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Review

Extracellular Vesicles as a Potential Therapy for Stroke

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Abstract: Although thrombolytic therapy has enjoyed relative success, limitations remain, such as a narrow therapeutic window and inconsistent efficacy. Consequently, there is a pressing need to develop novel therapeutic approaches. In recent years, extracellular vesicles (EVs) have garnered increasing attention as a potential alternative to stem cell therapy. Because of their ability to cross the blood–brain barrier and exert neuroprotective effects in cerebral ischemia and hemorrhage, the exploration of EVs for clinical application in stroke treatment is expanding. EVs are characterized by high heterogeneity, with their composition closely mirroring that of their parent cells. This property enables EVs to distinguish between cerebral ischemia and hemorrhage, thus facilitating a more rapid and accurate diagnosis. Additionally, EVs can be engineered to carry specific molecules, such as miRNAs, targeting them to specific cells, potentially enhancing the therapeutic outcome and improving stroke prognosis. In this review, we will also explore the methodologies for the isolation and extraction of EVs, critically evaluating the advantages and disadvantages of various commonly employed separation techniques. Furthermore, we will briefly address current EV preservation and administration methods, providing a comprehensive overview of the state of EV-based therapies in stroke treatment.

Keywords: stroke; extracellular vesicles; neuroprotective; ischemia; hemorrhage

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1. Introduction

In a study of the Global Burden of Disease, Injuries, and Risk Factors 2021, stroke was the greatest contributor globally to the neurological burden, and its mortality rate had increased by 44.1% compared with that in 1990 [1]. Stroke include two groups: ischemic stroke, caused by the blockage of blood vessels and hemorrhagic stroke, caused by the rupture of blood vessels. At present, the most common type is acute ischemic stroke (AIS). In AIS, each minute saved in onset-to-treatment time can add an average of 4.2 days of extra healthy life, and every 20 min decrease in treatment delays can lead to a gain of an average of 3 months of disability-free life equivalency [2]. Thus, quick, precise and early diagnosis is paramount for treating patients with AIS to preserve brain issues and ultimately improve functional outcomes. Currently, relatively effective therapeutic methods simply include intravenous tissue plasminogen activator (tPA) to induce thrombolysis and endovascular thrombectomy to physically remove the blockage [3]. Furthermore, hemorrhagic stroke tends to have significant risk as the increased intracranial pressure causes a mass effect, such as midline shift and cerebral hernia, which can threaten a patient's life. The treatment measures include pharmacological and surgical therapies to control intracranial pressure and cerebral edema. However, the use of tPA may induce or worsen the situation of

hemorrhagic stroke [4]. Likewise, the therapeutic way of lowering intracranial pressure will increase the potential risk for those who suffer from ischemic stroke [5]. Therefore, medical imaging, like computed tomography (CT) or magnetic resonance imaging (MRI), is used to distinguish ischemic stroke from hemorrhagic stroke, but the examination will require a certain amount of time. Therefore, for quick diagnosis and subsequent neuroprotective therapies, extracellular vesicles (EVs) might be employed as an ideal biomarker. As discussed below, it holds true that the ideal biomarker is one that is able to pass through the BBB freely. In addition, it would be of CNS origin and correlate with distinct cell types in order to offer a window into the physiologic processes occurring in the brain during stroke. In this review, we will focus on the cutting-edge research to address current progress on EVs, providing a comprehensive overview of the state of EV-based therapies in stroke treatment.

2. Stroke Pathophysiology

A hallmark of ischemic stroke, interrupted blood flow, mainly caused by cerebral embolism or vascular disease, depletes the brain of oxygen and glucose [6]. First, generated reactive oxygen species result in mitochondrial dysfunction and oxidative stress injury [7]. Second, the depletion can induce electrolyte disturbance, and an imbalance of ions, such as sodium, potassium, and calcium, could contribute to the depolarization of brain tissue and the release of glutamate, which, in excess, stimulates *N*-methyl-d-aspartate receptors (NMDARs), causing neuronal excitotoxicity [8,9]. This excitotoxicity can lead to pathological changes in brain neural tissue, including cerebral edema [10], neuroinflammation [11], and cell death [12], ultimately resulting in severe neurological dysfunction and deficits [13]. These derangements, including excitotoxicity, mitochondrial dysfunction, neuroinflammation, disruption of the blood–brain barrier, and cell death, ultimately contribute to ischemic stroke [14].

During a hemorrhagic stroke, the rupture of blood vessels leads to a rapid increase in intracranial pressure, causing brain tissue deformation and mutual compression, ultimately resulting in tissue necrosis and reduced perfusion [15]. This process is driven by the release of glutamate, which activates NMDARs and induces excitotoxicity, leading to cell death [9]. Necrotic cells will activate damage-associated molecular patterns (DAMPs), which could be detected by microglia [16]. Influenced by shear force, microglia would engage ameboid form and release inflammatory factors, such as NF-κB and IL-1, triggering the recruitment of inflammatory cells, such as neutrophils and macrophages [17]. Meanwhile, the permeability of endothelial cells at the blood–brain barrier will increase to achieve the kind of recruitment, allowing many enzymes and antibodies to penetrate the parenchyma tissue, impair ion homeostasis, and amplify the oxidative stress response [18]. Astrocytes participate in ferroptosis, and increase the expression of AQP4, disrupting the water homeostasis of brain tissue [19]. Collectively, these processes result in substantial neurological damage and deficits, characteristic of hemorrhagic stroke [20].

3. Extracellular Vesicles

Extracellular vesicles refer to cell-derived and membrane-bound vesicles [21]. EVs can play important roles in waste removal and cell-to-cell communication via the extracellular secretory properties of all types of cells. EVs can display specific surface markers based on their original cell, which perform powerful functions in their intrinsic homing and can be taken up by cells through various endocytic pathways, such as endocytosis and micropinocytosis [22]. After uptake, EVs release their cargo, which is composed of proteins, lipids and nucleic acids [23]. According to their physical characteristics and biochemical composition, EVs can be divided into two major categories: exosomes and microvesicles.

Exosomes are the smallest types of EVs, ranging from 30–150 nm in diameter [24], and are formed by the inward budding of early endosomes, which then mature into multivesicular bodies (MVBs). MVBs are either sent to be degraded by lysosomes or fuse with plasma membranes to release their contents [25]. Exosomes contain various and sundry proteins and nucleic acids, like the family of HSP and Rabs [26]. However, they tend to include proteins associated with their endosomal secretion pathway, higher levels of glycoproteins, and proteins involved in inter-organelle crosstalk [27]. Furthermore, nuclear EVs are generated by membrane budding at the inner nuclear membrane [28]. They are passaged across the cytosol and released into the extracellular space. Nuclear EVs are rich in pre-microRNAs. Pre-microRNAs need to be processed to microRNAs to exert biological roles. Microvesicles (MVs) are larger irregularly shaped vesicles, ranging 50–1000 nm in diameter [29], and are formed by the direct outward budding of a cellular plasma membrane. MVs generally also display some of the original cell's surface markers and contain cytosolic proteins, heat shock proteins, and integrins [30]. Apoptotic bodies with diameters larger than 500 nm are released by the outward budding of larger plasma membrane fractions as part of a cellular decomposition process in apoptotic cell death [31]. Apoptotic bodies are typically phosphatidyl-serine (PS)-decorated on their outer membrane surface, which predisposes them for clearing by phagocytes [32]. Thus, apoptotic cells may also release EVs in the exosome size that confer pro-inflammatory signals to myeloid leucocytes [33].

As said above, extracellular vesicles are heterogeneous in size, content and origin, which makes isolation difficult [34]. Therefore, to efficiently enrich EVs, different isolation methods are selected for different purposes and applications, among which ultracentrifugation, size-based isolation techniques, polymer precipitation, and immunoaffinity capture techniques are more commonly used (Table 1).

Ultracentrifugation (UC) is currently the most widely used isolation technique and the criterion standard for extraction and separation. UC mainly harvests the re-quired components based on the size and density differences of each component in the original solution [35]. This method does not need to label EVs, which can avoid cross-contamination. However, it is not conducive to downstream analysis because of its time demand, high cost, cause of structural damage, formation of aggregates, and co-separation with lipoproteins [36]. The separation principle of size-exclusion chromatography (SEC) is that macromolecules cannot enter the gel pores and that they are eluted along the gaps between the porous gels with the mobile phase, while small molecules remain in the gel pores and are finally eluted by the mobile phase. The application of SEC is quick, easy, and low-cost. Still, they may be doped with other particles of similar size, resulting in reduced purity [37]. Immunoaffinity chromatography (IAC) is a separation and purification technology based on the specific binding of antibodies and ligands to separate desired substances from heterogeneous mixtures [38]. The binding efficiency is closely related to the biological affinity pairs, elution conditions and matrix carriers.

According to different derivations and purposes, the methods described above have various prospects. For body fluids, such as plasma and cerebrospinal fluid, and conditional cell medium, there are five purification methods commonly used to date: precipitation, membrane affinity-based separation, SEC, iodixanol gradient ultracentrifugation, and phosphatidylserine affinity isolation. For the conditioned medium, precipitation isolated the highest number of EV proteins, followed by membrane affinity-based separation. For the plasma samples, membrane affinity-based separation isolated many EV proteins and a few non-EV proteins, while precipitation isolated the most EV proteins [39]. However, it is more challenging to isolate EV from complex tis-sues, such as the brain [40]. To liberate the EVs from the extracellular matrix (ECM), the frozen or fresh tissue first must suffer an

initial mechanical disruption, generally followed by enzymatic digestion to disrupt the network of glycosaminoglycans, proteoglycans, glycoproteins, and fibrous proteins that compose the ECM.

Table 1. The advantages and disadvantages of the main methods of isolation.

Isolation	Advantages	Disadvantages	Citation
Ultracentrifugation	Suitable for separating large-dose sample components	Low purity and recovery rate	[35,36,41]
Size-exclusion Chromatography	(1) Quick, easy, and low-cost; (2) Isolated EVs have complete structure and uniform size	Easily doped with other particles of similar size, low purity	[37]
Immunoaffinity Chromatography	Relative high purity	High-cost and troublesome	[38]

4. The Role of Extracellular Vesicles

The composition of EVs closely defines their biological roles. EVs abundantly contain membrane-organizing proteins, especially including tetraspanins. Tetraspanins are a family of 34 transmembrane proteins in mammals which contain four trans-membrane domains and two extracellular loops [42]. Although each tetraspanins exhibit different tissue and subcellular distributions, they are detected in nearly all cell-types as components of plasma membranes, endosomes and exosomes. Clustering with transmembrane integrins, selectins, cell adhesion molecules, cadherins and receptor proteins, tetraspanins regulate biological processes including cell adhesion, motility, proliferation and immune cell activation when stroke occurs. Associated with glycosylphosphatidylinositol (GPI)-anchored proteins and binding proteins on the outer membrane leaflet, EVs could carry various protein cargos, including cytokines, cytokine receptors, enzymes, enzyme inhibitors, ephrin, ephrin receptors, death receptor ligands and major histocompatibility complex (MHC) proteins/complexes [43–45]. These cargos have immunomodulatory properties and control cell proliferation, migration and guidance, as well as axonal growth. Additionally, EVs may contain RNAs, namely microRNAs, pre-microRNAs, long non-coding RNAs and mRNAs, as well as DNA [46]. What's more, important functions of EVs have been attributed to lipids, like phosphatidic acid, phosphatidylserine (PS), and sphingolipids [31]. PS serves as signal for phagocyte removal when exposed on apoptotic cells [47], while the sphingolipids sphingomyelin and sphingosine-1 phosphate (S1P) crucially control EVs budding and release and modulate cell migration and differentiation upon target cell binding [48].

Stem cell therapy has received much attention in the past few years. However, it is undeniable that there are still many obvious safety concerns, such as the possibility of tumorigenic and immune rejection of cell transplantation [49]. EVs, as cell-free replacement therapy, have the advantage over cell therapy. Still, those derived from stem cells and other differentiated cells could show anti-inflammatory regenerative proper-ties in different tissue cells, such as the brain, lung and heart [50–52]. Moreover, EVs have been shown to reduce neuropathology and improve behavioral and cognitive deficits in several animal models of neurological diseases [53,54]. EVs can be enucleated by brain microvascular endothelial cells, allowing them to cross the blood–brain barrier and import foreign molecules directly into the nervous system, making EVs useful as an attractive potential treatment for neurological disorders [55–57]. For example, in amyotrophic lateral sclerosis, neurons can transport EV-encapsulated miR-124a to astrocytes, which elevates the glutamate transporter GLT1 by transcriptional regulation, reducing extracellular glutamate levels and reversing synaptic over-activation [58]. Additionally, in the inflamed brain, EVs are released by

macro-phages, which can further transport functional NADPH oxidase NOX2 and miRNAs to neuronal axons, from which they are retrogradely carried to the perikaryon, inducing axonal regeneration via PTEN deactivation [59].

5. Extracellular Vesicles in Stroke Diagnosis

As previously discussed, the effectiveness of stroke treatment diminishes over time, making it critical to minimize delays in diagnosis and intervention. Currently, stroke diagnosis depends on the ability to distinguish it from conditions such as migraines and seizures, as well as to differentiate between hemorrhagic and ischemic strokes. This latter distinction typically necessitates the use of CT or MRI, both of which are time-consuming and have limitations unlikely to be resolved soon. However, recent reports have highlighted the potential of EVs as a diagnostic tool for stroke. EVs can be rapidly measured and offer promise as an acute diagnostic tool that differentiates between ischemic and hemorrhagic strokes. The discussion below further explores this potential.

Extracellular vesicles have a unique molecular profile that presents the phenotypic composition of their original cell. The current studies show that several types of cells could release EVs into the blood during stroke, including neural cells, endothelial cells, platelets, erythrocytes, and leukocytes [60–62]. In 2006, Simak reported a correlation between the severity of stroke and EVs originating from endothelial cells [61]. Among them, CD54/ICAM-1 positive EVs (CD105+, CD54+, CD45−) had the strongest correlation with ischemic lesion volume, while endothelial cell-derived EVs (CD105+, CD41a−, CD45−) more strongly correlated with long-term clinical outcome. Similarly, Chiva-Blanch et.al observed that in AIS patients, neural progenitor cells (CD34+, CD56+), platelets (CD61+), endothelial cells (CD146+), erythrocytes (CD235ab+), and leucocytes (CD45+) were all increased [63]. However, none of these studies could distinguish between minor–moderate and severe stroke. In short, these studies show promising results and increase the anticipation of the diagnostic potential of peripheral blood EVs in AIS.

In another study, investigators found EVs from endothelial cells (CD105+, CD106+, CD54+, or CD62e+), leucocytes (CD45+), and erythrocytes (CD235+) were elevated in patients with ICH [64]. Interestingly, Sanborn et al. not only obtained similar results but also found that the concentration of EVs from neutrophils (CD66b+), erythrocytes (CD235a+), and endothelial cells (CD146+) tended to increase [65]. These studies show a clear diagnostic potential of EVs in hemorrhagic stroke. By contrast, in one group with ischemic stroke, for example, the EVs positive for CD235a+ erythrocytes and CD105+ endothelial cells are similar to the EV population above, making it difficult to distinguish stroke subtypes.

Moreover, the elevation of EVs for several days or even months might contribute to continuous inflammatory response, edema, or a leaky blood–brain barrier, which is not relevant to acute events. Instead, these EV changes could be valuable as prognostic markers of long-term clinical outcomes or serve as treatment-monitoring biomarkers.

6. Extracellular Vesicles in Ischemic Stroke Treatment

Extracellular vesicles have several therapeutic functions on ischemic stroke (Figure 1), including ameliorating neurological function, reducing permeability of the blood–brain barrier, brain edema, regulating inflammatory and immune response, attenuating neural apoptosis, adjusting autophagy and modulating angiogenesis and neuro-genesis [66].

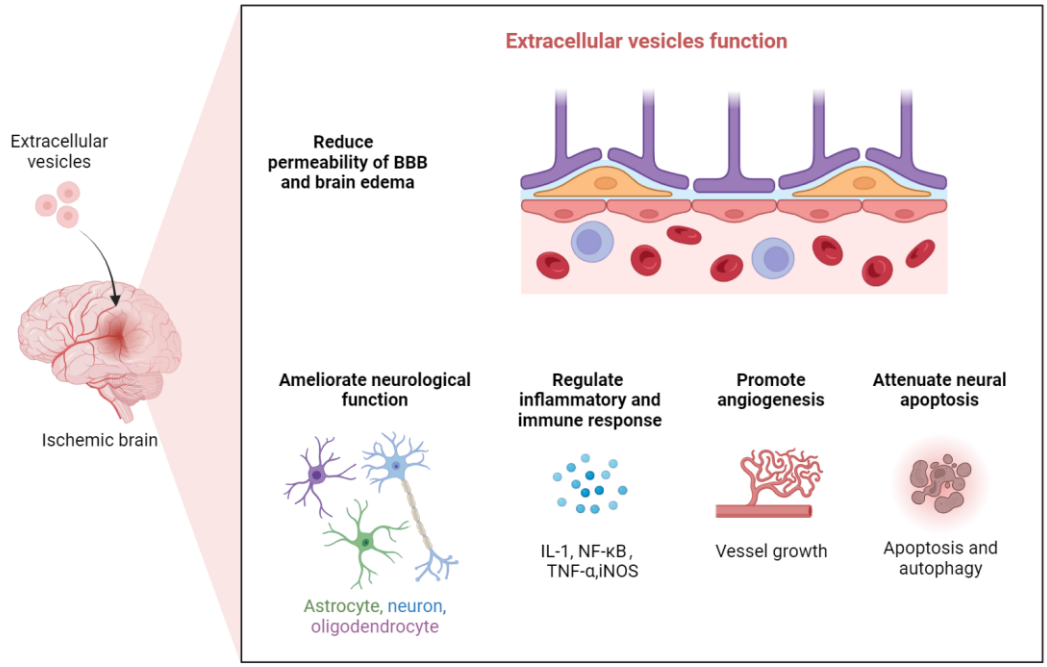

Figure 1. The function of extracellular vesicles in ischemic brain. Extracellular vesicles have several therapeutic functions on ischemic stroke, including ameliorating neurological function, reducing permeability of BBB and brain edema, regulating inflammatory and immune response, attenuating neural apoptosis, adjusting autophagy, and modulating angiogenesis and neurogenesis. Portrayed by Bio Render.

The most important effect of EVs on the pathological progression of cerebral ischemia is the restoration of neurological function and the protection of brain cells. In the mice transient middle cerebral artery occlusion (t-MCAO) model, it is reported that EVs derived from stromal cells, neurons, and astrocytes, respectively, could improve nerve severity scores by reducing the production and activation of ion-calcium-binding receptor molecule (lba1)-positive microglia [67,68]. Additionally, EVs have been shown to have the same effect in large mammalian models. For example, after intravenous injection of EVs from human neural progenitor cells in a pig permanent MCAO model, it was found that the lesion volume was significantly reduced and nerve function was greatly improved [69]. In addition, EVs can play a therapeutic role in the gray matter and cortex and enhance white matter function. In the rat MCAO model, intravenous injection of EVs derived from mesenchymal stem cells can regulate the number of oligodendrocytes and astrocytes, thereby promoting axon and myelin recovery, reducing infarct size, and improving nerve function [70]. Furthermore, a combination of EVs and drugs has been proven to have a more positive effect on is-chemic stroke than using EVs alone. Rosuvastatin via oral administration combined with EVs derived from BMSCs by stereotaxic injection markedly reduced infarct volume and brain edema, and attenuated cell death in ischemic areas poststroke by down-regulating the apoptosis-associated NLRP1/3 gene and countering lipid peroxidation [71].

The second most important effect is associated with reducing blood–brain barrier permeability and cerebral edema. As mentioned above, after ischemic stroke, the structure of the blood–brain barrier will be destroyed, and the permeability of the blood–brain barrier will in-crease, resulting in leukocyte infiltration and brain edema, aggravating brain injury, and neuronal death. Intravenous administration of hypoxia-induced MSC-derived EVs can reduce the infiltration of polymorphonuclear neutrophils in the brain tissue of MCAO mice [72]. In addition, the combination of adipose stem cells and adipose-derived EVs

can reduce the expression of AQP-4 and some oxidative stress molecules, such as MMP-9, TNF-α, and iNOS [73].

The significant response of brain inflammation in ischemic stroke is the activation of microglia and astrocytes and the subsequent upregulation of proinflammatory cytokines and chemokines, such as NF-κB and IL-1. Recently, some studies have found that EVs can inhibit this process. In hypoxic SHSY5Y cell models, IL-6, IL-1β, and TNF-α mRNA levels were significantly decreased after EV treatment of endothelial cells or stem cells [74]. A similar trend was observed in mouse MCAO models when administering EVs derived from astrocytes, bone marrow mesenchymal stem cells, or ADSCs [68,75,76]. In addition, EVs can inhibit the polarization of microglia toward proinflammatory (M1) or promote their anti-inflammatory (M2), polarization, thereby improving neurological outcomes [77].

Cell apoptosis occurs mainly in the penumbra during reperfusion after ischemic stroke and can further aggravate brain injury and nerve function [78]. There are primarily two apoptosis pathways: one is the endogenous pathway that activates caspa-se-3 through mitochondrial dysfunction, and the other is the exogenous pathway mediated by membrane surface apoptosis receptors that subsequently stimulate caspase-8 and ultimately directly or indirectly activate caspase-3, leading to apoptosis [79]. Mesenchymal stem cell-derived EVs can downregulate the expression of Bax and caspase-3 and the levels of IL-6, IL-1β, and TNF-α, and upregulate the expression of B-cell lym-phoma-2 (Bcl-2) and cyclin-dependent kinase 4, cyclin D1, and cyclin E, inhibiting apoptosis of astrocytes and neurons, both in OGD-induced (Oxygen and Glucose Deprivation-induced) astrocytes and in mouse MCAO models, in turn relieving injury after reperfusion [80,81]. Apart from apoptosis, autophagy plays an important role in ischemic stroke as another mechanism for cellular auto-degradation. The level of autophagy mainly depends on autophagy-related protein markers, like immunoglobulin protein (BIP) [82]. EVs derived from endothelial cells attenuated ER stress against apoptosis, regulating the crosstalk of pathways between ER stress-mediated autophagy and apoptosis after ischemic stroke [83].

After ischemia, angiogenesis can restore the metabolism of injured neurons, re-move necrotic debris, increase neuronal remodeling and promote the migration of NSCs, aggravating brain injury [84]. EVs have been shown to interfere with angiogenesis after ischemia. In the model of rat MCAO and OGD-induced endothelial cells, EVs derived from ADSCs can promote angiogenesis by evaluating endothelial cell migration and capillary formation, or by measuring vascular density in the ischemic boundary region with positive expression of endothelial cell markers vWF, the effect of which is related to endothelial microRNAs, such as miR-126 and miR-134, which may represents a novel therapeutic approach for stroke recovery [85]. Furthermore, EVs can also target neurogenesis, which is marked by Ki67 and DCX [86]. In the OGD-induced endothelial cells model, after treatment of intravenous EVs from MSCs, it suggested elevated expressions of Ki67, MOG, myelin basic protein, and synaptophysin in the infarct area of mice with subcortical ischemia, representing the enhancement of neurogenesis [87].

7. Extracellular Vesicles in Hemorrhagic Stroke Treatment

The treatment of cerebral hemorrhage and stroke mainly focuses on removing the hematoma and protecting the surrounding tissue of the hematoma, the latter referring to repairing the damaged nerve [88]. There are two main pathways in which EVs play a role in the treatment of cerebral hemorrhage: (1) direct action: the surface proteins on EVs recognize the receptors of target cells, inducing signal transduction, and thus transmit intercellular information; (2) indirect effect: EVs, loaded with drugs which can treat cerebral hemorrhage, fuse with target cells to deliver the loaded bioactive factors into the cell, interacting with target cell surface receptors and then achieving information transport [89]. As

for the treatment of cerebral ischemia, nerve repair after cerebral hemorrhage comprises five main aspects: anti apoptosis, inflammation reduction, angiogenesis, immunomodulation, and carriers for drug delivery [90].

Neuronal cell damage caused by intracerebral hemorrhage-induced programmed cell-death is an important cause of high mortality after intracerebral hemorrhage [91]. Therefore, anti-apoptosis plays a vital role in alleviating adverse consequences and improving prognosis after intracerebral hemorrhage. Some studies have shown that in mouse models of subarachnoid hemorrhage, bone marrow mesenchymal stem cell-derived EVs are transferred to neurons via miRNA-21, promoting neuron survival and alleviating cognitive impairment after subarachnoid hemorrhage [92]. Meanwhile, miRNA-133b-modified EVs derived from MSCs can reduce neuronal apoptosis after intracerebral hemorrhage by inhibiting RhoA expression and activating ERK1/2/CREB in vitro [93]. Additionally, downregulated miRNA-206-modified EVs derived from umbilical cord MSCs, by way of targeting BDNF, mediate the TrkB/CREB signaling pathway, inhibiting apoptosis and significantly improving nerve function and brain edema, thus preventing early brain injury caused by subarachnoid hemorrhage [94].

Brain hemorrhage can cause widespread neuroinflammation, leading to expanded nerve damage. EVs can deliver miRNA, proteins, or other contents to have an anti-inflammatory effect [95]. Studies have shown that EVs derived from bone marrow mesenchymal stem cells (BMSCs) can not only reduce neuroinflammation in brain tissue after subarachnoid hemorrhage by inhibiting NF-κB and activating the AMPK pathway, acting as neuroprotective agents but also downregulate the expression of IL-1b, CD16, CD11b, and iNOS and upregulating CD206 to control the polarization of microglia toward an M2 phenotype, finally suppressing inflammation [96,97]. Another study from the same researchers found that the same type of EV can also carry miRNA 183-5p into the ICH location of rat brain, inhibiting the NLRP3 pathway by targeting PDCD4, then reducing neuroinflammation after diabetic ICH [98]. In addition, miRNA-193b3p-modified EVs can acetylate NF-κB, inhibiting HDAC3 expression and inflammation [99].

With the occurrence of cerebral hemorrhage, the vascular endothelium is destroyed, and adjacent tissues and blood vessels will also be subjected to ischemic stress, aggravating brain injury [100]. Therefore, angiogenesis plays an important role in hemorrhagic stroke prognosis. It has been reported that BMSC-derived EVs can significantly improve the angiogenesis and neurogenesis function in mice with hemorrhagic stroke, thus promoting the recovery of neurological function [101]. In addition, it has been reported that mouse brain endothelial EVs can significantly increase the axon growth of primary cortical neurons and promote endothelial capillary formation, thereby increasing axon density, myelin density, vessel density, and artery diameter, and finally improving neurocognitive function [102].

As mentioned above, EVs can be loaded with proteins or miRNA and act as mediators of intercellular communication; therefore, they are biocompatible. Transfusion of human mesenchymal stem cell EVs into immunocompetent mouse models of acute myocardial ischemia is therapeutic without significant adverse effects [103]. In addition, it has been found that MSC-EVs loaded with peroxidase can successfully cross the blood–brain barrier and improve the disease state of Parkinson's disease [104]. However, there are no relevant reports on the treatment of cerebral hemorrhage.

8. Pretreatment and Clinical Trials

EV therapy has become increasingly widespread and widely used in models of central nervous system diseases in vivo [105]. Currently, EVs are routinely administered intravenously to treat experimental stroke in models in vivo. However, the ad-ministration results in a large distribution of EVs in peripheral organs (such as the liv-er, spleen, and

kidneys), making EVs less likely to migrate to the brain, clear quickly delaying their effects, and causing organ toxicity [106]. Although they can penetrate the blood–brain barrier, it is difficult for EVs to migrate to the specific injury area after stroke. Some investigators have used arterial, intranasal, and direct intraventricular injections to avoid the drawbacks of intravenous injections. Intra-arterial EV injection can reduce neuroinflammation caused by focal brain injury [107]. Furthermore, direct intraventricular administration of EVs can effectively reduce microglial density and neuronal apoptosis, thereby steadily improving functional recovery in MCAO [67]. In addition, researchers have found that in the treatment of Batten's disease, intranasal administration can better reduce astrocyte proliferation and promote neuronal recovery [108]. A study of BMSCs showed that in the rat model of MCAO, the intra-arterial pathway showed the greatest degree and speed of neural function recovery and could improve neural function more effectively [109]. However, it has not been determined which route of administration can maximize the therapeutic effect and reduce adverse reactions.

Because of the small size and low density of EVs, they usually coexist with various particles with similar properties, resulting in a difficult separation. Several standard separation methods are used, but it remains difficult to purify the EVs. However, pre-paring exosomes using these methods usually requires longer processing times and specialized equipment.

Currently, the main technologies to protect stored EVs are freezing, freeze drying, and spray-drying [110]. The most frequently used technology, cryogenic storage, may change EV shape and physical characteristics. Repeated freeze–thaw cycles may result in changes in the biological characteristics, content, and marker composition of the EV surface molecules. However, a freeze-drying method can be used to preserve EVs using trehalose as a protective agent. Trehalose has the cryoprotective effect of stabilizing protein, cell membrane, and liposomes, preventing the aggregation of proteins and EVs, reducing ice formation during freezing, and reducing the loss of extracellular vesicles during separation and preservation [111]. Even so, more research is needed to optimize these methods and explore other approaches.

To our knowledge, there are some clinical and preclinical trials on stroke patients (shown in Tables 2 and 3). However, a large number of clinical trials are still needed to confirm the efficacy and safety of EVs.

Table 2. List of clinical trials on clinicaltrials.gov investigating the application of EVs for stroke.

NCT Number	Sources	Country	Conditions
NCT05370105	Circulating EVs in the serum	Italy	Stroke
NCT05524506	Microparticles	Sweden	TIA and ischemic stroke
NCT04266639	RBC-derived EVs	Denmark	Acute ischemic stroke
NCT05645081	endothelial derived EVs	UK	TIA
NCT06612710	EVs derived from neural stem cell	China	Ischemic stroke

Table 3. List of part of preclinical trials in recent five years investigating EVs for stroke in vivo.

Animal Model	Stroke Model	Source of EV	Methods	Key Results	Ref.
65 healthy male SD rats (8 weeks)	OGD	mesenchymal stem cells and brain endothelial cells	Ultracentrifuge	BMSC-EVs exerted similar antagonistic efficacy to BEC-EVs on Cav-1-dependent ZO-1 and Claudin-5 endocytosis.	[112]
448 adult male C57BL/6J mice (8-10 weeks)	MCAO	circSCMH1	Centrifuge	circSCMH1-EVs afford protection in the rodent and the nonhuman primate ischemic stroke models.	[113]

Table 3. *Cont.*

Animal Model	Stroke Model	Source of EV	Methods	Key Results	Ref.
124 young male C57BL/6 mice (8-12 weeks)	OGD	brain endothelial cells	Ultracentrifuge	(PEG-DET/HSP27)/EV and EV/HSP27 mixtures increase BEC metabolic function and tight junction integrity.	[114]
122 ICR male mice (8-10 weeks)	MCAO	microglial BV2 cells	Ultracentrifuge	M2 microglia could communicate to OPCs through M2-EVs and promote white matter repair via miR-23a-5p after ischemic stroke.	[115]
36 male SD rat (250-300g)	MCAO/R	Que/mAb GAP43-Exo	Surface modification	Que/mAb GAP43-Exo can improve survival of neurons by inhibiting ROS production.	[116]
56 male SD rat (10 weeks)	MCAO	RBP-Exo *	Transfection engineered	RBP-Exo was a hypoxia-specific carrier for nose-to-brain delivery of AMO181a-chol in an ischemic stroke model.	[117]
60 male SD rat (8 weeks)	MCAO	BDNF-hNSC-Exo **	Coculture	BDNF-hNSC-Exo inhibited the activation of microglia and promoted the differentiation of endogenous NSCs.	[118]
45 male SD rat (8 weeks)	tMCAO	EXO-Hep ***	Ultrasonic	EXO-Hep mitigated ischemic injury in a model of tMCAO.	[119]
60 adult male C57BL/6 mice (8–10 weeks)	OGD/R	OGD/R-ADEXs	Centrifuge	Nampt released by OGD/R-ADEXs ameliorated acute ischemic stroke during neuronal injury to induce autophagy.	[120]
45 rat (8 weeks)	OGD	astrocyte-derived EVs	Ultracentrifuge	miR-29a in astrocyte-derived EVs inhibits BIRI by downregulating TP53INP1 and the NF-κB/NLRP3 axis.	[121]

* RBP: RAGE-binding-peptide; ** hNSC-Exo: human neural stem cell-derived exosomes; *** EXO-Hep: heptapeptide-loaded macrophage-derived exosomes.

9. Conclusions

The uses of EVs for diagnostic, prognostic, and therapeutic purposes, and as drug delivery tools have been well demonstrated and continue to be a subject of intense study based on the increasing number of articles on the topic. In ischemic stroke, EVs have the function to ameliorate neurological function, reduce the permeability of BBB and brain edema, regulate inflammatory and immune response, attenuate neural apoptosis and adjust autophagy, and modulate angiogenesis and neurogenesis. As for hemorrhage stroke, there're direct effects of EVs on stroke: anti-apoptosis, reducing inflammation, angiogenesis and immunomodulation. Additionally, it has become a trending issue to make EVs carriers for drug delivery. However, treatment with extracellular vesicles is still a nascent therapy. Many aspects remain to be resolved before being translated to the clinic as a typical practice, such as the time of administration, the most effective route, and a dose–response study. More studies are needed to evaluate the long-term biological safety, possible adverse effects, and efficacy of exosome administration in patients with stroke.

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References

1. Steinmetz, J.D.; Seeher, K.M.; Schiess, N.; Nichols, E.; Cao, B.; Servili, C.; Cavallera, V.; Cousin, E.; Hagins, H.; E Moberg, M.; et al. Global, regional, and national burden of disorders affecting the nervous system, 1990-2021: A systematic analysis for the Global Burden of Disease Study 2021. *Lancet Neurol.* **2024**, *23*, 344–381. [CrossRef]
2. Wassélius, J.; Arnberg, F.; von Euler, M.; Wester, P.; Ullberg, T. Endovascular thrombectomy for acute ischemic stroke. *J. Intern. Med.* **2022**, *291*, 303–316. [CrossRef] [PubMed]
3. Goyal, M.; Yu, A.Y.; Menon, B.K.; Dippel, D.W.; Hacke, W.; Davis, S.M.; Fisher, M.; Yavagal, D.R.; Turjman, F.; Ross, J.; et al. Endovascular Therapy in Acute Ischemic Stroke: Challenges and Transition From Trials to Bedside. *Stroke* **2016**, *47*, 548–553. [CrossRef] [PubMed]
4. Kase, C.S.; Furlan, A.J.; Wechsler, L.R.; Higashida, R.T.; Rowley, H.A.; Hart, R.G.; Molinari, G.F.; Frederick, L.S.; Roberts, H.C.; Gebel, J.M.; et al. Cerebral hemorrhage after intra-arterial thrombolysis for ischemic stroke: The PROACT II trial. *Neurology* **2001**, *57*, 1603–1610. [CrossRef]
5. Bath, P.M.; Woodhouse, L.J.; Krishnan, K.; Appleton, J.P.; Anderson, C.S.; Berge, E.; Cala, L.; Dixon, M.; England, T.J.; Godolphin, P.J.; et al. Prehospital Transdermal Glyceryl Trinitrate for Ultra-Acute Intracerebral Hemorrhage: Data From the RIGHT-2 Trial. *Stroke* **2019**, *50*, 3064–3071. [CrossRef]
6. Qin, C.; Yang, S.; Chu, Y.H.; Zhang, H.; Pang, X.W.; Chen, L.; Zhou, L.Q.; Chen, M.; Tian, D.S.; Wang, W. Signaling pathways involved in ischemic stroke: Molecular mechanisms and therapeutic interventions. *Signal Transduct. Target. Ther.* **2022**, *7*, 215. [CrossRef]
7. Orellana-Urzúa, S.; Rojas, I.; Líbano, L.; Rodrigo, R. Pathophysiology of Ischemic Stroke: Role of Oxidative Stress. *Curr. Pharm. Des.* **2020**, *26*, 4246–4260. [CrossRef]
8. Paoletti, P.; Bellone, C.; Zhou, Q. NMDA receptor subunit diversity: Impact on receptor properties, synaptic plasticity and disease. *Nat. Rev. Neurosci.* **2013**, *14*, 383–400. [CrossRef]
9. Lai, T.W.; Zhang, S.; Wang, Y.T. Excitotoxicity and stroke: Identifying novel targets for neuroprotection. *Prog. Neurobiol.* **2014**, *115*, 157–188. [CrossRef]
10. Stokum, J.A.; Gerzanich, V.; Simard, J.M. Molecular pathophysiology of cerebral edema. *J. Cereb. Blood Flow. Metab.* **2016**, *36*, 513–538. [CrossRef]
11. Jurcau, A.; Simion, A. Neuroinflammation in Cerebral Ischemia and Ischemia/Reperfusion Injuries: From Pathophysiology to Therapeutic Strategies. *Int. J. Mol. Sci.* **2021**, *23*, 14. [CrossRef] [PubMed]
12. Tuo, Q.Z.; Zhang, S.T.; Lei, P. Mechanisms of neuronal cell death in ischemic stroke and their therapeutic implications. *Med. Res. Rev.* **2022**, *42*, 259–305. [CrossRef]
13. Moskowitz, M.A.; Lo, E.H.; Iadecola, C. The science of stroke: Mechanisms in search of treatments. *Neuron* **2010**, *67*, 181–198. [CrossRef]
14. Barthels, D.; Das, H. Current advances in ischemic stroke research and therapies. *Biochim. Biophys. Acta Mol. Basis Dis.* **2020**, *1866*, 165260. [CrossRef]
15. Puy, L.; Parry-Jones, A.R.; Sandset, E.C.; Dowlatshahi, D.; Ziai, W.; Cordonnier, C. Intracerebral haemorrhage. *Nat. Rev. Dis. Primers* **2023**, *9*, 14. [CrossRef]
16. Shi, K.; Tian, D.C.; Li, Z.G.; Ducruet, A.F.; Lawton, M.T.; Shi, F.D. Global brain inflammation in stroke. *Lancet Neurol.* **2019**, *18*, 1058–1066. [CrossRef]
17. Au, N.P.B.; Ma, C.H.E. Neuroinflammation, Microglia and Implications for Retinal Ganglion Cell Survival and Axon Regeneration in Traumatic Optic Neuropathy. *Front. Immunol.* **2022**, *13*, 860070. [CrossRef]
18. Umlauf, B.J.; Shusta, E.V. Exploiting BBB disruption for the delivery of nanocarriers to the diseased CNS. *Curr. Opin. Biotechnol.* **2019**, *60*, 146–152. [CrossRef]
19. Stokum, J.A.; Shim, B.; Negoita, S.; Tsymbalyuk, N.; Tsymbalyuk, O.; Ivanova, S.; Keledjian, K.; Bryan, J.; Blaustein, M.P.; Jha, R.M.; et al. Cation flux through SUR1-TRPM4 and NCX1 in astrocyte endfeet induces water influx through AQP4 and brain swelling after ischemic stroke. *Sci. Signal* **2023**, *16*, eadd6364. [CrossRef]
20. Magid-Bernstein, J.; Girard, R.; Polster, S.; Srinath, A.; Romanos, S.; Awad, I.A.; Sansing, L.H. Cerebral Hemorrhage: Pathophysiology, Treatment, and Future Directions. *Circ. Res.* **2022**, *130*, 1204–1229. [CrossRef]
21. Doyle, L.M.; Wang, M.Z. Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. *Cells* **2019**, *8*, 727. [CrossRef] [PubMed]
22. Jeppesen, D.K.; Zhang, Q.; Franklin, J.L.; Coffey, R.J. Extracellular vesicles and nanoparticles: Emerging complexities. *Trends Cell Biol.* **2023**, *33*, 667–681. [CrossRef] [PubMed]
23. Abels, E.R.; Breakefield, X.O. Introduction to Extracellular Vesicles: Biogenesis, RNA Cargo Selection, Content, Release, and Uptake. *Cell Mol. Neurobiol.* **2016**, *36*, 301–312. [CrossRef] [PubMed]
24. Huotari, J.; Helenius, A. Endosome maturation. *EMBO J.* **2011**, *30*, 3481–3500. [CrossRef]

25. Cun, Y.; Jin, Y.; Wu, D.; Zhou, L.; Zhang, C.; Zhang, S.; Yang, X.; Zuhong, W.; Zhang, P. Exosome in Crosstalk between Inflammation and Angiogenesis: A Potential Therapeutic Strategy for Stroke. *Mediators Inflamm.* **2022**, *2022*, 7006281. [CrossRef]
26. Sullivan, J.A.; Schoch, K.; Spillmann, R.C.; Shashi, V. Exome/Genome Sequencing in Undiagnosed Syndromes. *Annu Rev Med* **2023**, *74*, 489–502. [CrossRef]
27. Zhang, Q.; Jeppesen, D.K.; Higginbotham, J.N.; Graves-Deal, R.; Trinh, V.Q.; Ramirez, M.A.; Sohn, Y.; Neininger, A.C.; Taneja, N.; McKinley, E.T.; et al. Supermeres are functional extracellular nanoparticles replete with disease biomarkers and therapeutic targets. *Nat. Cell Biol.* **2021**, *23*, 1240–1254. [CrossRef]
28. Hagen, C.; Dent, K.C.; Zeev-Ben-Mordehai, T.; Grange, M.; Bosse, J.B.; Whittle, C.; Klupp, B.G.; Siebert, C.A.; Vasishtan, D.; Bäuerlein, F.J.; et al. Structural Basis of Vesicle Formation at the Inner Nuclear Membrane. *Cell* **2015**, *163*, 1692–1701. [CrossRef]
29. Zhang, X.; Xu, Q.; Zi, Z.; Liu, Z.; Wan, C.; Crisman, L.; Shen, J.; Liu, X. Programmable Extracellular Vesicles for Macromolecule Delivery and Genome Modifications. *Dev. Cell* **2020**, *55*, 784–801.e789. [CrossRef]
30. Ollen-Bittle, N.; Roseborough, A.D.; Wang, W.; Wu, J.D.; Whitehead, S.N. Mechanisms and Biomarker Potential of Extracellular Vesicles in Stroke. *Biology* **2022**, *11*, 1231. [CrossRef]
31. Dixson, A.C.; Dawson, T.R.; Di Vizio, D.; Weaver, A.M. Context-specific regulation of extracellular vesicle biogenesis and ca rgo selection. *Nat. Rev. Mol. Cell Biol.* **2023**, *24*, 454–476. [PubMed]
32. Kamps, J.A.; Morselt, H.W.; Scherphof, G.L. Uptake of Liposomes Containing Phosphatidylserine by Liver Cells In Vivo and by Sinusoidal Liver Cells in Primary Culture:In Vivo–In Vitro Differences. *Biochem. Biophys. Res. Commun.* **1999**, *256*, 57–62. [CrossRef]
33. Park, S.J.; Kim, J.M.; Kim, J.; Hur, J.; Park, S.; Kim, K.; Shin, H.J.; Chwae, Y.J. Molecular mechanisms of biogenesis of apoptotic exosome-like vesicles and their roles as damage-associated molecular patterns. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E11721–E11730. [PubMed]
34. Brenna, S.; Altmeppen, H.C.; Mohammadi, B.; Rissiek, B.; Schlink, F.; Ludewig, P.; Krisp, C.; Schluter, H.; Failla, A.V.; Schneider, C.; et al. Characterization of brain-derived extracellular vesicles reveals changes in cellular origin after stroke and enrichment of the prion protein with a potential role in cellular uptake. *J. Extracell. Vesicles* **2020**, *9*, 1809065. [CrossRef] [PubMed]
35. Livshits, M.A.; Khomyakova, E.; Evtushenko, E.G.; Lazarev, V.N.; Kulemin, N.A.; Semina, S.E.; Generozov, E.V.; Govorun, V.M. Isolation of exosomes by differential centrifugation: Theoretical analysis of a commonly used protocol. *Sci. Rep.* **2015**, *5*, 17319. [CrossRef]
36. Cvjetkovic, A.; Lötvall, J.; Lässer, C. The influence of rotor type and centrifugation time on the yield and purity of extracellular vesicles. *J. Extracell. Vesicles* **2014**, *3*, 23111. [CrossRef]
37. Théry, C.; Amigorena, S.; Raposo, G.; Clayton, A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr. Protoc. Cell Biol.* **2006**, *30*, 3–22. [CrossRef]
38. Fitzgerald, J.; Leonard, P.; Darcy, E.; Sharma, S.; O'Kennedy, R. Immunoaffinity Chromatography: Concepts and Applications. *Methods Mol. Biol.* **2017**, *1485*, 27–51. [CrossRef]
39. Veerman, R.E.; Teeuwen, L.; Czarnewski, P.; Akpinar, G.G.; Sandberg, A.; Cao, X.; Pernemalm, M.; Orre, L.M.; Gabrielsson, S.; Eldh, M. Molecular evaluation of five different isolation methods for extracellular vesicles reveals different clinical applicability and subcellular origin. *J. Extracell. Vesicles* **2021**, *10*, e12128. [CrossRef]
40. D'Acunzo, P.; Kim, Y.; Ungania, J.M.; Pérez-González, R.; Goulbourne, C.N.; Levy, E. Isolation of mitochondria-derived mitovesicles and subpopulations of m icrovesicles and exosomes from brain tissues. *Nat. Protoc.* **2022**, *17*, 2517–2549. [CrossRef]
41. Oh, D.K.; Hyun, C.K.; Kim, J.H.; Park, Y.H. Production of penicillin in a fluidized-bed bioreactor: Control of cell growth and penicillin production by phosphate limitation. *Biotechnol. Bioeng.* **1988**, *32*, 569–573. [CrossRef] [PubMed]
42. Hemler, M.E. Tetraspanin functions and associated microdomains. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 801–811. [CrossRef] [PubMed]
43. Buzás, E.I.; Tóth, E.Á.; Sódar, B.W.; Szabó-Taylor, K.É. Molecular interactions at the surface of extracellular vesicles. *Semin. Immunopathol.* **2018**, *40*, 453–464. [CrossRef] [PubMed]
44. Rai, A.; Fang, H.; Claridge, B.; Simpson, R.J.; Greening, D.W. Proteomic dissection of large extracellular vesicle surfaceome unravels interactive surface platform. *J. Extracell. Vesicles* **2021**, *10*, e12164. [CrossRef]
45. Gong, J.; Körner, R.; Gaitanos, L.; Klein, R. Exosomes mediate cell contact–independent ephrin-Eph signaling during axon guidance. *J. Cell Biol.* **2016**, *214*, 35–44. [CrossRef]
46. Hermann, D.M.; Xin, W.; Bähr, M.; Giebel, B.; Doeppner, T.R. Emerging roles of extracellular vesicle-associated non-coding RNAs in hypoxia: Insights from cancer, myocardial infarction and ischemic stroke. *Theranostics* **2022**, *12*, 5776–5802. [CrossRef]
47. Miyanishi, M.; Tada, K.; Koike, M.; Uchiyama, Y.; Kitamura, T.; Nagata, S. Identification of Tim4 as a phosphatidylserine receptor. *Nature* **2007**, *450*, 435–439. [CrossRef]
48. Yusuf, A.M.; Hagemann, N.; Zhang, X.; Zafar, M.; Hussner, T.; Bromkamp, C.; Martiny, C.; Tertel, T.; Börger, V.; Schumacher, F.; et al. Acid sphingomyelinase deactivation post-ischemia promotes brain angiogenesis and remodeling by small extracellular vesicles. *Basic Res. Cardiol.* **2022**, *117*, 1–22. [CrossRef]

49. Konoshenko, M.Y.; Lekchnov, E.A.; Vlassov, A.V.; Laktionov, P.P. Isolation of Extracellular Vesicles: General Methodologies and Latest Trends. *BioMed Res. Int.* **2018**, *2018*, 8545347. [CrossRef]
50. Zhu, Y.-G.; Feng, X.-M.; Abbott, J.; Fang, X.-H.; Hao, Q.; Monsel, A.; Qu, J.-M.; Matthay, M.A.; Lee, J.W. Human Mesenchymal Stem Cell Microvesicles for Treatment of *Escherichia coli* Endotoxin-Induced Acute Lung Injury in Mice. *Stem Cells* **2014**, *32*, 116–125. [CrossRef]
51. Lai, R.C.; Arslan, F.; Lee, M.M.; Sze, N.S.K.; Choo, A.; Chen, T.S.; Salto-Tellez, M.; Timmers, L.; Lee, C.N.; El Oakley, R.M.; et al. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem Cell Res.* **2010**, *4*, 214–222. [CrossRef] [PubMed]
52. Xin, H.; Li, Y.; Cui, Y.; Yang, J.J.; Zhang, Z.G.; Chopp, M. Systemic Administration of Exosomes Released from Mesenchymal Stromal Cells Promote Functional Recovery and Neurovascular Plasticity After Stroke in Rats. *J. Cereb. Blood Flow Metab.* **2013**, *33*, 1711–1715. [CrossRef] [PubMed]
53. An, K.; Klyubin, I.; Kim, Y.; Jung, J.H.; Mably, A.J.; O'Dowd, S.T.; Lynch, T.; Kanmert, D.; Lemere, C.A.; Finan, G.M.; et al. Exosomes neutralize synaptic-plasticity-disrupting activity of Aβ assemblies in vivo. *Mol. Brain* **2013**, *6*, 47. [CrossRef] [PubMed]
54. Zhang, Y.; Chopp, M.; Meng, Y.; Katakowski, M.; Xin, H.; Mahmood, A.; Xiong, Y. Effect of exosomes derived from multipluripotent mesenchymal stromal cells on functional recovery and neurovascular plasticity in rats after traumatic brain injury. *J. Neurosurg.* **2015**, *122*, 856–867. [CrossRef]
55. Chen, C.C.; Liu, L.; Ma, F.; Wong, C.W.; Guo, X.E.; Chacko, J.V.; Farhoodi, H.P.; Zhang, S.X.; Zimak, J.; Ségaliny, A.; et al. Elucidation of Exosome Migration Across the Blood–Brain Barrier Model In Vitro. *Cell. Mol. Bioeng.* **2016**, *9*, 509–529. [CrossRef]
56. García-Romero, N.; Carrión-Navarro, J.; Esteban-Rubio, S.; Lázaro-Ibáñez, E.; Peris-Celda, M.; Alonso, M.M.; Guzmán-De-Villoria, J.; Fernández-Carballal, C.; de Mendivil, A.O.; García-Duque, S.; et al. DNA sequences within glioma-derived extracellular vesicles can cross the intact blood-brain barrier and be detected in peripheral blood of patients. *Oncotarget* **2016**, *8*, 1416–1428. [CrossRef]
57. Rufino-Ramos, D.; Albuquerque, P.R.; Carmona, V.; Perfeito, R.; Nobre, R.J.; de Almeida, L.P. Extracellular vesicles: Novel promising delivery systems for therapy of brain diseases. *J. Control. Release* **2017**, *262*, 247–258. [CrossRef]
58. McCluskey, G.; Morrison, K.E.; Donaghy, C.; Rene, F.; Duddy, W.; Duguez, S. Extracellular Vesicles in Amyotrophic Lateral Sclerosis. *Life* **2022**, *13*, 121. [CrossRef]
59. Canseco-Rodriguez, A.; Masola, V.; Aliperti, V.; Meseguer-Beltran, M.; Donizetti, A.; Sanchez-Perez, A.M. Long Non-Coding RNAs, Extracellular Vesicles and Inflammation in Alzheimer's Disease. *Int. J. Mol. Sci.* **2022**, *23*, 13171. [CrossRef]
60. He, Z.; Tang, Y.; Qin, C. Increased circulating leukocyte-derived microparticles in ischemic cerebrovascular disease. *Thromb. Res.* **2017**, *154*, 19–25. [CrossRef]
61. Simak, J.; Gelderman, M.P.; Yu, H.; Wright, V.; Baird, A.E. Circulating endothelial microparticles in acute ischemic stroke: A link to severity, lesion volume and outcome. *J. Thromb. Haemost.* **2006**, *4*, 1296–1302. [CrossRef] [PubMed]
62. Jung, K.; Chu, K.; Lee, S.; Park, H.; Bahn, J.; Kim, D.; Kim, J.; Kim, M.; Lee, S.K.; Roh, J. Circulating endothelial microparticles as a marker of cerebrovascular disease. *Ann. Neurol.* **2009**, *66*, 191–199. [CrossRef] [PubMed]
63. Chiva-Blanch, G.; Suades, R.; Crespo, J.; Peña, E.; Padró, T.; Jiménez-Xarrié, E.; Martí-Fàbregas, J.; Badimon, L. Microparticle Shedding from Neural Progenitor Cells and Vascular Compartment Cells Is Increased in Ischemic Stroke. *PLoS ONE* **2016**, *11*, e0148176. [CrossRef]
64. Lackner, P.; Dietmann, A.; Beer, R.; Fischer, M.; Broessner, G.; Helbok, R.; Marxgut, J.; Pfausler, B.; Schmutzhard, E. Cellular Microparticles as a Marker for Cerebral Vasospasm in Spontaneous Subarachnoid Hemorrhage. *Stroke* **2010**, *41*, 2353–2357. [CrossRef]
65. Sanborn, M.R.; Thom, S.R.; Bohman, L.-E.; Stein, S.C.; Levine, J.M.; Milovanova, T.; Maloney-Wilensky, E.; Frangos, S.; Kumar, M.A. Temporal dynamics of microparticle elevation following subarachnoid hemorrhage. *J. Neurosurg.* **2012**, *117*, 579–586. [CrossRef]
66. Li, Y.; Liu, B.; Chen, Y.; Quan, X.; Han, Y.; Zheng, Y.; Zhao, Y. Extracellular Vesicle Application as a Novel Therapeutic Strategy for Ischemic Stroke. *Transl. Stroke Res.* **2021**, *13*, 171–187. [CrossRef]
67. Mahdavipour, M.; Hassanzadeh, G.; Seifali, E.; Mortezaee, K.; Aligholi, H.; Shekari, F.; Sarkoohi, P.; Zeraatpisheh, Z.; Nazari, A.; Movassaghi, S.; et al. Effects of neural stem cell-derived extracellular vesicles on neuronal protection and functional recovery in the rat model of middle cerebral artery occlusion. *Cell Biochem. Funct.* **2019**, *38*, 373–383. [CrossRef]
68. Geng, W.; Tang, H.; Luo, S.; Lv, Y.; Liang, D.; Kang, X.; Hong, W. Exosomes from miRNA-126-modified ADSCs promotes functional recovery after stroke in rats by improving neurogenesis and suppressing microglia activation. *Am. J. Transl. Res.* **2019**, *11*, 780–792.
69. Webb, R.L.; Kaiser, E.E.; Jurgielewicz, B.J.; Spellicy, S.; Scoville, S.L.; Thompson, T.A.; Swetenburg, R.L.; Hess, D.C.; West, F.D.; Stice, S.L. Human Neural Stem Cell Extracellular Vesicles Improve Recovery in a Porcine Model of Ischemic Stroke. *Stroke* **2018**, *49*, 1248–1256. [CrossRef]

70. Otero-Ortega, L.; Laso-García, F.; Gómez-de Frutos, M.C.; Diekhorst, L.; Martínez-Arroyo, A.; Alonso-López, E.; García-Bermejo, M.L.; Rodríguez-Serrano, M.; Arrúe-Gonzalo, M.; Díez-Tejedor, E.; et al. Low dose of extracellular vesicles identified that promote recovery after ischemic stroke. *Stem Cell Res. Ther.* **2020**, *11*, 70. [CrossRef]
71. Safakheil, M.; Safakheil, H. The Effect of Exosomes Derived from Bone Marrow Stem Cells in Combination with Rosuvastatin on Functional Recovery and Neuroprotection in Rats After Ischemic Stroke. *J. Mol. Neurosci.* **2020**, *70*, 724–737. [CrossRef] [PubMed]
72. Wang, C.; Börger, V.; Sardari, M.; Murke, F.; Skuljec, J.; Pul, R.; Hagemann, N.; Dzyubenko, E.; Dittrich, R.; Gregorius, J.; et al. Mesenchymal Stromal Cell–Derived Small Extracellular Vesicles Induce Ischemic Neuroprotection by Modulating Leukocytes and Specifically Neutrophils. *Stroke* **2020**, *51*, 1825–1834. [CrossRef]
73. Chen, K.-H.; Chen, C.-H.; Wallace, C.G.; Yuen, C.-M.; Kao, G.-S.; Chen, Y.-L.; Shao, P.-L.; Chen, Y.-L.; Chai, H.-T.; Lin, K.-C.; et al. Intravenous administration of xenogenic adipose-derived mesenchymal stem cells (ADMSC) and ADMSC-derived exosomes markedly reduced brain infarct volume and preserved neurological function in rat after acute ischemic stroke. *Oncotarget* **2016**, *7*, 74537–74556. [CrossRef]
74. Yu, Y.; Zhou, H.; Xiong, Y.; Liu, J. Exosomal miR-199a-5p derived from endothelial cells attenuates apoptosis and inflammation in neural cells by inhibiting endoplasmic reticulum stress. *Brain Res.* **2019**, *1726*, 146515. [CrossRef]
75. Pei, X.; Li, Y.; Zhu, L.; Zhou, Z. Astrocyte-derived exosomes suppress autophagy and ameliorate neuronal damage in experimental ischemic stroke. *Exp. Cell Res.* **2019**, *382*, 111474. [CrossRef]
76. Deng, Y.; Chen, D.; Gao, F.; Lv, H.; Zhang, G.; Sun, X.; Liu, L.; Mo, D.; Ma, N.; Song, L.; et al. Exosomes derived from microRNA-138-5p-overexpressing bone marrow-derived mesenchymal stem cells confer neuroprotection to astrocytes following ischemic stroke via inhibition of LCN2. *J. Biol. Eng.* **2019**, *13*, 1–18. [CrossRef]
77. Zheng, Y.; He, R.; Wang, P.; Shi, Y.; Zhao, L.; Liang, J. Exosomes from LPS-stimulated macrophages induce neuroprotection and functional improvement after ischemic stroke by modulating microglial polarization. *Biomater. Sci.* **2019**, *7*, 2037–2049. [CrossRef]
78. Brouns, R.; De Deyn, P. The complexity of neurobiological processes in acute ischemic stroke. *Clin. Neurol. Neurosurg.* **2009**, *111*, 483–495. [CrossRef]
79. Radak, D.; Katsiki, N.; Resanovic, I.; Jovanovic, A.; Sudar-Milovanovic, E.; Zafirovic, S.; Mousad, S.A.; Isenovic, E.R. Apoptosis and Acute Brain Ischemia in Ischemic Stroke. *Curr. Vasc. Pharmacol.* **2017**, *15*, 115–122. [CrossRef]
80. Jakubec, M.; Maple-Grødem, J.; Akbari, S.; Nesse, S.; Halskau, Ø.; Mork-Jansson, A.E. Plasma-derived exosome-like vesicles are enriched in lyso-phospholipids and pass the blood-brain barrier. *PLoS ONE* **2020**, *15*, e0232442. [CrossRef] [PubMed]
81. Kahl, A.; Blanco, I.; Jackman, K.; Baskar, J.; Mohan, H.M.; Rodney-Sandy, R.; Zhang, S.; Iadecola, C.; Hochrainer, K. Cerebral ischemia induces the aggregation of proteins linked to neurodegenerative diseases. *Sci. Rep.* **2018**, *8*, 2701. [CrossRef]
82. Ajoolabady, A.; Wang, S.; Kroemer, G.; Penninger, J.M.; Uversky, V.N.; Pratico, D.; Henninger, N.; Reiter, R.J.; Bruno, A.; Joshipura, K.; et al. Targeting autophagy in ischemic stroke: From molecular mechanisms to clinical therapeutics. *Pharmacol. Ther.* **2021**, *225*, 107848. [CrossRef] [PubMed]
83. Song, S.; Tan, J.; Miao, Y.; Zhang, Q. Crosstalk of ER stress-mediated autophagy and ER-phagy: Involvement of UPR and the core autophagy machinery. *J. Cell. Physiol.* **2017**, *233*, 3867–3874. [CrossRef] [PubMed]
84. Ergul, A.; Alhusban, A.; Fagan, S.C. Angiogenesis: A harmonized target for recovery after stroke. *Stroke* **2012**, *43*, 2270–2274.
85. Yang, Y.; Cai, Y.; Zhang, Y.; Liu, J.; Xu, Z. Exosomes Secreted by Adipose-Derived Stem Cells Contribute to Angiogenesis of Brain Microvascular Endothelial Cells Following Oxygen–Glucose Deprivation In Vitro Through MicroRNA-181b/TRPM7 Axis. *J. Mol. Neurosci.* **2018**, *65*, 74–83. [CrossRef]
86. Bond, A.M.; Ming, G.-L.; Song, H. Adult Mammalian Neural Stem Cells and Neurogenesis: Five Decades Later. *Cell Stem Cell* **2015**, *17*, 385–395. [CrossRef]
87. Bang, O.Y.; Kim, E.H. Mesenchymal Stem Cell-Derived Extracellular Vesicle Therapy for Stroke: Challenges and Progress. *Front. Neurol.* **2019**, *10*, 211. [CrossRef]
88. Greenberg, S.M.; Ziai, W.C.; Cordonnier, C.; Dowlatshahi, D.; Francis, B.; Goldstein, J.N.; Hemphill, J.C.; Johnson, R.; Keigher, K.M.; Mack, W.J.; et al. 2022 Guideline for the Management of Patients With Spontaneous Intracerebral Hemorrhage: A Guideline From the American Heart Association/American Stroke Association. *Stroke* **2022**, *53*, E282–E361. [CrossRef]
89. Schlunk, F.; Greenberg, S.M. The Pathophysiology of Intracerebral Hemorrhage Formation and Expansion. *Transl. Stroke Res.* **2015**, *6*, 257–263. [CrossRef]
90. Zou, Y.; Liao, L.; Dai, J.; Mazhar, M.; Yang, G.; Wang, H.; Dechsupa, N.; Wang, L. Mesenchymal stem cell-derived extracellular vesicles/exosome: A promising therapeutic strategy for intracerebral hemorrhage. *Regen. Ther.* **2023**, *22*, 181–190. [CrossRef]
91. Zhang, L.; Wei, W.; Ai, X.; Kilic, E.; Hermann, D.M.; Venkataramani, V.; Bähr, M.; Doeppner, T.R. Extracellular vesicles from hypoxia-preconditioned microglia promote angiogenesis and repress apoptosis in stroke mice via the TGF-β/Smad2/3 pathway. *Cell Death Dis.* **2021**, *12*, 1068. [CrossRef] [PubMed]
92. Gao, X.; Xiong, Y.; Li, Q.; Han, M.; Shan, D.; Yang, G.; Zhang, S.; Xin, D.; Zhao, R.; Wang, Z.; et al. Extracellular vesicle-mediated transfer of miR-21-5p from mesenchymal stromal cells to neurons alleviates early brain injury to improve cognitive function via the PTEN/Akt pathway after subarachnoid hemorrhage. *Cell Death Dis.* **2020**, *11*, 1–16. [CrossRef] [PubMed]

93. Shen, H.; Yao, X.; Li, H.; Li, X.; Zhang, T.; Sun, Q.; Ji, C.; Chen, G. Role of Exosomes Derived from miR-133b Modified MSCs in an Experimental Rat Model of Intracerebral Hemorrhage. *J. Mol. Neurosci.* **2018**, *64*, 421–430. [CrossRef]
94. Zhao, H.; Li, Y.; Chen, L.; Shen, C.; Xiao, Z.; Xu, R.; Wang, J.; Luo, Y. HucMSCs-Derived miR-206-Knockdown Exosomes Contribute to Neuroprotection in Subarachnoid Hemorrhage Induced Early Brain Injury by Targeting BDNF. *Neuroscience* **2019**, *417*, 11–23. [CrossRef]
95. Harrell, C.R.; Jovicic, N.; Djonov, V.; Arsenijevic, N.; Volarevic, V. Mesenchymal Stem Cell-Derived Exosomes and Other Extracellular Vesicles as New Remedies in the Therapy of Inflammatory Diseases. *Cells* **2019**, *8*, 1605. [CrossRef]
96. Han, M.; Cao, Y.; Guo, X.; Chu, X.; Li, T.; Xue, H.; Xin, D.; Yuan, L.; Ke, H.; Li, G.; et al. Mesenchymal stem cell-derived extracellular vesicles promote microglial M2 polarization after subarachnoid hemorrhage in rats and involve the AMPK/NF-κB signaling pathway. *Biomed. Pharmacother.* **2021**, *133*, 111048. [CrossRef]
97. Ding, H.; Jia, Y.; Lv, H.; Chang, W.; Liu, F.; Wang, D. Extracellular vesicles derived from bone marrow mesenchymal stem cells alleviate neuroinflammation after diabetic intracerebral hemorrhage via the miR-183-5p/PDCD4/NLRP3 pathway. *J. Endocrinol. Investig.* **2021**, *44*, 2685–2698. [CrossRef]
98. Elia, C.A.; Losurdo, M.; Malosio, M.L.; Coco, S. Extracellular Vesicles from Mesenchymal Stem Cells Exert Pleiotropic Effects on Amyloid-β, Inflammation, and Regeneration: A Spark of Hope for Alzheimer's Disease from Tiny Structures? *Bioessays* **2019**, *41*, e1800199. [CrossRef]
99. Lai, N.; Wu, D.; Liang, T.; Pan, P.; Yuan, G.; Li, X.; Li, H.; Shen, H.; Wang, Z.; Chen, G. Systemic exosomal miR-193b-3p delivery attenuates neuroinflammation in early brain injury after subarachnoid hemorrhage in mice. *J. Neuroinflammation* **2020**, *17*, 1–13. [CrossRef]
100. Li, H.-T.; Zhou, H.-J.; Zhong, J.-H.; Tang, T.; Cui, H.-J.; Zhang, Q.-M.; Zhou, J.-H.; Zhang, Q. 2-methoxyestradiol inhibits intracerebral hemorrhage-induced angiogenesis in rats. *Turk. Neurosurg.* **2016**, *28*, 241–247. [CrossRef]
101. Xu, R.; Bai, Y.; Min, S.; Xu, X.; Tang, T.; Ju, S. In vivo Monitoring and Assessment of Exogenous Mesenchymal Stem Cell-Derived Exosomes in Mice with Ischemic Stroke by Molecular Imaging. *Int. J. Nanomed.* **2020**, *ume 15*, 9011–9023. [CrossRef]
102. Venkat, P.; Cui, C.; Chopp, M.; Zacharek, A.; Wang, F.; Landschoot-Ward, J.; Shen, Y.; Chen, J. MiR-126 Mediates Brain Endothelial Cell Exosome Treatment–Induced Neurorestorative Effects After Stroke in Type 2 Diabetes Mellitus Mice. *Stroke* **2019**, *50*, 2865–2874. [CrossRef] [PubMed]
103. Vallhov, H.; Gutzeit, C.; Johansson, S.M.; Nagy, N.; Paul, M.; Li, Q.; Friend, S.; George, T.C.; Klein, E.; Scheynius, A.; et al. Exosomes containing glycoprotein 350 released by EBV-transformed B cells selectively target B cells through CD21 and block EBV infection in vitro. *J. Immunol.* **2011**, *186*, 73–82. [CrossRef] [PubMed]
104. Haney, M.J.; Klyachko, N.L.; Zhao, Y.; Gupta, R.; Plotnikova, E.G.; He, Z.; Patel, T.; Piroyan, A.; Sokolsky, M.; Kabanov, A.V.; et al. Exosomes as drug delivery vehicles for Parkinson's disease therapy. *J. Control. Release* **2015**, *207*, 18–30. [CrossRef] [PubMed]
105. Reed, S.L.; Escayg, A. Extracellular vesicles in the treatment of neurological disorders. *Neurobiol. Dis.* **2021**, *157*, 105445. [CrossRef]
106. Takahashi, Y.; Nishikawa, M.; Shinotsuka, H.; Matsui, Y.; Ohara, S.; Imai, T.; Takakura, Y. Visualization and in vivo tracking of the exosomes of murine melanoma B16-BL6 cells in mice after intravenous injection. *J. Biotechnol.* **2013**, *165*, 77–84. [CrossRef]
107. Dabrowska, S.; Andrzejewska, A.; Strzemecki, D.; Muraca, M.; Janowski, M.; Lukomska, B. Human bone marrow mesenchymal stem cell-derived extracellular vesicles attenuate neuroinflammation evoked by focal brain injury in rats. *J. Neuroinflammation* **2019**, *16*, 1–15. [CrossRef]
108. Haney, M.J.; Zhao, Y.; Jin, Y.S.; Batrakova, E.V. Extracellular Vesicles as Drug Carriers for Enzyme Replacement Therapy to Treat CLN2 Batten Disease: Optimization of Drug Administration Routes. *Cells* **2020**, *9*, 1273. [CrossRef]
109. Zhang, H.-L.; Xie, X.-F.; Xiong, Y.-Q.; Liu, S.-M.; Hu, G.-Z.; Cao, W.-F.; Wu, X.-M. Comparisons of the therapeutic effects of three different routes of bone marrow mesenchymal stem cell transplantation in cerebral ischemic rats. *Brain Res.* **2018**, *1680*, 143–154. [CrossRef]
110. Zhang, Y.; Bi, J.; Huang, J.; Tang, Y.; Du, S.; Li, P. Exosome: A Review of Its Classification, Isolation Techniques, Storage, Diagnostic and Targeted Therapy Applications. *Int. J. Nanomed.* **2020**, *15*, 6917–6934. [CrossRef]
111. Görgens, A.; Corso, G.; Hagey, D.W.; Wiklander, R.J.; Gustafsson, M.O.; Felldin, U.; Lee, Y.; Bostancioglu, R.B.; Sork, H.; Liang, X.; et al. Identification of storage conditions stabilizing extracellular vesicles preparations. *J. Extracell. Vesicles* **2022**, *11*, e12238. [CrossRef] [PubMed]
112. Li, Y.; Liu, B.; Zhao, T.; Quan, X.; Han, Y.; Cheng, Y.; Chen, Y.; Shen, X.; Zheng, Y.; Zhao, Y. Comparative study of extracellular vesicles derived from mesenchymal s tem cells and brain endothelial cells attenuating blood-brain barrier permeability via regulating Caveolin-1-dependent ZO-1 and Claudin-5 en docytosis in acute ischemic stroke. *J. Nanobiotechnology* **2023**, *21*, 70.
113. Yang, L.; Han, B.; Zhang, Z.; Wang, S.; Bai, Y.; Zhang, Y.; Tang, Y.; Du, L.; Xu, L.; Wu, F.; et al. Extracellular Vesicle–Mediated Delivery of Circular RNA SCMH1 Promotes Functional Recovery in Rodent and Nonhuman Primate Ischemic Stroke Models. *Circulation* **2020**, *142*, 556–574. [CrossRef] [PubMed]

114. Dave, K.M.; Stolz, D.B.; Venna, V.R.; Quaicoe, V.A.; Maniskas, M.E.; Reynolds, M.J.; Babidhan, R.; Dobbins, D.X.; Farinelli, M.N.; Sullivan, A.; et al. Mitochondria-containing extracellular vesicles (EV) reduce mouse brain infarct sizes and EV/HSP27 protect ischemic brain endothelial cultures. *J. Control. Release* **2023**, *354*, 368–393. [CrossRef]
115. Li, Y.; Liu, Z.; Song, Y.; Pan, J.-J.; Jiang, Y.; Shi, X.; Liu, C.; Ma, Y.; Luo, L.; Mamtilahun, M.; et al. M2 microglia-derived extracellular vesicles promote white matter repair and functional recovery via miR-23a-5p after cerebral ischemia in mice. *Theranostics* **2022**, *12*, 3553–3573. [CrossRef]
116. Guo, L.; Huang, Z.; Huang, L.; Liang, J.; Wang, P.; Zhao, L.; Shi, Y. Surface-modified engineered exosomes attenuated cerebral ischemia/reperfusion injury by targeting the delivery of quercetin towards impaired neurons. *J. Nanobiotechnology* **2021**, *19*, 1–15. [CrossRef]
117. Kim, M.; Lee, Y.; Lee, M. Hypoxia-specific anti-RAGE exosomes for nose-to-brain delivery of anti-miR-181a oligonucleotide in an ischemic stroke model. *Nanoscale* **2021**, *13*, 14166–14178. [CrossRef]
118. Chen, L.-K.; Zhu, Z.-H.; Jia, F.; Ahmed, W.; Zhang, G.-L.; Wang, H.; Lin, C.-Q.; Chen, W.-H. Neural stem cell-derived exosome as a nano-sized carrier for BDNF delivery to a rat model of ischemic stroke. *Neural Regen. Res.* **2022**, *18*, 404–409. [CrossRef]
119. Liu, W.; Su, C.; Qi, Y.; Liang, J.; Zhao, L.; Shi, Y. Brain-targeted heptapeptide-loaded exosomes attenuated ischemia-reperf usion injury by promoting the transfer of healthy mitochondria from as trocytes to neurons. *J. Nanobiotechnology* **2022**, *20*, 242. [CrossRef]
120. Deng, Y.; Duan, R.; Ding, W.; Gu, Q.; Liu, M.; Zhou, J.; Sun, J.; Zhu, J. Astrocyte-derived exosomal nicotinamide phosphoribosyl-transferase (Nam pt) ameliorates ischemic stroke injury by targeting AMPK/mTOR signalin g to induce autophagy. *Cell Death Dis.* **2022**, *13*, 1057. [CrossRef]
121. Liu, X.; Lv, X.; Liu, Z.; Zhang, M.; Leng, Y. MircoRNA-29a in Astrocyte-derived Extracellular Vesicles Suppresses Br ain Ischemia Reperfusion Injury via TP53INP1 and the NF-κB/NLRP3 Axis. *Cell. Mol. Neurobiol.* **2022**, *42*, 1487–1500.

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