Progress of Research on Exosomes in the Protection Against Ischemic Brain Injury

Xianhui Kang1,2†, Ziyi Zuo1†, Wandong Hong4†, Hongli Tang1* and Wujun Geng1*

1 Department of Anesthesiology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China, 2 Department of Anesthesiology, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China, 3 The First Clinical College, Wenzhou Medical University, Wenzhou, China, 4 Department of Gastroenterology and Hepatology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China

Exosomes, as a type of extracellular vesicle (EV), are lipid bilayer vesicles 20–100 nm in diameter that can cross the blood-brain barrier. Exosomes are important transport vesicles in the human body that participate in many conduction pathways and play an important physiological role. Because of their high biocompatibility and low immunogenicity and toxicity, exosomes have attracted increasing attention as an attractive drug delivery system. This article reviews the relevant studies that have shown that exosomes play an important role in protective mechanisms against ischemic brain injury.

Keywords: exosomes, brain protection, ischemic brain injury, stroke, drug delivery

INTRODUCTION

For ischemic brain injury, pharmacological and non-pharmacological brain protection methods are commonly used in the clinic. Pharmacological methods include ion channel blockers, lipid peroxidation inhibitors, excitatory amino acid (EAA) antagonists, blood sugar reduction, barbiturates, and traditional Chinese medicine, whereas non-pharmacological methods include mild hypothermia treatment and acupuncture. Research has examined both ischemic postconditioning and ischemic preconditioning.

In recent years, an increasing number of studies have shown that exosomes can act on the central nervous system through crossing the blood-brain barrier due to their own properties and contents and protect brain tissues through various mechanisms; these findings suggest that exosomes from various sources can protect the brain through cerebral ischemic preconditioning and ameliorate nervous system diseases in the clinic. Exosomes are derived from the intracellular lysosome pathway. Intracellular lysosome particles invade and form multivesicular bodies (MVBs). Then, the extracellular membrane of these vesicles fuses with the cell membrane and secretes them to the extracellular matrix (Colombo et al., 2014). Exosomes, which are between 20 and 100 nm in diameter, are important transport vesicles that can cross the blood-brain barrier and participate in multiple signaling pathways. Exosomes play an important role in the normal physiological function of cells and the occurrence and development of diseases, but research on exosomes is relatively new. Exosomes have been found to mediate the occurrence and development of related diseases such as Alzheimer’s disease and Parkinson’s disease by participating in the production, secretion, aggregation and uptake of related “toxic” proteins, suggesting that exosomes may be an important marker for the early diagnosis of related diseases. This article reviews the latest progress of research on exosomes in the field of ischemic brain injury protection.
OVERVIEW OF EXOSOMES

Discovery of Exosomes
Pan and Johnstone (1983) studied the transformation of sheep reticulocytes to mature erythrocytes in vitro. Through ultracentrifugation, a small vesicle was isolated from the supernatant of sheep erythrocytes. Under electron microscopy, the vesicle was found to be composed of a lipid bilayer with a round or concave cup-like structure and was later named an exosome. For some time afterward, exosomes were considered carriers of waste transported by cells to the outside world. In Raposo et al. (1996) discovered that B lymphocyte-derived exosomes have multiple functions, including antigen presentation, T lymphocyte activation, and immune cell function regulation. Related functions of exosomes began to be discovered gradually. After further study, exosomes were found to be widely present in human blood, cerebrospinal fluid, saliva, urine and so on. In Valadi et al. (2007) discovered for the first time that exosomes contained both RNA and microRNA and confirmed that the RNA carried by exosomes had certain biological activities. With the gradual discovery of substances carried by exosomes, the important roles of proteins, lipids and RNA carried by exosomes in intercellular information exchange and genetic material transfer have increasingly become hot research subjects in the fields of disease occurrence, disease treatment and disease prevention.

Biogenesis and Composition of Exosomes
Extracellular vesicles (EVs) include exosomes with a diameter of 20–100 nm, microvesicles with a diameter of 20–1000 nm and apoptotic bodies with a diameter of 500–2000 nm. Exosomes originate from the endolysosome pathway, whereas microvesicles originate from the direct germination of cells, making the composition of microvesicles much simpler than that of exosomes. The exosome formation process mainly includes early endosomal formation by invagination of the cytoplasmic membrane and early endosomal formation by regulation of the endosomal sorting complex (ESCRT) to form multiple intraluminal vesicles (ILVs), which then constitute MVBs. MVBS mature and fuse with lysosomes for lysosome degradation or fuse with plasmalemma, releasing ILVs to the cell surface to form exosomes (Samanta et al., 2018).

The composition of exosomes has been examined by trypsin digestion, mass spectrometry, Western blot and fluorescence-activated cell sorting (FACS). Exosomes are lipid bilayer vesicles rich in cholesterol, ceramide, sphingomyelin and phospholipids with long saturated ester chains. Exosomes contain a variety of proteins: protein membrane transport fusion proteins (GTPases, annexins, flotillin), transmembrane proteins (CD9, CD63, CD81 and CD82), heat shock proteins (Hsp70, Hsp60, Hsp20, Hsp90) (Gupta and Knowlton, 2007; Zhang et al., 2012) and other proteins (Alix, TSG101), lipoproteins and phospholipases (Roucourt et al., 2015) involved in the formation of vesicles. In addition, exosomes contain many microRNAs, RNAs and other non-coding RNAs, which can be transferred between cells and then regulate the expression of related genes (Pegtel et al., 2010). Many scholars are now focusing on the RNA contained in exosomes and its corresponding regulatory role. An increasing number of scholars are examining the mechanisms of exosomes in mediating disease and tissue protection. The biogenesis and composition of exosomes as shown in Figure 1 (Shahabipour et al., 2017).

Regulation of Exosome Secretion
Precise regulation of exosome secretion is important for various cell functions. The molecular mechanisms that directly regulate exosome secretion have been studied in recent year. Increasing numbers of studies have shown that some essential regulators of exosome biogenesis and secretion in diverse cell types (Hessvik et al., 2016; Wei et al., 2017). Endosomal sorting complexes required for transport proteins (e.g., HRS and Tsg101), tetraspanins (e.g., CD81 and CD9), lipids (e.g., ceramide) and Rab GTPases (e.g., Rab11, Rab27, and Rab35) have been identified to regulate exosome secretion and release (Hsu et al., 2010; Ostrowski et al., 2010; Colombo et al., 2013; Sims et al., 2018). However, the upstream platform for exosome regulators is not well understood. Song L. et al. (2019) revealed that KIBRA controls exosome secretion via inhibiting the proteasomal degradation of Rab27a. Given that Exosomes play a vital role in intercellular communication and numerous biological processes, the exact molecular mechanisms implicated in Exosomes secretion warrant further exploration.

Purification of Exosomes
Separation of exosomes is the first step in functioning as a carrier, and thus, appropriate separation is the key to maintenance of their physical, chemical, and biological functions. Given the substantial differences in exosome size and surface markers, the methods for separation of exosomes must have high specificity and high efficiency. The commonly used methods of separation include ultracentrifugation, ultrafiltration, precipitation, immunoaffinity procedures and microfluidics (Ayala-Mar et al., 2019).

Ultracentrifugation
An effective method for separation of exosomes is the key to the value of exosomes, and therefore, it is essential to reserve the physical, chemical, and biological functions, structure and content of the exosomes to the greatest extent. At present, the golden standard for separation of exosomes is ultracentrifugation (Li P. et al., 2017). By utilizing the differences in the sedimentation rates of components of different molecular weights in a homogeneous suspension and by increasing the centrifugal force gradually, this technique separates cells, cell debris, vesicles, and proteins of different molecular weights and thus purifies exosomes. Distinguishing exosomes, small vesicles and some proteins following ultracentrifugation is difficult. Purification is usually achieved by sucrose density gradient centrifugation combined with ultracentrifugation (Gupta et al., 2018).

Owing to the intrinsically exosome-like nature of EVs, it is easy to confuse EVs with exosomes. The difference between the two lies in their biological functions. EVs are membrane vesicles with a diameter of 20–1000 nm released by cells and contain proteins, lipids, and nucleic acids. Exosomes are EVs with a diameter of 20–100 nm, which are isolated from extracellular fluids by ultracentrifugation and contain proteins, lipids, and nucleic acids. Therefore, EVs include exosomes, and exosomes are a type of EVs.

Keywords: Extracellular vesicles, exosomes, biogenesis, composition, regulation, secretion, purification, ultracentrifugation.
**Ultrafiltration**

Ultrafiltration allows EVs to pass or remain on a selective membrane based on their different sizes through application of different forces, thereby achieving the purpose of isolating the exosomes of a specific size.

**Precipitation**

Exosomes are precipitated by mixing the sample with a highly hydrophilic polymer to change the solubility or dispersibility of the exosomes. Polyethylene glycol (PEG) is commonly used for this process and has extensive applications, including the extraction of exosomes from serum, plasma, ascites, and urine.

**Imunoaffinity Procedures**

Immunoblotting based on immunoaffinity is an effective means for identifying the separated exosomes. In addition, immunoaffinity techniques can be used to selectively separate exosomes in complex liquid environments. Exosomes separated using this method have high quality and purity. At present, the magnetic beads are coated with monoclonal antibody microparticles and then specifically bound to the exosome surface proteins to achieve the separation.

**Microfluidics**

Microfluidic techniques, an emerging separation method, include immunoaffinity, screening, and porous structure capture (Liga et al., 2015). Because this method requires a much smaller volume of samples and reagents than other methods, these techniques can complete the processing of small samples in a short period of time and thus have been widely applied in biomedicine, analytical chemistry and other fields.
Relevance of Exosomes for Occurrence and Development of Diseases

Related studies have discovered that exosomes contain many miRNAs, mRNAs, and other non-coding RNAs, and therefore, as a new form of intercellular communication, these molecules play a very important role in the information transfer between cells (Salem and Fan, 2017). In recent years, the role of exosomes in the development of various diseases has been discovered gradually. For their biological effects, exosomes transfer information from the original cells to the recipient cells mainly through the information transfer between cells and simultaneously release the encoded information into the intercellular fluid or blood circulation, thereby inducing corresponding changes in the recipient cells. Therefore, the occurrence of many diseases is closely related to exosomes. In the course of diabetes development, a variety of miRNAs carried by exosomes, including miR-155 and miR-204, can facilitate the occurrence of diabetes by causing insulin resistance, reducing the sensitivity of the body to insulin, and activating mitochondrial apoptosis in β cells.

Among the exosome-mediated diseases related to the central nervous system, Alzheimer's disease has been extensively studied. Dinkins et al. found that astrocyte-derived exosomes can aggravate cognitive dysfunction by enriching and blocking the degradation of Aβ42 as a component of the senile plaques of Alzheimer’s disease (Dinkins et al., 2016). Moreover, microglia can internalize and release Tau protein through exosomes. Exosomes can carry overphosphorylated Tau protein into peripheral cells, causing damage to the functions of cells when intracellular regulatory functions are dysfunctional.

Exosomes also play a vital role in the development of various blood-related diseases. In recent years, many studies have found that exosomes are closely linked to hypertension (Pironti et al., 2015), atherosclerosis (Moreno et al., 2013), cardiac hypertrophy and other diseases and can carry and transfer miR-21-3p, miR-133b and other miRNAs, playing an important role in the occurrence and development of the above cardiovascular diseases.

Advantages of Exosomes as a Natural Carrier System

As an important barrier to isolate plasma and cerebrospinal fluid, the blood-brain barrier plays a crucial role in preventing harmful substances from entering the brain and maintaining the basic stability of the brain environment. However, the restriction of the transport of macromolecule proteins by the blood-brain barrier makes entering the brain through the blood-brain barrier difficult for some macromolecule drugs that would be otherwise effective for the treatment of nervous system diseases, thereby limiting their clinical application. To enable these drugs to be used effectively in the clinic, an effective carrier system is needed to participate in the delivery of drugs.

Currently, carrier systems including liposomes and nanoparticles are widely used, but their high immunogenicity, low biocompatibility, short half-life and lack of specificity are limiting. As a natural carrier system, exosomes have a low immunogenicity, high biocompatibility, long half-life (Ha et al., 2016), and strong targeting ability (Lakhal and Wood, 2011). Exosomes can freely cross the blood-brain barrier (Zhuang et al., 2011) and maintain high activity during long-term storage, giving them major advantages as an ideal drug delivery system. A large number of studies have shown that exosomes can deliver different pharmacological molecules to target cells or tissues. These molecules can be further modified and reinserted into exosomes for different therapeutic applications (see Figure 2), opening up a new method for clinical drug delivery for central nervous system diseases (Samanta et al., 2018).

Brain Tissue Protection and Treatment in Central Nervous System Diseases

A series of studies have shown that exosomes play a therapeutic role in ischemic diseases of the central nervous system, creating treatment options. Exosomes have a variety of sources (endothelial cells, adipose tissue-derived mesenchymal stem cells, astrocytes, etc.) and can protect against and repair ischemic central nervous system injury. A key characteristic of exosomes is their ability to penetrate the blood-brain barrier and release the associated RNA, protein, etc. into the central nervous system (Valadi et al., 2007) and then pass through it. There are many pathways that promote the growth and repair of blood vessels, inhibit the apoptosis of nerve cells, and promote the repair and regeneration of nerve cells. Long et al. (2017) found that the adult exudate of mesenchymal stem cells administered through the nasal spray administration route can be absorbed by neurons and microglia in the motor cortex, thereby alleviating neuronal inflammation, indicating that exosomes can penetrate the blood-brain barrier and play a therapeutic role in relevant regions of the brain. Among all the sources of exosomes, mesenchymal
stem cell-derived exosomes have been extensively studied for their ability to promote the protection and repair of the central nervous system. The neuroprotective effect of mesenchymal stem cell-derived exosomes was found to be related to their dose and number of generations. The smaller the generation, the stronger the neuroprotective effect of the exosomes is. Low-dose exosomes could inhibit neuronal injury through antiapoptotic effects and oxidation, while high-dose exosomes had the opposite effect on neurons (Venugopal et al., 2017).

**Protective and Reparative Effects of Exosomes on Brain Tissue Injury**

Relevant studies on the protection and repair of brain tissue mediated by exosomes have shown that exosomes can protect and repair neurons by (1) improving the microenvironment and regulating the corresponding immune function; (2) inhibiting neuronal apoptosis and mediating axon reconstruction and neurogenesis; (3) promoting vascular regeneration and remodeling; and (4) alleviating inflammation. Exosomes also play a role in the sexual response and immunosuppression. Dosage and route of administration of exosomes in animal experiments (see Table 1).

**Improving the Microenvironment and Regulating Immune-Mediated Tissue Protection and Repair**

In acute brain injury, insufficient cerebral perfusion can lead to ischemic stroke. White et al. (2000) showed that during stroke, high levels of glutamate accumulate in cells, which opens voltage-dependent and glutamate-regulated calcium channels, resulting in a substantial calcium influx and activation and production of large quantities of nitric oxide synthase. Excessive consumption of superoxide dismutase (SOD) leads to an accumulation of free radicals in cells, causing cell apoptosis, DNA damage, and brain tissue damage. Wei et al. (2016) showed that in a glutamate-induced neuronal injury model, adipose-derived mesenchymal stem cell-derived exosomes can protect brain tissue from glutamate-induced neuronal injury by transporting and releasing cytokines such as insulin-like growth factor (IGF) and hepatocyte growth factor (HGF), which are potentially associated with activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. In addition, Kalani et al. (2016) found that exosomes could reduce the infarct volume and degree of edema in the brain of ischemia-reperfusion mice by reducing the expression of the glutamate receptor N-methyl-D-aspartate receptor (NMDAR) in the central nervous system.

Neurons, as permanent cells, are difficult to repair after damage. Although neural stem cells provide hope for regeneration of neurons through self-differentiation, this process is very difficult due to the poor peripheral microenvironment of damaged tissues. Han et al. (2019) showed that intravenous injection of mesenchymal stem cell-derived exosomes into a intracerebral hemorrhage rat model improved the brain microenvironment through crossing the blood-brain barrier and promoting vascular remodeling and neurological function by regulating angiogenesis and neuron regeneration. These results indicated that mesenchymal stem cell-derived exosomes could enter the brain microenvironment and improve it to promote the repair of damaged neurons.

**Promoting Vascular Regeneration and Reconstruction**

The protective effect of exosomes is also reflected in their ability to promote the regeneration and reconstruction of blood vessels. Du et al. (2018) co-cultured mesenchymal stem cell-derived exosomes with high expression of microRNA-132-3p and bEnd.3 with mouse brain microvascular endothelial cells damaged by glucose and oxygen deprivation/reoxygenation (H/R). The mesenchymal stem cell-derived exosomes expressing high levels of microRNA-132-3p effectively improved the proliferation and migration function of H/R-induced damaged cerebrovascular endothelial cells and reduced Akt phosphorylation levels, thereby promoting blood vessel regeneration through the PI3K/Akt pathway. These observations indicated that exosomes can promote the regeneration of cerebrovascular endothelial cells by promoting the proliferation of cerebrovascular endothelial cells, providing a new method for stem cell therapy for the treatment of cerebrovascular injury.

**Inhibiting Neuronal Apoptosis and Mediating Axon Remodeling and Neurogenesis**

Shen et al. (2018) showed that the number of apoptotic and degenerative neurons in the rat brain was significantly reduced by transfecting exosomes with microRNA-133b and transfusing them back into the rat tail vein after intracerebral hemorrhage, indicating that microRNA-133b-containing exosomes had a protective effect on the brain tissue after intracerebral hemorrhage. Similarly, Li et al. (2018) found that neural stem cell (NSC)-derived exosomes could inhibit neuronal apoptosis and promote neuronal survival by studying the role of NSC-derived exosomes in a cobalt chloride (CoCl₂)-induced hypoxia

---

**TABLE 1** | Dosages and routes of administration of exosomes in animal experiments.

| Administration route | Protein dosage | Quantity | References |
|---------------------|----------------|----------|------------|
| Intranasal (IN)     | 15 µg          | 7.5 × 10⁶ exosomes | Long et al., 2017 |
| Intranasal (IN)     | 10 µg          | –        | Kalani et al., 2016 |
| Tail vein injection (TV) | 100 µg         | 3 × 10⁶ exosomes | Zhang et al., 2017c |
| Intravenous injection (IV) | 250 µL        | EVs released by 2 × 10⁶ MSCs | Doeppner et al., 2015 |
| Tail vein injection (TV) | 100 µg         | –        | Xin et al., 2013 |
| Tail vein injection (TV) | 100 µg/day for 3 days | –        | Song Y. et al., 2019 |
| Tail vein injection (TV) | 100 µg         | –        | Shen et al., 2018 |
Antagonizing Immunosuppression and the Inflammatory Response

Li Y. et al. (2017) showed that exosomes of dental pulp mesenchymal stem cells could inhibit the neuroinflammatory response induced by traumatic brain injury. Huang et al. (2018) also showed that under traumatic brain injury, an increase in microglial exosome miRNA-124-3p not only reduced the occurrence of the inflammatory response but also promoted the growth of axons. Similarly, Zhang et al. (2017c) found that exosomes derived from mesenchymal stem cells can protect neurons by releasing miRNA-124 target proteins, which inhibited neurological deficits and neuronal apoptosis in the mouse model of stroke, thereby increasing the survival rate of neuronal cells.

Protection of Brain Tissue Mediated by Secretion of Exosome in Ischemic Preconditioning

As research on the function of exosomes has been performed and our knowledge of their role has deepened, researchers’ views on exosomes have changed. At an early stage, exosomes were considered a medium for cells to discharge waste to the outside world. In recent years, the above studies showed that exosomes play an important role in alleviating or even preventing brain tissue damage caused by ischemia and hypoxia. At present, non-pharmacological approaches such as mild hypothermia and ion channel blockers are mostly used to prevent ischemic brain damage in the clinic, as well as other pharmacological approaches such as ion channel blockers. In recent years, an increasing number of researchers have found that exosomes may play an important role in brain protection mediated by ischemic preconditioning.

Ischemic preconditioning refers to a process involving short-term blockade and reperfusion of blood flow to activate various endogenous protective mechanisms and alleviate tissue damage. This method is widely used in various cardiovascular operations. However, because the protection of brain tissue using this process requires the separation of the brain tissue for blood flow blockage and reflow, the process has many advantages. In recent years, the concept of ischemic conditioning has been extended to remote ischemic conditioning (RIC), i.e., a short series of blood flow blockage and reperfusion in the distal limbs through cuff suppression, which has also been found to have protective effects on brain tissue after multiple cycles. Although ischemic preconditioning and RIC have great potential for development as effective, low-cost and simple methods, few researchers have studied the mechanisms of ischemic preconditioning.

Xiao et al. (2017) showed that ischemic cell-derived exosomes could be induced by upregulation of transcription and translation through sugar deprivation/reoxygenation in the SH-SY5Y nerve cell line. In this process, the expression of Bcl-2 inhibits the expression of Bax, thus alleviating nerve cell apoptosis and achieving a protective effect. The mechanism of this process may be related to the Janus kinase 2 (JAK2)/signal transducer and activator of transcription-3 (STAT3) pathway (Cheng et al., 2014) and the PI3K/Akt pathway (Zhang et al., 2017a); the latter pathway has been extensively studied, and the former requires further testing.

In Xiao et al. (2017), the CD63, HSP70 and TSG101 expression levels in exosomes in the hippocampus of the RIP group did not increase, but the expression in plasma increased, indicating that RIP can promote the release of exosomes. This finding indicates that in light of the spatial distribution of exosomes in a model of acute cerebral ischemia, exosomes are extensively distributed in the blood circulation. Moreover, the brain protection mediated by remote ischemic preconditioning also indicates that exosomes are extensively distributed in the whole body through the blood circulation, and due to the size of the exosomes themselves and the specificity of their physical and chemical properties, these molecules can further mediate brain protection by passing through the BBB and releasing miRNAs and other substances. Furthermore, based on the elevated blood exosomes induced by remote ischemic preconditioning, these molecules are likely associated with the protection of other organs. The dependent interaction of exosomes in the damage of organism has been outlined above. In recent years, increasing numbers of studies have shown that in addition to the brain protection mediated by exosomes, exosomes can act on tissues such as the myocardium in
a similar manner and mediate the corresponding tissue protective functions, indicating that the effect of exosomes is not specific to brain tissue. Given the diversity of these mechanisms, multiple organ protective mechanisms may be present simultaneously, and further exploration of these mechanisms is needed.

OUTLOOK

As a new therapeutic carrier, exosomes have attracted increasing attention because of their unique biological characteristics. Our understanding of exosomes has also changed from an excreta carrier in earlier years to a new therapeutic carrier with tremendous research potential in recent years, with an ability to mediate the repair process of multiple brain tissue injuries. Many studies have shown that exosomes can ameliorate ischemic and hypoxic brain injury by improving the microenvironment, regulating the corresponding immune effects, inhibiting neuronal apoptosis, mediating axon reconstruction and neurogenesis, promoting vascular regeneration and remodeling, alleviating the inflammatory response and immune suppression, etc. Moreover, these repairing effects of exosomes also suggest that they can play a protective role in preventing ischemic brain necrosis by improving the resistance of brain tissue to acute ischemic injury. Through the study of animal models of acute ischemia, we see that RIC can produce exosomes and transfer them to brain tissue to play a protective role, not only indicating that exosomes can play a protective role in preventing ischemic brain necrosis but also showing tremendous research value in the study of their involvement in mediating brain protection. Research has also shown that exosomes, with their high biocompatibility, low immunogenicity and toxicity, can effectively participate in brain protection. In addition to traditional non-pharmacological approaches such as mild hypothermia and pharmacological approaches, exosomes can protect against cerebral ischemia injury. At the same time, the protective effect mediated by exosomes found in remote ischemic preconditioning experiments indicates that exosomes are related to the traditional preconditioning mechanism.

However, our knowledge of the protective effect of exosomes on brain tissue is limited; most experiments are limited to the protective effect of exosomes on the tissue itself, and the interaction between exosomes and signaling pathways is not discussed in detail. In addition to studies on the use of exosomes as an effective therapeutic approach, more research examining the specific mechanism of exosome-mediated brain tissue protection is needed.

AUTHOR CONTRIBUTIONS

WG and HT were the guarantor of integrity of the entire study. WG and WH contributed to study concepts. XK and WH contributed to manuscript preparation. XK and ZZ contributed to manuscript editing. HT helped to manuscript review.

FUNDING

This study was funded by the Natural Science Foundation of China (81774109 and 81973620), Natural Science Foundation of Zhejiang Provincial (Y19H310028), Zhejiang Public Welfare Technology Research Plan (GD20H290004), and Wenzhou Science and Technology project (ZY2019105 and Y20180496).

REFERENCES

Ayala-Mar, S., Donoso-Quezada, J., Gallo-Villanueva, R. C., Perez-Gonzalez, V. H., and Gonzalez-Valdez, J. (2019). Recent advances and challenges in the recovery and purification of cellular exosomes. Electrophoresis doi: 10.1002/elps.201800526 [Epub ahead of print].

Cheng, Z., Li, L., Mo, X., Zhang, L., Xie, Y., Guo, Q., et al. (2014). Non-invasive remote limb ischemic postconditioning protects rats against focal cerebral ischemia by upregulating STAT3 and reducing apoptosis. Int. J. Mol. Med. 34, 957–966. doi: 10.3892/ijmm.2014.1873.

Colombo, M., Moita, C., van Niel, G., Kowal, J., Vigneron, J., Benaroch, P., et al. (2013). Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. J. Cell Sci. 126, 5553–5565. doi:10.1242/jcs.128868.

Colombo, M., Raposo, G., and Thery, C. (2014). “Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles,” in Annual Review of Cell and Developmental Biology, Vol. 30, eds R. Schedman and R. Lehmann (California, CA: Annual Reviews), 255–289. doi:10.1146/annurev-cellbio-101512-122326.

Dinkins, M. B., Enasco, J., Hernandez, C., Wang, G., Kong, J., Helwa, L., et al. (2016). Neutral sphingomyelinase-2 deficiency ameliorates Alzheimer’s Disease pathology and improves cognition in the SXFAD Mouse. J. Neurosci. 36, 8653–8667. doi:10.1523/jneurosci.1429-16.2016.

Doepner, T. R., Herz, J., Goergens, A., Schlechter, J., Ludwig, A.-K., Radtke, S., et al. (2015). Extracellular vesicles improve post-stroke neuroregeneration and prevent postischemic immunosuppression. Stem Cells Transl. Med. 4, 1131–1143. doi: 10.5966/sctm.2015-2078.
Huang, S., Ge, X., Yu, J., Han, Z., Yin, Z., Li, Y., et al. (2018). Increased miR-124-3p in microglial exosomes following traumatic brain injury inhibits neuronal inflammation and contributes to neurite outgrowth via their transfer into neurons. FASEB J. 32, 512–528. doi: 10.1096/fj.20170673R

Kalan, A., Chaturvedi, P., Kumat, P. K., Maldonado, C., Bauer, P., Joshuaa, I. C. G., et al. (2016). Circumventing load embryonic stem cell exosomes restored neurovascular unit following ischemia-reperfusion injury. Int. J. Biochem. Cell Biol. 79, 360–369. doi: 10.1016/j.biocel.2016.09.002

Lakhal, S., and Wood, M. J. A. (2011). Exosome nanotechnology: an emerging delivery. J. Cell. Physiol. doi: 10.1002/jcp.22040

Long, Q., Upadhya, D., Hattiangady, B., Kim, D.-K., An, S. Y., Shuai, B., et al. (2017). Rab27a and Rab27b control different steps of the exosome secretion pathway. Nat. Cell Biol. 19, 12–30. doi: 10.1038/ncl.2016.119

Pironti, G., Strachan, R. T., Abraham, D., Yu, S. M.-W., Chen, M., Chen, W., et al. (2018). Role of exosomes in cardiovascular diseases: mediating diabetic cardiomyopathy, “in Exosomes in Cardiovascular Diseases: Biomarkers, Pathological and Therapeutic Effects,” in Proceedings of the 8th International Conference on Exosomes in the Cardiovascular System. Bioessays, 33, 737–741. doi: 10.1002/bies.201100076

Valadil, H., Ekstrom, K., Bossios, A., Sjostrand, M., Lee, J. J., and Lotvall, J. O. (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat. Cell Biol. 9, 654–659. doi: 10.1038/nccb1596

Wei, J., Chen, Y., Xue, C., Li, M., Lu, J., and Li, A. (2017). Endothelial cell-derived exosomes protect the mouse brain from ischaemia-reperfusion injury via exosomal miR-124. Theranostics 9, 2910–2923. doi: 10.7150/thno.30879

Wei, J., Chen, Y., Xue, C., Li, M., Lu, J., and Li, A. (2017). Protection of nerve injury with Exosome extracted from mesenchymal stem cell. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 38, 33–36. doi: 10.3881/jissn.1000-503X.2016.01.006

Wei, Y., Wang, D., Jin, F., Bian, Z., Li, L., Liang, H., et al. (2017). Pyruvate kinase type M2 promotes tumour cell exosome release via phosphorylating synaptophysin-associated protein 23. Nat. Commun. 8:4041. doi: 10.1038/ncomms4041

White, B. C., Sullivan, J. M., DeGracia, D. J., O’Neil, B. J., Neuman, R. W., Grosman, L. L., et al. (2000). Brain ischemia and reperfusion: molecular mechanisms of neuronal injury. J. Neurosci. 19, 1–33. doi: 10.1172/jnl.2000.48-645

Xiao, B., Chai, Y., Lv, S., Ye, M., Wu, M., Xie, L., et al. (2017). Endothelial cell-derived exosomes protect SH-SYSY nerve cells against ischaemia/reperfusion injury. Int. J. Mol. Med. 40, 1201–1209. doi: 10.3892/ijmm.2017.3106

Zhang, X., Wang, X., Zhu, H., Kranias, E. G., Tang, Y., Peng, T., et al. (2012). Hsp20 functions as a novel Cardiokine in promoting angiogenesis via activation of functional Angiotensin II Type 1 receptors. Circulation 125, 2120–2130. doi: 10.1161/circulationaha.115.015687

Zhang, X., Wang, X., Zhu, H., Kranias, E. G., Tang, Y., Peng, T., et al. (2012). Hsp20 functions as a novel Cardiokine in promoting angiogenesis via activation of functional Angiotensin II Type 1 receptors. Circulation 125, 2120–2130. doi: 10.1161/circulationaha.115.015687