happen, which may lead to BBB damage and normal neuron functioning disruption (Jiang et al., 2018). Head injuries often cause neurological disorders (Wright, 2017), CNS inflammatory response (Russo and McGavern, 2016), and can accelerate neurodegeneration and increase the risk of AD, PD development (McKee and Daneshvar, 2015). Some infectious agents, causing encephalitis (Venkatesan and Murphy, 2018), neuroborreliosis, neurosyphilis (Halperin, 2018), streptococcal meningoencephalitis (Gres et al., 2019), can also overcome BBB and lead to acute inflammation, changes in the brain immune cells and neuron damage. Some lysosomal storage diseases can also lead to neurodegeneration, causing accumulation of pathogenic compounds in nerve cells, such as cholesterol and sphingolipids in Niemann-Pick disease (Strauss et al., 2010), GM2 ganglioside in Tay-Sachs disease (Solovyeva et al., 2018) and Sandhoff disease (Hooper et al., 2017).
The main limitation in the treatment of nervous system diseases is the selective permeability of the BBB. Conventional treatment methods involve therapeutic agent delivery using invasive methods, including neurosurgery (Timbie et al., 2015), BBB osmotic opening (Bhattacharjee et al., 2001) etc. However, such methods result in reduction in the treatment effectiveness and risks for patients.
EVs are a promising vector for therapeutic agent delivery into the nervous system, as they are protected from degradation, retain their original state, and, most importantly, are able to overcome the BBB (Kourembanas, 2015). In addition, it was found that brain endothelial cell-released exosomes help white blood cells to overcome the BBB. Exosomes are able to transfer claudin-5 (CLN-5) protein to the surface of leukocytes, which provides tight contacts between cells and make up the BBB. With the help of CLN-5, leukocytes can pass BBB using “zipper mechanism” (Paul et al., 2016).
The use of exosomes and MVs is a promising alternative to cell therapy, which can prevent some side effects, such as cell oncogenic transformation, undesirable differentiation etc (Chulpanova et al., 2018b). At the same time, EVs have several advantages noted during the cell therapy. First, their use prevents the risk of transplantation of cells with damaged DNA, which is one of the main cell therapy disadvantages. Secondly, EVs are small and easily circulate in the blood, while large cells can lead to blockage of vessels. Third, the use of EVs makes it possible to distribute therapeutic agents much better, and also to reach CNS (Phinney and Pittenger, 2017). Therapeutic molecules

inside EVs are protected by natural lipid bilayer, which ensures stability, biocompatibility, low immunogenicity, ability to overcome body biological barriers (for example, BBB), as well as targeted drug delivery ability (Rufino-Ramos et al., 2017).
Despite promising prospects for the use of EVs, there are a number of limitations that need to be considered when constructing treatment strategies. It was found that EVs may contribute to the progression of certain diseases by spreading pathogenic agents into healthy cells (Bakhshandeh et al., 2017).
Native MSCs-derived exosomes are used for the regeneration of nervous tissue. For example, the exosomes were shown to stimulate the restoration of damaged axons. It is believed that this effect is due to growth factors such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), epidermal growth factor (EGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), which are necessary for neuron growth and recovery (Lopez-Verrilli et al., 2016). Similar results were shown for native exosomes from Schwann cells (Lopez-Verrilli et al., 2013).
Constant inflammatory processes lead to chronic neurodegeneration, which is associated with the activation of microglia. Microglia can play a dual role, on the one hand, it responds to certain stimuli with a set of pro-inflammatory molecules, on the other hand, it cleans damaged cells and stimulates tissue repair. The use of native MSCs-released MVs leads to a modulation of microglia, reducing the transcription of genes associated with inflammation and thereby preventing microglia activation (Jaimes et al., 2017). EVs can be used in therapy both in their native form and loaded with specific molecules using various methods (Table 1).
Modification can be carried out after EV isolation or by changing parental cells (Rufino-Ramos et al., 2017). Native EVs typically express lipids, cell adhesion molecules and ligands that naturally target specific types of recipient cells. Molecules that determine targeting can also be changed, most often by parental cell modification with genes encoding the molecule of interest (Luan et al., 2017). For example, targeted drug delivery with EVs to nervous system cells can be achieved by EV surface modification with a rabies virus glycoprotein, by which EVs selectively target neurons and endothelial brain cells by binding to nicotinic acetylcholine receptors (Cui et al., 2018).

Parkinson's disease

Parkinson's disease is the second most common neurodegenerative disease (Tomiyama et al., 2015). PD is characterized by the presence of Lewy bodies formed due to α-synuclein aggregation and the death of dopamine neurons (Fan et al., 2017). Dopamine deficiency leads to motor impairment, particularly tremor, rigidity and bradykinesia (Tysnes and Storstein, 2017). It is known that oxidative stress aggravates neurodegeneration in PD patients. Intranasal administration of exosomes loaded with catalase into PD model mice resulted in significant neuroprotective effect. Exosomes isolated from monocytes and macrophages were used in the work, this vesicles avoid capture by the immune cells, are able to overcome the BBB and effectively bind with brain cells. Various methods were used for catalase loading, the most effective were the use of saponin, sonication and extrusion (Haney et al., 2015; Kojima et al., 2018). Exosomes, isolated from stem cells from human exfoliated deciduous teeth (SHEDs) 3D culture, possess neuroprotective potential and prevent apoptosis in dopaminergic neurons by approximately 80%. It is noteworthy that exosomes obtained from cells cultured under standard conditions do not show such an effect (Jarmalaviciute et al., 2015).

Alzheimer's disease

Alzheimer's disease is a degenerative disease of the CNS, one of the most common causes of dementia, characterized by the formation of two major protein aggregates: senile (amyloid) plaques and neurofibrillary tangles (NFTs), which are involved in processes leading to progressive neurodegeneration and death (Thei et al., 2018). Senile plaques are formed by the deposition of Aβ peptide fibrils in the human brain is the main component of paired helical filaments (PHFs), which

form compact filamentous structures called neurofibrillary tangles. *In vitro* experiments with hippocampal cells have shown a relationship between amyloid fibrils and signaling pathways which cause excessive phosphorylation of tau protein, which leads to destabilization of microtubules and axonal transport blocking (Dhiman et al., 2019). It was also shown that abnormal phosphorylation of tau protein involves two protein kinases: cyclin-dependent kinase 5 (Cdk5) and glycogen synthase kinase 3β (GSK3β). *In vitro* studies of brain cells and neuroblastoma cells showed that Cdk5 is involved in the processes of cortex maturation and neuron migration, and also plays an important role in normal brain development. Deregulation of this protein kinase leads to excessive tau protein phosphorylation, thereby causing a sequence of molecular events leading to neuron degeneration (Liu et al., 2016). A number of studies showed that oxidative stress is a major factor in normal signaling pathway altering in neurons, which leads to their biochemical, structural abnormalities and degeneration. The main genes involved in the development of Alzheimer's disease encode proteins such as APP, presenilins 1 and 2, alpha-2-macroglobulin and apoliprotein E (Qu et al., 2019).
MicroRNAs play an important role in the regulation of various inflammatory responses. It is known that miR-21 controls the balance between pro-inflammatory, immunoregulatory and anti-inflammatory reactions. Dysregulation of miR-21 causes a chronic inflammation. Hypoxia-preconditioned MSCs have increased miR-21 expression. Injection of exosomes isolated from hypoxia-preconditioned MSCs to AD model animals (APP/PS1) led to improvement in their memory and ability to learn, and also reduced the accumulation of Aβ-peptide. The results confirmed the ability of native MSC-derived exosomes without surface molecule modification to penetrate into the CNS (Cui et al., 2018). In AD glyceraldehyde 3-phosphate dehydrogenase (GAPDH) glycolytic enzyme is involved in neurodegenerative processes and in apoptotic cell death (El Kadmiri et al., 2014). The administration of GAPDH siRNA into mice using exosomes isolated from autologous dendritic cells modified with neuron-specific RVG peptide for targeting resulted in specific gene knockdown in neurons, microglia, and oligodendrocytes in the brain. Exosomes were loaded with exogenous siRNA by electroplating (Alvarez-Erviti et al., 2011).

Huntington's Disease

Huntington's disease is an autosomal dominant neurodegenerative disease which leads to impaired motor and cognitive functions. Neurodegeneration is caused by the accumulation of mutant huntingtin protein, which negatively affects many cell processes. Mutant protein occurs due to CAG repeats, the CAG repeat length is inversely correlated with the disease severity and age of onset (Pagan et al., 2017). It is known that mutations in the huntingtin protein are the main cause of the Huntington's disease. Hydrophobically modified small interfering RNAs (hsiRNAs) aimed at huntingtin mRNA were used to eliminate the toxic protein. During joint incubation, hsiRNAs were loaded into exosomes isolated from the human U87 glioblastoma cell. It was shown that the use of such exosomes improved hsiRNA spread in the brain of model animals, due to which huntingtin gene silence was achieved. The authors recognize that use of glioblastoma cell-derived exosomes can provoke tumor formation, due to which it is necessary to optimize methods for obtaining exosome from other cell types in order to introduce this approach into clinical practice (Didiot et al., 2016).

Epilepsy

Epilepsy is a widespread chronic neurological disorder characterized by recurrent convulsive seizures. Variety of brain damages, including injuries, CNS infections and tumors can lead to epilepsy (Vezzani et al., 2016). The seizures cause an increase in extracellular glutamate level which contributes to cell damage and changes in neuronal signaling (Barker-Haliski and White, 2015). Epilepsy can result in mental disorders and mental retardation (Guerreiro, 2016).
Chronic hippocampus dysfunction is another one consequence of epilepsy. In order to prevent it, the use of exosomes from human bone marrow-derived MSCs, which have strong anti-inflammatory

and neuroprotective properties was proposed. Intranasal administration of exosomes to model mice led to a neuronal loss decrease, inflammation reduction, normal neurogenesis maintenance, cognitive functions and memory preservation (Long et al., 2017).

Multiple sclerosis

Multiple sclerosis is a chronic inflammatory CNS disease leading to demyelination and neurodegeneration (Correale et al., 2017). MS etiology remains unclear, but it is assumed that MS is an autoimmune disease. Progressive MS leads to the loss of axons and trophic support (Nicholas and Rashid, 2013). It was shown that native exosomes and MVs from human periodontal ligament stem cells (hPDLSCs) exhibit regenerative and immunomodulating properties in the treatment of multiple sclerosis. After their administration to MS model mice a decrease in proinflammatory cytokines interleukin 17 (IL-17), interferon γ (IFN-γ), IL-1β, IL-6, tumor necrosis factor α (TNF-α), induction of anti-inflammatory IL-10 and expression attenuation of signal transducer and activator of transcription 1 (STAT1), p53, Caspase 3 and Bcl-2-associated X protein (BAX), which are associated with cell apoptosis were observed (Rajan et al., 2016).

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis is characterized by the degeneration of the upper and lower motor neurons, which leads to muscle weakness, convulsions, paralysis, and death (Hardiman et al., 2017). The causes of ALS remain unknown, but the genes associated with the disease have been identified (Chia et al., 2018). Neuronal cytoplasmic inclusions of TDP-43, FUS, C9orf72, TDP-43 are characterized of ALS (Saberi et al., 2015). The use of native exosomes from adipose tissue-derived stem cells (ADSCs) on mouse neuronal cell culture ALS model showed dismutase 1 (SOD1) aggregation relief, it is assumed that the effect is achieved due to the restoration of mitochondrial functions (Lee et al., 2016).

Conclusion

Exosomes and microvesicles are membrane nanoparticles of endosomal and membrane origin, which provide intercellular communication through the transport of biological molecules. It was found that exosomes and MVs are released by many CNS cells and play an important role in its functioning, as well as contribute to the spread of pathogenic agents in various diseases. Due to the identification of novel biomarkers we face the prospects of exosomes and MVs application for the early diagnosis of the nervous system diseases. The ability to overcome the blood-brain barrier, protect therapeutic agents from degradation, lack of immunoreactivity, biosafety and the possibility of targeted delivery make exosomes and MVs promising tools for use in clinical practice for cell-mediated therapy of CNS diseases.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

This study was supported by the Russian Government Program of Competitive Growth of Kazan Federal University. Albert A. Rizvanov was supported by state assignment 20.5175.2017/6.7 of the Ministry of Education and Science of Russian Federation.

Table 1. Examples of the use of extracellular vesicles for the treatment of CNS diseases

Disease	EV type	Therapeutic effect	Reference
Parkinson's disease	Exosomes from monocytes and macrophages loaded with catalase	Neuroprotection	(Haney et al., 2013; Haney et al., 2015; Kojima et al., 2018)
	Exosomes from MSC 3D culture	Neuroprotection, protection of dopaminergic neurons	(Jarmalaviciute et al., 2015)
	Exosomes from macrophages transfected with GDNF encoding plasmid	Neuroprotection, protection of dopaminergic neurons	(Zhao et al., 2014)
Alzheimer's disease	Exosomes with increased miR-21 expression isolated from hypoxia-preconditioned MSCs	Memory and learning ability improvement, Aβ-peptide accumulation reduction	(Cui et al., 2018)
	Exosomes from dendritic cells with *GAPDH* siRNA and RVG peptide	GAPDH gene knockdown, reduction of neurodegeneration and apoptotosis	(Alvarez-Erviti et al., 2011)
Huntington's Disease	Exosomes from human U87 glioblastoma cell with huntingtin-targeted siRNA	Huntingtin gene knockdown	(Didiot et al., 2016)
Epilepsy	Native exosomes from bone marrow MSCs	Neuronal loss and inflammation reduction, neurogenesis normalization, preservation of cognitive functions and memory	(Long et al., 2017)
Multiple sclerosis	Native exosomes and MVs from hPDLSCs	Regeneration and immunomodulation	(Rajan et al., 2016)
	Plasma exosomes after environmental enrichment	Myelination increase	(Pusic et al., 2016)
Amyotrophic lateral sclerosis	Native exosomes from MSCs	SOD1 aggregation relief	(Lee et al., 2016)

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