Exosomes — beyond stem cells for restorative therapy in stroke and neurological injury

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Abstract | Stroke is a leading cause of disability worldwide, and brain injuries devastate patients and their families, but currently no drugs on the market promote neurological recovery. Limited spontaneous recovery of function as a result of brain remodelling after stroke or injury does occur, and cell-based therapies have been used to promote these endogenous processes. Increasing evidence is demonstrating that the positive effects of such cell-based therapy are mediated by exosomes released from the administered cells and that the microRNA cargo in these exosomes is largely responsible for the therapeutic effects. This evidence raises the possibility that isolated exosomes could be used alone as a neurorestorative therapy and that these exosomes could be tailored to maximize clinical benefit. The potential of exosomes as a therapy for brain disorders is therefore being actively investigated. In this Review, we discuss the current knowledge of exosomes and advances in our knowledge of their effects on endogenous neurovascular remodelling events. We also consider the opportunities for exosome-based approaches to therapeutic amplification of brain repair and improvement of recovery after stroke, traumatic brain injury and other diseases in which neurorestoration could be a viable treatment strategy.

Stroke is the leading cause of adult-onset disability worldwide[1]. Most survivors of stroke experience some degree of spontaneous functional recovery, but the regain of function tends to be modest and is often insufficient for survivors to be independent in their daily lives. Consequently, disability after stroke imposes many social and economic burdens on society. Similarly, people with traumatic brain injury (TBI) can experience spontaneous recovery, but many are left permanently disabled, and no current medical intervention can aid or improve upon spontaneous restoration of neurological function.

Patients who survive a stroke often improve rapidly in the following weeks (the acute phase), after which their recovery slows but can continue for months (the chronic phase)[2]. This spontaneous improvement of neurological function, which is also observed after TBI, suggests that limited remodelling can occur in the adult brain to compensate for injury and loss of tissue. Long-term neurological recovery is thought to depend on remodelling of neuronal circuitry in healthy tissue to compensate for dead and damaged tissue that is not recoverable. For example, in rodents, spontaneous remodelling of axons after experimental ischaemic stroke has been observed for at least 8 weeks without any intervention, and this remodelling was necessary for neurological recovery[3]. In this context, a possible therapeutic strategy to improve recovery from brain insults such as stroke and TBI is amplification of intrinsic restorative processes by targeting intact CNS tissue to promote neurite remodelling in the brain and spinal cord, angiogenesis at the site of damage, and functional restorative changes in the glia (astrogliosis, oligodendrogenesis and induction of a reparative microglial phenotype).

Initial attempts at such a strategy were cell-based therapies, particularly with mesenchymal stromal cells (MSCs) derived from bone marrow[4–8]. This approach has proved to be safe and has improved recovery of neurological function in animal models; early-phase clinical evidence for MSC therapy is also promising[7,9]. The rationale for such cell-based therapies was the replacement of dead neurons by differentiation of grafted MSCs, but overwhelming evidence from preclinical studies demonstrates that MSC therapy and other cell-based therapies promote endogenous neuronal rewiring and increase angiogenesis and neurogenesis in the ischaemic brain by secreting factors that trigger signalling pathways that amplify brain remodelling[4,10]. Emerging data suggest that exosomes, a subtype of extracellular vesicles, that are released from the MSCs contribute substantially to the beneficial effects of cell therapies, including MSC therapy for stroke, TBI and other neurodegenerative diseases[9,11,12].

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Key points

- Exosomes are involved in many aspects of normal brain physiology and facilitate communication between brain cells and between the brain and the periphery.
- Increasing evidence suggests that exosomes from mesenchymal stromal cells (MSCs) mediate the beneficial effects of cell therapy for stroke and traumatic brain injury (TBI).
- The effects of MSC-derived exosomes alone have the potential to improve neurological outcomes in animal models of stroke, TBI and other neurological diseases.
- Of the cargo in exosomes, microRNA (miRNA) is of prime importance in mediating the therapeutic effects.
- Compared with naive MSC-derived exosomes, engineered MSC-derived exosomes that contain selected miRNA have more potent therapeutic effects in stroke and TBIs.

In this Review, we discuss recent advances that demonstrate the importance of exosomes in intercellular signalling in the brain and consider the use of exosomes for treatment of acute stroke and for amplification of brain remodelling to improve recovery of neurological function after stroke and TBI. We discuss evidence that shows how unmodified and modified exosomes can affect outcomes of stroke and TBI when used as therapy, and we examine cellular and molecular mechanisms that might underlie the benefits of exosome therapy. We also outline opportunities for and challenges in translation of exosome-based therapy to clinical applications.

Properties and functions of exosomes

Exosomes are endosome-derived membrane-bound vesicles with diameters of ~30–150 nm, and they are released by cells in all living systems in physiological and pathophysiological conditions[13–15]. The sizes of other subtypes of extracellular vesicles — microvesicles and apoptotic bodies — overlap with those of exosomes, but each subtype has a distinct biogenesis pathway[16–18]; the biogenesis of exosomes is reviewed in detail elsewhere[16–18].

Exosomes are uniform lipid bilayer spheroids, and their membranes contain tetraspanin proteins, including CD63, CD81 and CD9, and the endosome membrane proteins flotillin and ALIX (also known as PDCD6-interacting protein). The endosome membrane proteins have been used as exosomal markers[19,20], but distinguishing exosomes from other extracellular vesicles with overlapping size and density solely on the basis of these markers is difficult[14].

Exosome cargo. Exosomes carry proteins, lipids and nucleic acids, including mRNA, microRNA (miRNA) and long non-coding RNA. In physiological conditions, exosomes routinely transfer these functional biomolecules from cell to cell to facilitate intercellular communication[14,17,18]. Of the cargo that exosomes carry, miRNAs have been investigated most fully in terms of their functional importance, and studies have indicated that they are central to the therapeutic effects of exosomes.

miRNAs derive from primary transcripts that are processed to form mature duplex miRNAs[21,22]. One or both of the mature RNA strands binds to argonaute 2 (Ago2) and is incorporated into the RNA-induced silencing complex (RISC), which targets mRNA for cleavage or translational repression[21,23,24]. Novel miRNAs arise frequently but are rarely lost[25], and miRNA diversity within an organism correlates with morphological complexity even though genomic density does not[26]. This phenomenon might reflect the fact that miRNAs each have several or even hundreds of target genes, making them highly efficient regulators of gene networks. miRNAs are therefore extremely powerful in the regulation of complex networks.

Exosomes in the brain. Neurons contain large numbers of multivesicular bodies, where generation of exosomes initially occurs[27], and brain cells secrete exosomes in physiological conditions[28] (FIG. 1). These brain exosomes have an important role in the heterocellular communication that regulates brain function[27,29,30]. Glutamatergic synaptic activity in cultured mature neurons increases exosomal release from these cells, and these neuron-derived exosomes carry the neuronal-specific protein L1 cell adhesion molecule (L1CAM) and the GluR2 and GluR3 subunits of glutamate receptors[31–33], suggesting that neuronal exosomes have a role in regulating neuronal function. Exosomes derived from neurons also communicate with cerebral endothelial cells to regulate the blood–brain barrier (BBB)[34].

Oligodendrocytes and astrocytes also communicate with neurons and each other via exosome release[27]. Exosome release is regulated by potassium chloride concentrations in astrocytes and by cytosolic calcium levels in oligodendrocytes. Glutamate from activated neurons also stimulates oligodendrocytes to release exosomes[35,36]. Multivesicular bodies from oligodendrocytes have been detected in the periaxonal space, and oligodendrocyte exosomes carry the myelin proteins proteolipid protein (PLP), 2'3'-cyclic-nucleotide-phosphodiesterase (CNP) and myelin basic protein (MBP)[36,37]; these observations suggest that oligodendrocyte exosomes coordinate the myelination of axons by oligodendrocytes[38]. In addition, oligodendrocyte exosomes improve neuronal viability in conditions of oxygen–glucose deprivation (OGD) by transferring their cargo, which includes superoxide dismutase and catalase that provide resistance to oxidative stress, into neurons[37].

Astrocytes have diverse roles in brain physiology, from maintaining BBB integrity to synaptic regulation, and several lines of evidence show that many of their functions are mediated by exosomes. For example, some evidence suggests that synaptic plasticity depends in part on the release of miR-26-containing exosomes from astrocytes[39], an effect that might result from miR-26-mediated inhibition of glycogen synthase kinase 3β[40], a potent suppressor of axonal remodelling and synaptic plasticity. In addition, one study has shown that astrocyte exosomes protect against OGD-induced neuronal death via transfer of exosomal cargo prion protein (PrP) into oxygen–glucose-deprived neurons[41]. Furthermore, owing to the unique property of astrocytes that the end feet of a single cell often contact the BBB and the synaptic space, internalization and release of exosomes by astrocytes might bridge signals from neurons to the periphery and vice versa[42]. Much is left to discover about the functions of exosomes from astrocytes, given that

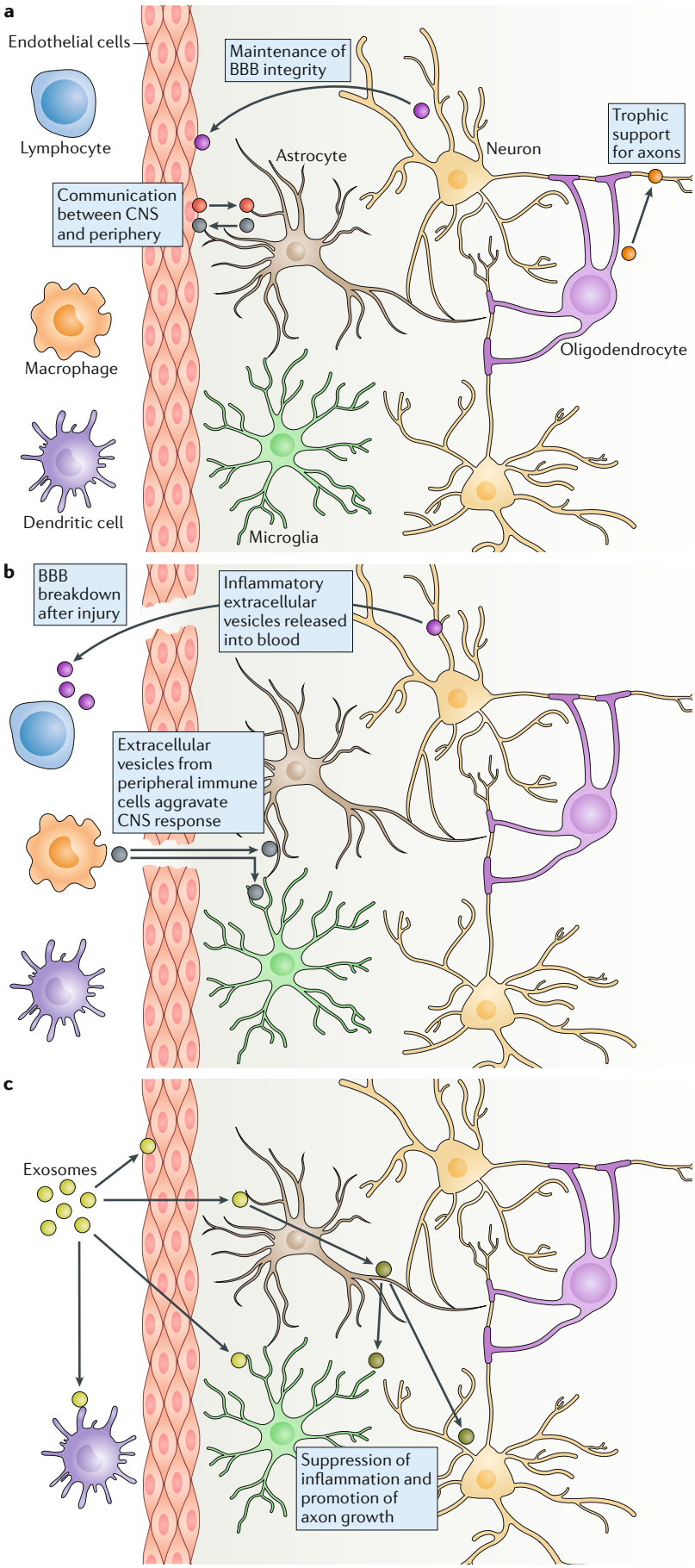

Fig. 1 | **Roles of exosomes in the brain in physiology, after an insult and in exosome therapy. a** | In normal physiology, exosomes mediate communication between cells within the CNS and between the CNS and the periphery. Oligodendrocytes provide trophic support to axons, and neurons transfer miR-132 via exosomes to endothelial cells, where it helps to maintain blood–brain barrier (BBB) integrity. Exosomes also mediate dynamic crosstalk between astrocytes and endothelial cells. **b** | After a neural insult, the BBB is disrupted, and exosomes and other extracellular vesicles from distressed or dying cells in the brain enter the bloodstream and the cerebrospinal fluid, where they stimulate inflammatory responses in peripheral immune cells in addition to microglia in the brain. Furthermore, exosomes from inflammatory peripheral immune cells can infiltrate the CNS, where they exacerbate the response to injury. **c** | When exosomes are introduced intravenously, they target several cell types, including peripheral and CNS immune cells, and help to induce an anti-inflammatory phenotype in these cells. Furthermore, they target neurons and glia, which in turn release their own exosomes that promote CNS repair, including axonal sprouting.

miRNA diversity is high in exosomes from astrocytes and distinct from that of the parent cells[43]. Given the numerous roles that astrocytes have in normal physiology and pathophysiology, the specificity of miRNA abundance in astrocyte exosomes could signify that the functions these exosomes have in the brain are as varied as those of the parent cell themselves.

Exosomes as a potential therapy

Since the earliest investigations of MSC therapy for brain injuries, the cell therapy community has sought to isolate the specific paracrine factors that mediate recovery. The only constituents of the MSC secretome that have successfully been used to recapitulate the response to therapy with the parent cells are exosomes[9,11,12,14,17,18,44–46], and several studies have been conducted in which exosomes (or extracellular vesicles) were used to treat stroke, TBI or intracerebral haemorrhage (ICH) in animals (TABLE 1).

The therapeutic potential of MSC-derived exosomes in rodent models of stroke and TBI was documented for the first time in 2013 and 2015, respectively[9,11,12]. These studies showed that intravenous administration of MSC-derived exosomes to rats that had been subjected to focal cerebral ischaemia or TBI substantially increased neurovascular remodelling and improved the neurological, behavioural and cognitive outcomes during recovery[9,11,12]. These improvements were comparable to those observed with MSC therapy[9,11,12]. A subsequent head-to-head comparison of the two treatments conducted in an independent laboratory demonstrated that the treatment of stroke with MSCs or MSC-derived exosomes in mice produced equal improvements in motor function and coordination[46]. Improved recovery of sensorimotor function as a result of treatment with MSC-derived exosomes has also been demonstrated in a rat model of subcortical stroke[47]. In another study in a mouse model of TBI, intravenous administration of human MSC-derived exosomes substantially preserved pattern separation and spatial learning ability[48].

Table 1 | **Studies published to date in which exosomes or extracellular vesicles have been used to treat cerebral injuries**

Disease	Animal model	Treatment time after insult	Source of extracellular vesicles	Extracellular vesicle modification	Proposed mechanism	Refs
Stroke	Rat	24 h	MSCs	None	White matter repair	11,43
	Rat	24 h	MSCs	miR-133b overexpressed	White matter repair	12
	Rat	24 h	MSCs	miR-17–92 cluster overexpressed	White matter repair	124
	Mouse	2 h, 14 h and 38 h	NSCs (iPSC-derived)	None	Immunomodulation	59
	Pig	2 h, 14 h and 24 h	NSCs (iPSC-derived)	None	Reduction in oedema	60
	Mouse	24 h, 3 d and 5 d	MSCs	None	Immunosuppression	50
	Rhesus monkey	24 h and 14 d	MSCs	None	Immunomodulation	52
TBI	Rat	24 h	MSCs	None	Angiogenesis and neurogenesis	12
	Rat	24 h	MSCs (grown on collagen scaffold)	None	Angiogenesis and neurogenesis	57
	Rat	1 h	MSCs	None	Angiogenesis and neurogenesis	48
TBI (polytrauma)	Pig	6 h	MSCs	None	Neuroprotection	51
Fetal hypoxia	Sheep	4 d and 8 d	MSCs	None	Neuroprotection	45
Intracerebral haemorrhage	Rat	24 h	MSCs	None	White matter repair and angiogenesis	49,50
Focal ischaemia	Mouse	24 h	MSCs	miR-124 overexpressed and RVG–LAMP2B fusion protein expressed	Neurogenesis	119

iPSC, induced pluripotent stem cell; MSC, mesenchymal stem cell; NSC, neural stem cell; RVG, rabies virus glycoprotein; TBI, traumatic brain injury.

Improvements in sensorimotor and cognitive function have also been reported as a result of treatment with MSC-derived exosomes in a rat model of ICH[49,50].

Following the initial studies in rodents, several studies of exosome therapy have involved large animal models of stroke and TBI. In a sheep model of hypoxic–ischaemic brain injury in ovine fetuses, in utero administration of MSC-derived extracellular vesicles reduced the frequency and duration of seizures and preserved the sensitivity of the baroreceptor reflex[45]. In a porcine model of polytrauma including TBI, administration of exosomes derived from human MCSs 9 h after injury promoted neurological recovery[51]. Similarly, treatment with MSC-derived exosomes in adult rhesus monkeys with cortical lesions led to faster and fuller recovery than without treatment; the treated animals attained a grasping pattern that did not differ significantly from that of uninjured animals[52]. This model of cortical injury is highly relevant to stroke in humans because the monkeys used have fine motor function of the hand and digits that is comparable to that in humans[53,54].

Collectively, the data from rodent and large animal studies suggest that MSC-derived exosomes contribute to, and probably mediate, the beneficial effects of MSC therapy. On the basis of the apparent efficacy of exosomes in these studies, preclinical and human studies have been conducted to assess safety, and no noticeable adverse immune reactions have been observed[55–57]. For example, exosomes derived from human MSCs were well tolerated and therapeutically efficacious in a rat model of TBI[48,51,57]. In a human with symptoms of graft versus host disease, multiple intravenous infusions of MSC-derived exosomes over a 2-week period were also well tolerated and did not cause adverse effects[58]. However, whether exosomes from MSCs express major histocompatibility complex molecules is unclear, so additional studies are warranted to systematically evaluate the effects of exosomes on host immune responses.

Exosomes derived from other cell types can also induce brain and CNS remodelling and can reduce infarct volume when employed to treat acute ischaemic stroke[18,59]. A direct comparison of the effects of exosomes from human pluripotent-stem-cell-induced MSCs and those of exosomes from human pluripotent-stem-cell-induced neural stem cells showed that exosomes from neural stem cells, but not those from MSCs, significantly reduced infarct volume and improved neurological function in a mouse model of ischaemic stroke when the treatment was initiated within 6 h after stroke. In mice aged 18–19 months, exosomes from neural stem cells did not reduce cerebral infarct volume but did improve functional outcome 14 days after stroke[59].

In a porcine model of ischaemic stroke, administration of exosomes from neural stem cells 2 h after stroke reduced the size of the ischaemic lesion at 1 day but not at 84 days after stroke, although neurological outcome was improved at 84 days after stroke[60]. In vitro experiments have shown that endothelial-derived exosomes can protect neurons from ischaemia–reperfusion injury[61]. Collectively, the in vivo and in vitro data discussed suggest that treatment for acute stroke with exosomes has a neuroprotective effect by suppressing expansion of the ischaemic core, but that without subsequent brain tissue perfusion, this neuroprotective effect does not persist.

Given that the standard of care for acute ischaemic stroke is thrombolysis with tissue plasminogen activator (tPA) within 4.5 h of onset or thrombectomy within 24 h of ischaemic stroke with large artery occlusion[6,62–68], exosomes could be used as an adjunctive treatment. Indeed, in one study of a rabbit model of multiple small clot embolic stroke, administration of exosomes from human cardiosphere-derived cells in combination with tPA 1 h after embolization significantly improved functional outcome compared with tPA monotherapy[69].

The type of cell-derived exosomes that are most effective for treatment of stroke and TBI is yet to be determined[14,17]. Transcriptomic and proteomic analysis of MSCs derived from human embryonic stem cells and those derived from human bone marrow demonstrates that the two MSC types are functionally equivalent[70]. However, exosome content is determined by the parent cell[14,23], and whether the cargo of exosomes from human pluripotent-stem-cell-induced MSCs is comparable to that of exosomes from adult MSCs has not been investigated. Nevertheless, given that MSC therapies have been intensively studied and are currently in clinical trials for stroke and TBI, exosomes from MSCs are attractive candidates for therapeutic application. Furthermore, use of exosomes rather than cells could overcome some safety problems with MSCs. Systemic administration of exogenous MSCs in a rat model of stroke caused obstruction of lung vessels[71], and transplanted cells can induce malignant transformation[72,73]. Naive exosomes that derive from healthy MSCs provide the same therapeutic effects as cell-based therapy but are not associated with these adverse effects.

Exosome therapy and the BBB

Preclinical studies suggest that systemic administration of exosomes facilitates endogenous rewiring of neuronal circuitry, white matter remodelling, oligodendrogenesis, angiogenesis and neurogenesis in the injured brain[74–77], all of which are associated with better neurological outcomes[74–77]. For example, exosomes from MSCs increase synaptogenesis, axonal sprouting and angiogenesis in peri-infarct regions, and increase neurogenesis in the hippocampus[44,47,78]. However, whether exosomes cross the BBB and have direct effects or remain in the periphery and have indirect effects remains an unanswered question. Several techniques, described below, have been used to answer this question, and the findings collectively suggest that systemically administered exosomes cross the BBB to interact with target cells within the brain.

In several studies, intravenous or intranasal administration of exosomes that were labelled with lipophilic dyes or carried CD63–GFP has resulted in fluorescent signals in neurons, glia and cerebral endothelial cells[47,79]. Lipophilic dye labelling can produce nonspecific artefacts, but CD63 is a specific marker of exosomes; therefore, the GFP fusion protein is a more reliable indication of exosome location[80–82].

Non-invasive in vivo CT has shown that MSC-derived exosomes that are labelled with gold nanoparticles enter the mouse brain after intranasal administration in non-ischaemic conditions[83]. After focal cerebral ischaemia, these exosomes accumulated around the ischaemic lesion[83].

Radiolabelling has also been used to study the ability of exosomes from macrophages and from neural stem cells to cross the BBB after intravenous administration. In one study, technetium-radiolabelled nanovesicles from macrophages did not cross the BBB[84], although these exosome mimics were produced by forcing suspended macrophages through an extruder to make artificial exosomes, therefore their equivalence to native exosomes is unclear. A subsequent study demonstrated that macrophage-derived exosomes that were labelled with iodine could cross the BBB in healthy and inflamed brains; concentrations of exosomes were approximately sixfold higher in the inflamed brain than in the healthy brain[85]. Finally, another study demonstrated that exosomes derived from neural stem cells and labelled with inidium-111 were present in the penumbra of the ischaemic brain at 1 h after their administration when they were intravenously administered at 1 h after stroke onset[59].

Potential effects of exosome therapy

Direct effects. The primary aim of neurorestorative therapy is to act on relatively healthy resident brain cells to facilitate innate brain remodelling processes[4,64]. In vitro studies have shown that exosomes from MSCs or fibroblasts fulfil this aspect of a neurorestorative therapy by directly promoting dendritic and axonal outgrowth[86,87].

As an example of these direct effects, evidence suggests that exosomes from rodent and human cerebral endothelial cells and from neural stem cells in adult rodents contribute to the neurogenesis and angiogenesis that is observed during recovery from injury[88–91]. For example, in vitro experiments have shown that exosomes from ischaemic neural stem cells promote angiogenesis in non-ischaemic cerebral endothelial cells and that exosomes from ischaemic cerebral endothelial cells increase neurogenesis of adult neural stem cells[89].

Indirect effects. Evidence suggests that, in addition to the direct effects on brain parenchymal cell function, exogenously administered exosomes have indirect neurorestorative effects. In a rat model of focal cerebral ischaemia, administration of MSC-derived exosomes stimulated astrocytes to release native exosomes, and these astrocyte-derived exosomes increased neurite outgrowth of cultured cortical neurons, which suggests that they contribute to the therapeutic effect of MSC-derived exosomes on ischaemic brain plasticity[92]. Thus, exogenously administered exosomes seem to interact with recipient brain cells, which in turn release their own exosomes that mediate communication between recipient cells and other brain cells to promote neurorestoration (FIG. 1).

In agreement with these observations, the findings of another study suggest that the secretomes of recipient cells are important in the effects of exosome therapy. This study showed that the cargo profiles of exosomes released by GFAP-positive astrocytes in the ischaemic brain differ according to whether or not the astrocytes have been treated with MSC-derived exosomes.

Exosomes released from astrocytes that were treated with MSC-derived exosomes increased ischaemic brain plasticity to a greater extent than did exosomes from untreated astrocytes[92].

Effects on inflammation. Stroke and TBI trigger inflammation in the CNS and PNS, which exacerbates brain damage. Clinical studies suggest that endogenous exosomes in the blood might contribute to exacerbation of stroke-induced inflammation. Exosomes that have been isolated from the blood of patients with acute stroke contain elevated levels of C-reactive protein, and these exosomes activate macrophages and increase expression of cytokines and chemokines in macrophages[93]; high levels of these proteins have been associated with a worse prognosis after stroke[94].

By contrast, several preclinical studies have suggested that MSC-derived exosomes reduce inflammation. In one study in rats, MSC-derived exosomes suppressed lipopolysaccharide-induced microgliosis and reactive astrogliosis and ameliorated inflammation-induced preterm brain injury[95]. In a mouse model of stroke, treatment with MSC-derived exosomes attenuated post-ischaemic immunosuppression, although the exosomes did not affect cerebral immune cell infiltration[46]. In a mouse model of TBI, MSC-derived exosomes reduced levels of IL-1β in the injured brain[48]. In addition, exosomes from hypoxia-preconditioned MSCs reduced cognitive impairment in a mouse model of Alzheimer disease (AD), in part by decreasing brain inflammation[96].

Systemic effects. Although exosome therapy for stroke and TBI would be targeted to the brain, some evidence suggests that the effects might not only be local. At least one in vivo study has shown that endothelial exosomes from ischaemic rodent brains exacerbate heart dysfunction[97], highlighting the diverse functions that exosomes have within and between organ systems. Another study showed that endogenous exosomes released by hypothalamic neural stem cells affect ageing speed in mice[90]. In this study, suppression of Rab27A, which is a key molecule for exosome secretion, in hypothalamic neural stem cells accelerated ageing. Conversely, injection of the miRNAs that are contained in hypothalamic stem cell exosomes into the forebrains of aged mice slowed their ageing process. Whether this age-regulating pathway exists in other mammals is unclear, but the data show how the exosome system can control a diverse array of biofunctions and provides a suite of potential therapeutic targets.

Similarly, evidence indicates that exosomes from peripheral cells affect the interaction between peripheral and brain inflammation. The choroid plexus epithelium forms the blood–cerebrospinal fluid (CSF) barrier[98]. Peripheral inflammation triggers the choroid plexus epithelium to release exosomes into the CSF, which in turn cross the blood–CSF barrier to transfer their pro-inflammatory cargo into astrocytes and microglia, leading to brain inflammation. Blocking of this exosome-mediated communication by inhibition of exosome secretion attenuates brain inflammation[98], again demonstrating how the exosome system can affect organisms at a systemic level.

Mechanisms of exosome therapy

The molecular mechanisms that underlie exosome communication with recipient cells are unclear but are under investigation[17,78,99]. Exosomes fuse with the plasma or endocytic membranes of recipient cells and subsequently deliver their cargo of proteins, lipids and nucleic acids into the recipient cells. This cargo presumably alters cell functions[100].

The role of miRNA. The contents of exosomes are enriched with miRNAs[17,33,101,102]. Genetic approaches have demonstrated that miRNAs alter recipient cell functions and mediate the therapeutic effects of MSC-derived exosomes[17]. Knockout of Drosha — a ribonuclease involved in miRNA production — in MSCs depletes miRNAs from their exosomes, and use of these miRNA-deficient exosomes has demonstrated that they lose their therapeutic effects in acute kidney injury[103], providing direct evidence for the importance of miRNAs to exosome function. Studies in adult neural stem cells have also provided direct evidence for the importance of miRNAs in exosomes. In one study, ablation of Dicer — another ribonuclease involved in miRNA production — in adult neural stem cells in mice substantially decreased multiple miRNAs that are required for stem cell function and resulted in cognitive impairment, but administration of exosomes from healthy neural stem cells restored these key miRNAs and rescued cognitive function[104].

Further evidence for the importance of miRNAs in exosome-based therapeutics comes from perturbation of Ago2. Most miRNAs in exosomes are bound to Ago2 (REFS[17,33,101,102]), and exosomes from MSCs in which Ago2 has been deleted do not promote axonal growth as exosomes from wild-type cells do[86]. In addition, blocking Ago2 in recipient cells attenuates the effect of exosomes on axonal growth. In a model of optic nerve crush, intravitreal injection of MSC-derived exosomes normally promotes axonal growth, but this effect was abolished when MSC-derived exosomes that contained small interfering RNA (siRNA) against Ago2 were administered[105]. Collectively, the aforementioned studies suggest that the therapeutic effects of MSC-derived exosomes can largely be attributed to the miRNA cargo, although delivery of cargo proteins is also involved in MSC-derived exosome therapy to some extent[78,99,106–108] (BOX 1).

Specific targets. Specific targets of exosomal miRNA have been described in several neural cell types and could mediate the beneficial effects of exosomal therapy. These targets are discussed in this section.

Studies of exosomes that are released by neurons upon depolarization have shown that the putative target genes of many cargo miRNAs are involved in neurite plasticity[32,33]. Axonal outgrowth after injury is limited in the adult CNS owing to the presence of axonal and extracellular axonal inhibitors[109]. Pharmacological and genetic suppression of axonal phosphate and tensin homologue (PTEN; a negative regulator of the mTOR

Box 1 | **Exosomal proteins in brain repair**

Proteins, including growth factors, chemokines and cytokines, that are secreted from mesenchymal stromal cells (MSCs) are known to be involved in the brain repair process after injury[134]. Exosomes from MSCs also contain protein, but the role of these exosomal proteins in the therapeutic effects of exosome therapy is unclear and has been studied to a much lesser extent than that of microRNA. Proteomic analysis of exosomes from MSCs of young adult humans has shown that several exosomal proteins are involved in regulation of angiogenesis; these proteins include canonical angiogenic proteins, platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR) and fibroblast growth factor (FGF)[135]. These angiogenic proteins and proteins involved in the nuclear factor-κB (NF-κB) signalling pathway were further enriched in exosomes from MSCs exposed to ischaemic tissue-simulated conditions[135]. In vitro angiogenic assays showed that the exosomes from hypoxic MSCs promoted angiogenesis in human umbilical cord vein endothelial cells (HUVECs) and that pharmacological blockade of NF-κB signalling abolished this exosome-promoted angiogenesis[135]. These findings indicate that exosomal cargo proteins that are involved in NF-κB signalling promote angiogenesis and, more broadly, that proteins within exosomes can have functional effects in recipient cells. However, the pharmacological inhibition of NF-κB signalling might have blocked the endogenous NF-κB signal in HUVECs; therefore, the role of exosomal proteins in recipient cells remains unclear.

pathway) and other neurite inhibitors, such as RhoA and connective tissue growth factor (CTGF), promotes axonal growth after injury[86,109,110], and miRNA in exosomes has been used to suppress intrinsic axonal inhibitors. Delivery of miR-133b to neurons in MSC-derived exosomes suppresses RhoA, a target of miR-133b, leading to increased neurite outgrowth[78]. Exosomal delivery of miR-133b to astrocytes, however, reduces expression of another of its target proteins, CTGF[99,107], an observation that highlights the divergent, cell-specific and perhaps synergistic mechanisms by which a single miRNA can act in the system. Administration of MSC-derived exosomes in which miR-133b has been depleted increases levels of CTGF and RhoA in the ischaemic brain, leading to attenuation of neurological outcome after stroke in the rat; elevation of miR-133b in MSC-derived exosomes has the opposite effect[10,111]. Moreover, axonal application of MSC-derived exosomes that contain the miR-17–92 cluster promotes axonal growth by locally suppressing the axonal intrinsic inhibitor PTEN[86]. In addition, one study has demonstrated that fibroblast-derived exosomes promote axonal growth by activating the mTOR signalling pathway[87].

In addition to their effects on neurons, exosomes can also affect the integrity of the BBB, which is often disrupted after stroke and TBI. A study published in 2017 shows that exosomes derived from neurons help to maintain BBB integrity by delivery of miR-132 to cerebral endothelial cells in a zebrafish larvae model and in cultured mouse brain endothelial cells[34]. Blocking delivery of miR-132 from neurons to cerebral endothelial cells exacerbates BBB leakage[34]. miR-132 is mainly expressed in neurons and regulates neurite outgrowth and neuronal transmission[112,113], but when transferred to endothelial cells it regulates expression of eukaryotic elongation factor 2-kinase, a component of vascular endothelial cadherin that is found at adherens junctions[34]. These data explain the effect of miR-132 on BBB integrity and further show that miRNA interactions with targets are dependent on cell type[108].

Tailored exosomes as therapeutics

Exosomes hold great promise as vehicles for delivery of therapeutics[14,18,56,114,115]. A major challenge in developing exosome therapy for stroke or brain injury is to target specific recipient cells in the CNS more efficiently than can be achieved using naive cell-derived exosomes. Several strategies have been employed for targeting exosomes to specific cell types. Use of virus-derived peptides has enabled cell-specific targeting of exosomes. For example, exosomes that express a fusion protein of the neuron-specific rabies virus glycoprotein (RVG) peptide and the exosomal membrane protein LAMP2B (known as RVG exosomes) specifically target neurons, microglia and oligodendrocytes that express nicotinic acetylcholine receptors, to which RVG binds[116,117]. In a functional study in a mouse model of AD, systemic administration of RVG exosomes that carried siRNA against the gene that encodes β-site APP cleaving enzyme 1 (BACE1) substantially reduced levels of BACE1 mRNA and, consequently, total levels of amyloid-β $(Aβ)_{1-42}$ in the brain[116]. This effect is similar to that of BACE1 inhibitors, which have potential as disease-modifying drugs for AD[118]. This study demonstrated that RVG exosomes can cross the BBB and specifically target brain cells that express nicotinic acetylcholine receptors in the intact mouse brain.

Subsequent studies have provided additional evidence that RVG exosomes can be used for targeted delivery of miRNA and siRNA cargo to recipient brain cells in disease. In one study in mice, systemically administered RVG exosomes that carried siRNA against the μ-opioid receptor could cross the BBB and specifically inhibit μ-opioid receptor expression in the brain and prevent relapses of morphine addiction[40]. Similarly, in mice with focal cerebral ischaemia, intravenously administered RVG exosomes that were loaded with miR-124, which is enriched in neurons, enabled exosomes to reach the peri-ischaemic area and promote neurogenesis[119]. Furthermore, RVG exosomal delivery of siRNA seems to enable efficient mRNA knockdown in recipient cells[116]. For example, RVG exosomes that were loaded with siRNA against the μ-opioid receptor at a concentration of 0.14 pmol/μg reduced μ-opioid receptor mRNA levels by 75%[40]. These data demonstrate the potential value of engineered exosomes to specifically target therapeutics to brain cells.

Adeno-associated virus capsids have also been used to selectively target exosomes to neurons[120]. In this study, the exosomes rescued hearing loss in a mouse model of hereditary hearing loss[120]. However, viral peptides have the potential to induce adverse immunological responses[56], and chemically synthesized peptides might avoid this problem. Use of chemically synthesized peptides to modify the exosome surface has been assessed as a strategy for targeting exosomes to specific sites in a mouse model of transient cerebral ischaemia. For example, in mice with transient ischaemia, MSC-derived exosomes that were conjugated with a functional cyclo(Arg–Gly–Asp–D–Tyr–Lys) peptide (cRGD-Exo) more efficiently localized to the ischaemic lesion than did non-modified MSC-derived exosomes because the peptide has a high affinity for

αvβ3 integrin (REF.[121]). cRGD-Exo was also taken up by neurons and astrocytes. When these exosomes were loaded with the anti-inflammatory drug curcumin, they suppressed inflammatory responses and reduced ischaemic cell death[121]. Curcumin-loaded exosomes have also been administered intranasally in mice, and in this setting, the exosomes crossed the BBB and diminished lipopolysaccharide-induced brain inflammation[122]. Furthermore, in another study, exosomes that were from embryonic stem cells and were loaded with curcumin promoted neurovascular repair after cerebral ischaemia in mice[123]. The previous two studies did not provide evidence that curcumin is encapsulated in the exosomes[122,123], but the observations suggest that engineering of exosomes to express viral or chemical peptides improves their ability to cross the BBB and selectively target recipient cells.

The use of tailored exosomes to deliver specific miRNAs for the treatment of stroke is under active investigation. For example, in rat models of ischaemic stroke and ICH, treatment with tailored MSC-derived exosomes that carry high levels of miR-133b or the miR-17–92 cluster leads to stronger suppression of target proteins that inhibit axonal growth than does treatment with naive MSC-derived exosomes, thereby increasing axonal growth and neurogenesis, and leads to suppression of genes that promote neuronal death[11,124,125]. On the basis of this evidence, targeting of tailored exosomes that carry key regulatory miRNAs to specific brain recipient cells might be expected to increase the efficacy of exosome therapy for stroke and other brain diseases. Furthermore, tailoring of exosomes could enable multiple targets to be targeted at different times after a neural insult. For instance, exosomes loaded with a neuroprotective miRNA could be administered for acute stroke, and exosomes loaded with a restorative miRNA could be administered during subacute and chronic stroke phases. Such strategies can be developed further as methods for loading quantifiable doses of miRNAs and for precise cell targeting are refined.

In some studies, the therapeutic potential of exosomes that are tailored to carry other types of cargo has been investigated, but the data are limited. In one study, exosomes from dendritic cells were loaded with siRNA, and this siRNA remained functional in the brain after delivery[116]. However, direct loading of siRNA has rarely been used because the efficiency of this process is low. Engineering of functional proteins into exosomes has also been attempted[91,126]. In one of these studies, a tagged form of Cre recombinase was loaded into exosomes and was successfully delivered to reporter neurons in multiple brain regions, including the cortex and hippocampus, via intranasal administration of the exosomes[126]. With further development, these approaches to engineering of exosomes offer many possibilities for exosome-based medicines.

Exosome therapy for other neurological diseases

The potential of MSC-derived exosomes to treat a host of neurological diseases is becoming apparent. In the past few years, therapeutic benefits of MSC-derived exosomes have been reported in AD[106], Parkinson disease[127] and prenatal brain injury[95], among other neurological diseases.

In the APP/PS1 mouse model of AD, exosomes from MSCs slowed cognitive decline and reduced Aβ and plaque deposition. These effects were increased by pre-treatment of the MSCs with hypoxic stress[96], suggesting that 'alerting' the MSCs to harmful conditions primes them to better fight disease.

One study has indicated a benefit of tailored exosomes in Parkinson disease[128]. In this study, exosomes from macrophages were loaded with catalase. The catalase retained its enzymatic activity after exosome loading and the loaded exosomes were readily taken up by cultured PC12 cells (a neuronal cell line). In an animal model of Parkinson disease, direct injection of catalase-loaded exosomes into the brain protected neurons from death, and neuroinflammation was reduced compared with that in animals that received non-catalase-loaded exosomes or no treatment. This study differs from many exosome treatment studies in that exosomes were loaded with a protein rather than RNA, again highlighting that exosome-based therapies can take many forms that can be specific to the therapeutic target.

Treatment of prenatal brain injuries with MSC-derived exosomes has also been explored in two studies. In a sheep model of fetal hypoxia induced by umbilical cord occlusion, in utero infusion of MSC-derived exosomes reduced the incidence of seizures in the fetuses and prevented hypomyelination[45]. Similarly, in early

Table 2 | **Registered trials of exosomes as therapeutics in various diseases**

Trial identifier[a]	Institution	Disease	Source of exosomes	Trial phase
NCT02565264	Kumamoto University, Japan	Cutaneous wound healing and ulcers	Plasma	I
NCT01668849	James Graham Brown Cancer Center, University of Louisville, USA	Chemoradiation-induced oral mucositis	Grape	I
NCT01294072	James Graham Brown Cancer Center, University of Louisville, USA	Colon cancer	Curcumin-loaded plant	I
NCT03384433	Isfahan University of Medical Sciences, Iran	Ischaemic stroke	MSCs	I/II
NCT01159288	Gustave Roussy, Cancer Campus, Grand Paris, France	Non-small-cell lung cancer	Dendritic cells	II
NCT02138331	General Committee of Teaching Hospitals and Institutes, Egypt	Type 1 diabetes mellitus	MSCs	II/III

MSC, mesenchymal stromal cell. [a]At US National Library of Medicine Clinical Trials.

postnatal rats with inflammatory brain injury, injection of MSC-derived exosomes prevented myelin degradation, reduced astrogliosis and protected the ability to perform learning and motor tasks later in life[95]. Adverse effects of the exosomes were not observed in either study, lending confidence that MSC-derived exosomes are safe and tolerated by patients of sensitive ages.

These examples are not an extensive review of the potential applications of exosomes for therapy of neurological diseases and injuries, but they highlight the diversity of clinical problems that exosomes have the potential to address. We expect the field to expand in the coming years as new methods of exosome loading and manufacturing become available.

Conclusions and future perspectives

The field of exosomes as a potential therapy for stroke, TBI and other neurological disorders is rapidly growing. Most preclinical studies show that either naive or engineered exosomes have potent therapeutic effects in stroke, injury and disease. The precise cellular and molecular mechanisms that underlie the benefits of exosome therapy are not yet clear but are actively being investigated. In the meantime, multiple clinical trials of exosomes as a therapy have been registered for diseases such as cancer, wound healing, stroke and diabetes (TABLE 2). This list is currently small but will undoubtedly increase by orders of magnitude in the coming years.

Several challenges remain to enable translation of exosome-based therapy to clinical applications. Biologically, a greater understanding of the molecular mechanisms of exosomal action is needed to refine the engineering process to maximize clinical benefit in specific conditions. Practically, safety considerations need to be explored fully, and the manufacturing and quality control of clinical-grade exosomes needs to be developed and standardized[14,17]. Protocols for the manufacture of human extracellular-vesicle-based therapeutics for clinical use, in particular exosomes derived from human MCSs, are being developed[129,130]. For example, studies have shown that scaling up of exosome production for use in humans is achievable[51] and that lyophilization can be used to preserve exosomal cargo bioactivity[131]. However, standard quality control criteria remain to be established to ensure consistency and reproducibility of exosomal products, and this step is essential to develop exosome therapy for clinical treatment of brain disorders[14,132,133].

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