

MiRNAs in Astrocyte-Derived Exosomes as Possible Mediators of Neuronal Plasticity

Supplementary Issue: Brain Plasticity and Repair

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ABSTRACT: Astrocytes use gliotransmitters to modulate neuronal function and plasticity. However, the role of small extracellular vesicles, called exosomes, in astrocyte-to-neuron signaling is mostly unknown. Exosomes originate in multivesicular bodies of parent cells and are secreted by fusion of the multivesicular body limiting membrane with the plasma membrane. Their molecular cargo, consisting of RNA species, proteins, and lipids, is in part cell type and cell state specific. Among the RNA species transported by exosomes, microRNAs (miRNAs) are able to modify gene expression in recipient cells. Several miRNAs present in astrocytes are regulated under pathological conditions, and this may have far-reaching consequences if they are loaded in exosomes. We propose that astrocyte-derived miRNA-loaded exosomes, such as miR-26a, are dysregulated in several central nervous system diseases; thus potentially controlling neuronal morphology and synaptic transmission through validated and predicted targets. Unraveling the contribution of this new signaling mechanism to the maintenance and plasticity of neuronal networks will impact our understanding on the physiology and pathophysiology of the central nervous system.

KEYWORDS: microRNA, extracellular vesicles, astrocytes, neurons

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Astrocytes in Neuronal Plasticity

Astrocytes, a major glial cell type in the central nervous system, have emerged as powerful regulators of brain function where they maintain ion, metabolic, and neurochemical homeostasis. Astrocytes form organized networks that communicate through gap junctions formed by hemichannel proteins called connexins, potentially creating a functional syncytium.¹ The close structural and functional relationship between the presynaptic bouton and the postsynapse, both engulfed by astrocyte processes, has been named the tripartite synapse.² Thus, by extending processes that wrap around brain capillaries and synapses, astrocytes control neurovascular coupling, as well as synaptic transmission.^{3,4} Increasing evidence has specified their important functions in the nervous system, regulating blood-brain barrier permeability, cellular responses to brain injury, formation and elimination of synapses, synaptic maintenance and plasticity, as well as axon myelination in the adult brain, among others.^{5–8} Table 1 shows a summary of the core functions of astrocytes in relation to neuronal function. Though most of this evidence has been obtained in vitro, encouraging additional evidence comes from in vivo models, as recently revealed by measures of

astrocytic Ca²⁺ dynamics with high-resolution Ca²⁺ imaging in living mice.⁹ The nature of astrocyte-to-neuron interactions are mediated by direct cell-to-cell contact as well as by a complex array of astrocyte-derived molecules, ie, the astrocyte secretome, that varies according to neuronal activity and astrocyte activation and thus according to physiological or pathological conditions.^{3,10} It has been recently proposed and in part documented that the different cell types in the central nervous system could communicate by means of extracellular vesicles, among which small extracellular vesicles, also named exosomes, have received intense attention.¹¹ However, the involvement of exosomes in astrocyte-to-neuron communication has not been explored in depth. Thus, in this brief commentary, we will discuss the new exciting possibilities related to the interaction of astrocyte-derived exosomes with neurons in the nervous system, focusing on the content of microRNAs (miRNAs) present in these vesicles.

Exosome Release by Astrocytes

Cells release a variety of extracellular vesicles that differ in their subcellular origin and, possibly, in their biology. Among them, exosomes are defined by their endosomal origin, storage in

**Table 1.** Summary of neuron-related functions of astrocytes.

NEURON RELATED FUNCTION	EXPERIMENTAL EVIDENCE	REFERENCES
Metabolic support		
Lactate-shuttling	<i>In vivo/in vitro</i>	12–15
Excitatory/inhibitory control		
Glutamate buffering	<i>In vitro</i>	16,17
Glutamate/glutamine cycle	<i>In vitro/ex-vivo</i>	18,19
GABAergic activity	<i>In vitro/ex-vivo</i>	20–22
D-serine release for LTP	<i>In vitro</i>	23
Myelination	<i>In vivo/in vitro</i>	10
Cognitive impact/function	<i>In vivo</i>	24
Trophic support with gliotransmitters	<i>In vitro</i>	25,26
Axon regeneration	<i>In vivo</i>	27
Neurovascular coupling	<i>In vivo</i>	28,29
Blood-brain barrier permeability	<i>In vitro</i>	30
Regulation of neural oscillations	<i>In vivo</i>	31

multivesicular bodies, and characteristic size of ~30–120 nm. By fusion of multivesicular bodies with the plasma membrane, exosomes are released by exocytosis from most eukaryotic cells.³² These vesicles carry a large diversity of molecules, such as RNA species, proteins, and lipids, that can modify the physiology of nearby or distant target cells.

The current knowledge on the mechanisms involved in the biogenesis and secretion of exosomes, as well as their possible functional interaction with target cells, has been described in several excellent recent reviews.^{32–35} Most of this knowledge relies on the isolation of exosomes from extracellular fluids by differential ultracentrifugation and further characterization by size and presence of protein markers.³⁶ However, a major problem has more recently been identified: this isolated exosome-like small extracellular vesicle fraction contains a range of small vesicle subpopulations consisting of vesicles of larger size (>150 nm) or of nonendosomal origin, while in most *exosome* studies, their endosomal origin has not been demonstrated.³⁷ Thus, the identification of specific protein markers and general protein markers among vesicle subpopulations as well as the implementation of additional purification steps leading to homogeneous preparations are under active research. In this commentary, we will focus on the available literature in the field that uses mostly small extracellular vesicle preparations obtained by ultracentrifugation that are enriched in exosomes, as revealed by the current available experimental tools.

Despite the large amount of evidence about exosomes derived from cells of the nervous system,³⁸ specific research about astrocyte-derived exosomes is scarce. Some of the pioneers' evidences for the presence of exosomes in astrocyte cultures came from Milligan's and Agnati's group in 2007 and 2010,

respectively. They found the presence of classical exosome markers in a fraction obtained after the well-accepted differential centrifugation protocol.^{39,40} Three years later, it was shown that astrocyte-derived exosomes harbor synapsin-1, a glycoprotein that was released from these extracellular vesicles following an increase in extracellular potassium levels. Although they did not show a direct effect of these exosomes on neurons, it was suggested that released synapsin-1 could have a positive effect on neurite outgrowth and neuronal survival.⁴¹ Soon after, it was reported that astrocytes exposed to β -amyloid both *in vitro* and *in vivo* release exosomes that trigger apoptosis in other astrocytes, acting in an autocrine fashion.⁴² Using a different strategy, astrocytes may use extracellular vesicles, probably including exosomes, to transport the excitatory amino acid transporters EAAT-1 and EAAT-2, essential components in the maintenance of glutamate homeostasis. Interestingly, this extracellular vesicle fraction is able to actively take up aspartate, suggesting that in addition to their potential role in cell–cell communication, exosomes may participate in glutamate clearance.⁴³ Interestingly, members of the heat shock protein 70 family as well as astrocyte-specific glycolytic enzyme aldolase C have also been found in this fraction, raising the question about the potential transfer of these proteins with functional consequences to neurons.^{39,44} Accordingly, it was recently shown that astrocytes release exosomes containing nonpathogenic prion protein that can be transferred to neurons, inducing protection after exposure to a series of stressors such as hypoxia, hypoglycemia, and ischemia.⁴⁵ Thus, astrocyte-derived exosomes are capable of transferring proteins and lipids to target cells to modify their function.⁴² Strikingly, astrocyte-derived exosomes may have far-reaching consequences *in vivo*: they facilitate the outgrowth of metastatic cells in the brain by inhibiting the expression of the tumor suppressor phosphatase and tensin homolog (PTEN) through a functional transfer of miR-19a.⁴⁶ Nevertheless, as far as we know, there is no functional evidence of exosome-mediated transfer of astrocytic miRNAs to neurons.

After the first evidences were provided by Valadi et al, the functional transfer of exosomal components such as proteins and nucleic acids to elicit responses on target cells has been reported in several studies^{47,48} and has been summarized recently.³⁵ Next, we will focus here on the possible transfer of miRNAs from astrocytes to neurons by means of exosomes.

MiRNAs in Exosomes

Exosomes as well as other extracellular vesicles contain mRNAs and a plethora of noncoding RNAs, among which miRNAs stand out for their capacity of reprogramming protein expression in recipient cells. A long precursor miRNA molecule is transcribed in the nucleus, and after several sequential processing steps by RNase complexes, it is transported as pre-miRNA to the cytoplasm.^{49–51} Here, the pre-miRNA hairpin is cleaved by the RNase Dicer producing the ~22 nucleotides, double-stranded mature form of the miRNA.⁵² The mature form is



loaded onto the RNA-induced silencing complex (RISC), where one of the strands (ie, the passenger strand) is degraded after recruiting argonaute proteins while the other, called the leading strand, is held inside the mature complex carrying argonaute 2 (Ago2).⁵³ After this processing, a short sequence of 6–8 nucleotides or *seed sequence* of the miRNA is capable of targeting the 3' UTR region of mRNAs to inhibit translation by two mechanisms: (1) the direct degradation of the mRNA or (2) translational repression by reducing the recognition capacity of ribosomes to mRNA.⁵⁴ As each miRNA harbors target-recognition motifs in as many as hundreds of mRNAs, they are able to control complex biochemical processes in a coordinated manner and thus, disease and health states.

It is important to consider that genes coding for miRNAs tend to form clusters, defined as groups of genes that are separated by no more than 1 MB between them, in such a way that three to six miRNA genes are usually found in one cluster. All of these genes may be transcribed as polycistrons and, surprisingly, have preference for targeting functionally related genes.^{55,56} For example, one of the most studied mammalian clusters is miR-17-92, which is composed of six members: miR-17, miR-19b, miR-20a, miR-92, miR-18a, and miR-19a. All of them, except miR-18a, are demonstrated to downregulate PTEN, inducing axonal elongation.⁵⁷ Interestingly, all of these miRNAs have been previously described in astrocytes, and miR-19a is contained in exosomes.⁴⁶ In addition, miR-26a, although located outside this cluster, also favors neurite/axonal elongation by targeting PTEN as well as glycogen synthase kinase-3 β (GSK-3 β) (see below).^{58,59}

The loading of miRNAs into exosomes leads to a specific enrichment of some miRNAs in them in comparison to their originating cells, mediated by sorting mechanisms that include: (1) the presence of specific miRNA sequences (including uridylylated miRNAs and EXOmotifs), (2) miRNA binding to lipid raft-like regions in the cytoplasmic face of the multivesicular body limiting membrane, and (3) interactions with proteins such as sumoylated heterogeneous nuclear ribonucleoproteins or Ago2.^{60,61} The participation of Ago2 in miRNA loading is further demonstrated because its KRAS–MEK–ERK pathway-dependent phosphorylation decreases the sorting of Ago2 and its associated miRNAs to exosomes.^{62,63} Although miRNAs are selectively retained or released by different cell types,^{48,64–66} the mechanisms underlying selective packaging of miRNAs into astrocyte exosomes needs to be further explored.

Exploring Astrocyte's miRNA Content: Unraveling the Words to Understand Exosome-mediated Messages to Target Cells

Over the past few years several studies have provided a deeper insight into the miRNA species that are expressed or enriched in astrocytes, or even unique compared to other CNS cell types (eg, neurons, oligodendrocytes, and microglia).^{67–72} We will summarize some of the reported miRNAs and their functional relevance in different cellular contexts.

Some of the miRNAs found in astrocytes may be crucial to determine a glial fate (as opposed to a neuronal one) from progenitor cells.⁷³ miR-146a, for example, may prevent astrocytes from expressing neuronal proteins,⁶⁷ and provided that certain conditions are met, miR-34a can promote astroglialogenesis.⁷⁴ MiR-9 and miR-124 provide another example of a miRNA in regulating cellular differentiation fate. If the miR-124 binding site in the 3'UTR of the histone methyltransferase enhancer of zeste homolog 2 (EZH2) is manipulated as to prevent the action of this miRNA, astrocytic differentiation is enhanced (and neuronal differentiation diminished).⁷⁵ Consistent with these results, overexpression of miR-124a or miR-9 in neural precursor cells promotes neurogenesis and reduces gliogenesis.⁷⁶ Studies performed in different brain regions of humans show that many miRNAs are common to all astrocytes, regardless of the brain region analyzed, whereas others are unique to certain areas (eg, miR-129 and miR-181a were not found in interlaminar astrocytes, but were detected in astrocytes from deep cortical layers), suggesting their involvement in the regulation of factors specific to those regions. In line with the previously mentioned results, a low expression of miRNAs involved with inflammatory responses was observed in fetal compared to adult white-matter astrocytes. A differential expression was also observed between adult interlaminar astrocytes compared to those found at the fetal germinal matrix, as the latter contains miRNAs involved in neurogenesis with antiapoptotic properties (eg, miR-210, miR-129, and miR-214).⁷⁷

In various pathological contexts, astrocyte miRNAs have been shown to increase or decrease their levels, as summarized in Table 2. The highly expressed miR-29 family in astrocytes is transported with functional consequences in exosomes.⁸² When the expression of miR-29b increases by exposure to pathological conditions in the brain and in cultured astrocytes, its derived exosomes reduce neuronal viability by targeting the platelet-derived growth factor (PDGF-B).⁷⁸ Surprisingly, transfecting a mimic of miR-29a into cultured astrocytes protects them from oxygen-glucose deprivation.⁷⁹ As the targets of miR-29 are both pro- and antiapoptotic, this apparently controversial effect can be explained by different mRNAs being overexpressed and thus targeted under diverse physiological or pathological conditions.⁷⁰

Another frequently addressed neuropathological phenomenon is astrogliosis. For example, after spinal cord injury, the activation of astrocytes involving the overexpression of astrocyte-specific genes such as the glial fibrillary acid protein favors the repair and limits the inflammation of the damaged zone.⁸⁰ The concomitant increase of miR-21, however, attenuates the beneficial astrocytic response to injury and limits axon numbers in the scar zone. Although the targets of miR-21 explaining this effect are not clear, inhibiting miR-21 facilitates recovery and increases axon density.⁸¹ The expression of miR-146 is upregulated in tissue obtained from patients with intractable epilepsy, especially in areas of noticeable gliosis.

Table 2. Regulation of miRNAs in astrocytes.

CONDITION	miRNA IN ASTROCYTES	EFFECT	SOURCE	REF
Spinal cord injury	21	↑ around lesion	In situ (mouse)	81
	145	↓ around lesion	In situ (rat)	88
Glioneuronal lesions (from epileptic patients)	146a	↑	In situ (tissue from epileptic patients)	82
Forebrain ischemia	29a	↑ dentate gyrus	Hippocampus	79
		↓ CA1		
Multiple sclerosis lesions patients	155, 160	↑	Laser capture microdissection from white matter	83
Oxygen Glucose Deprivation	21, 29b, 30b, 107, 137, 210	↑	Cultured rat astrocytes	84
	7	↓	Cultured mice astrocytes	89
Transfection with human heme oxygenase 1	140, 17, 16	↑	Cultured rat astrocytes	68
	297, 206, 187, 181a, 138, 29c	↓		
Ammonia	26a, 30b, 30e, 125, 135, 145, 425... (43 in total)	↑	Cultured rat astrocytes	69
Aβ 42	146a	↑	Cultured human astrocytes	85
LPS	145	↓	Cultured rat astrocytes	88
LPS and IFN-γ	146a, 155	↑	Cultured mice astrocytes	73
	149, 455, 351, 298	↓		
	146a, 155	↑	Cultured marmoset astrocytes	
	149, 455, 125b	↓		
IL-10 or IL-4	145	↑	Cultured rat astrocytes	88
IL-β	146a	↑	Cultured human astrocytes	82,85
Inflammatory cytokines (e.g. IL-β, IFN-γ, TNF-α, etc)	23a, 146a, 155	↑	Cultured human astrocytes	83
IL-6	125b	↑	Cultured human astrocytes	86
IL-1/IFN-γ	155, 483-3p, 147, 27a, 147b, solexa-578-1915, 23a, 155, 29b-1, 33b, 146a	↑	Cultured human astrocytes	87
	296-3p, 767-3p	↓		

This miRNA regulates inflammation: in cultured astrocytes, its upregulation reduces the interleukin-1β (IL-1β) mediated increase of interleukin-6 (IL-6) and cyclooxygenase 2 (COX-2), two molecules associated with inflammatory gliosis.⁸² In tissue obtained from multiple sclerosis patients, most of the miRNAs induced by the lesions are present in astrocytes, with a subset of these being upregulated by cytokines (eg, miR-23a, miR-146a, and miR-155). Some of these miRNAs (miR-155, miR-326, and miR-34a) regulate the expression of CD47, leading to the release of macrophages and promoting myelin phagocytosis.⁸³ A differential expression of astrocytic miRNAs has been observed after oxygen-glucose deprivation, and this expression profile changed with time after deprivation, as was the case with miR-29b and miR-21, upregulated after 12 hours, or of miR-30b and miR-107, upregulated after 6 and 8 hours after oxygen-glucose deprivation, respectively.⁸⁴ The levels of miRNAs in astrocytes also change after exposure to various stressors that increase oxidative stress such as Aβ-42,⁸⁵ ammonia,⁶⁹ and transfection with

human heme oxygenase 1, an enzyme induced by oxidative stress that is overexpressed in a number of CNS disorders.⁶⁸ In line with these observations, exposing astrocytes to cytokines and pro-inflammatory molecules (eg, interleukins, tumor necrosis factors, and interferon gamma) has a profound impact on the expression of miRNAs.^{73,82,83,85-88}

Though still speculative, the prospect that some of the abovementioned miRNAs may be found in exosomes circulating in body fluids and thus amenable to be used as biomarkers, or the possibility of engineering exosomes with sequences that either mimic or antagonize these miRNAs to target astrocytes under pathological conditions remains an exciting topic of exploration.

The miRNA Content of Astrocyte-derived Exosomes: Focusing on miR-26a

An extensive RNA sequencing profile of astrocyte-derived exosomes under different physiological or pathological conditions is a must to predict the main functional pathways that exosomal miRNAs modify on target cells. On the next part of

this commentary, we will focus on the possible transfer of miR-26a within exosomes based on the following arguments: (1) it is highly expressed in astrocytes when compared with neurons,⁷² (2) we have found miR-26a to be present in astrocyte-derived exosomes (unpublished data), (3) this miRNA is dysregulated in several CNS diseases (see below), and (4) miR-26a can be sorted to exosomes and transported by these vesicles in the plasma, serum, whole blood, urine, or secreted in vitro by human umbilical vein endothelial cells.^{90–94}

Altered levels of miR-26 have been reported for some of the most prevalent disorders of the CNS. In human post-mortem tissue, this miRNA can be upregulated or downregulated, depending on the brain region, at different stages of Alzheimer's disease (AD)^{95–99} as well as in biofluids (eg, blood, serum, and cerebrospinal fluid) obtained from AD patients.^{100,101} Increased levels of miR-26b have been observed in the substantia nigra of patients with Parkinson's disease (PD),^{102,103} as well as in the blood of PD patients who underwent treatment compared with those who did not.¹⁰⁴ Levels of miR-26a are enhanced in the relapsing phase of patients with relapsing-remitting multiple sclerosis compared to the remitting phase and to healthy controls.¹⁰⁵ Patients diagnosed with major depression showed lower blood levels of miR-26a/b,¹⁰¹ while treatment with the antidepressant escitalopram increased its blood levels.¹⁰⁶ Patients diagnosed with schizophrenia showed lower blood levels of miR-26a/b,¹⁰¹ while the precursor and mature miR-26b was upregulated in postmortem tissue from the superior temporal gyrus and the dorsolateral prefrontal cortex¹⁰⁷ and downregulated in post-mortem tissue from the prefrontal cortex.¹⁰⁸

During neurogenesis, an increase in the level of some miR-26 family members has been reported,^{109–111} in contrast to pre-miR-26,⁷² which remains at constant levels.¹¹⁰ In zebrafish, miR-26b targets the mRNA of the C-terminal domain of the small phosphatases (*ctdsp2*) gene. As the sequence for miR-26b is localized in one of the introns of *ctdsp2*, this is a case where a gene is transcribed simultaneously with the miRNA that regulates its expression. For neuronal differentiation to occur, miR-26b has to inhibit *ctdsp2* translation, therefore allowing for genes containing the repressor element 1 (RE-1; otherwise inhibited by Ctspd2) sequence to be expressed. MiR-26a is also able to repress the expression of *ctdsp2* and shows a upregulation similar to miR-26b during progression of neurogenesis.^{110,112}

The predicted mRNA targets of a miRNA are commonly validated by tools such as quantitative real-time polymerase chain reaction (qRT-PCR), Western blots, or luciferase reporter assays. The first two do not exclude that the decrease of a given mRNA or protein is secondarily associated with the downregulation of the miRNA target sequence. However, the luciferase reporter assay provides direct evidence of mRNA targets, because the expression of the luciferase reporter 3'-UTR construct will decrease in the presence of a regulatory miRNA, which is able to interact with the

introduced 3'-UTR sequence.¹¹³ Figure 1 contains the proposal of the present commentary, in which astrocyte-derived exosomes carrying miR-26a will influence neuronal function and brain physiology through validated and predicted targets.

One of the validated targets of miR-26 members in neurons and other cell types under physiological/pathological conditions is GSK-3 β .^{59,114–116} GSK-3 β is a cytosolic protein that takes part in the canonical Wnt pathway, and it is capable of forming a complex with β -catenin and two other proteins: axin and adenomatous polyposis coli.¹¹⁷ In the absence of Wnt signaling, β -catenin is phosphorylated by GSK-3 β and degraded.¹¹⁸ However, under Wnt signaling, GSK-3 β is inhibited, allowing the stabilization of β -catenin and its translocation into the nucleus to activate the expression of Wnt target genes.^{119,120}

Another target of miR-26 detected in neurons is the phosphatase PTEN, a negative regulator of the phosphoinositide 3-kinase (PI3K)/AKT pathway.⁵⁸ PI3K/AKT inhibits the previously mentioned target of miR-26a GSK-3 β , by phosphorylation of a serine residue.¹²¹ Inhibition of PTEN increases neurite outgrowth both in vitro and in vivo,^{122–125} and these changes resemble those observed under miR-26a regulation, with the concomitant decrease in PTEN levels and increase in AKT protein levels.^{58,126}

Other targets validated by luciferase reporter assays include Wnt5a,^{127,128} a ligand of the noncanonical, β -catenin independent pathway,¹²⁹ and brain-derived neurotrophic factor (BDNF),¹³⁰ a growth factor that controls a wide range of mechanisms in the CNS, including plasticity and synaptic maturation.¹³¹

Potential and Demonstrated miR-26a Functional Consequences on Neurons

In hippocampal neurons, the exogenous addition of miR-26a mimics induces an increment in axonal length,¹¹¹ consistently, electroporation of an inhibitor of miR-26a prevents axonal regeneration in vivo after sciatic nerve crush.⁵⁹ Neurite outgrowth is also enhanced by the addition of miR-26a in dorsal root ganglia and in primary cortical neurons.^{58,126} In mature primary hippocampal neurons (ie, 14DIV), transfection with a miR-26a-expressing plasmid or a miR-26a sponge did not affect spine area or density, but miR-26a did inhibit the spine enlargement induced after 90 minutes of chemical long term potentiation (LTP). Reduction in the levels of miR-26a is necessary for maintenance of LTP but not for its induction, and the effects of miR-26a on both, spine remodeling and LTP, was mediated by the miR-26a targeting of ribosomal S6 kinase 3 (RSK3).¹³² This evidence suggests that this miRNA could have a differential effect in two different neuronal compartments: the dendritic and the axonal arborization.

Outside the CNS, other targets of miR-26a have been validated: plasminogen activator inhibitor 1 RNA-binding protein (serbp1),¹³³ phosphatidylinositol-4-phosphate 3-kinase C2 domain-containing alpha polypeptide

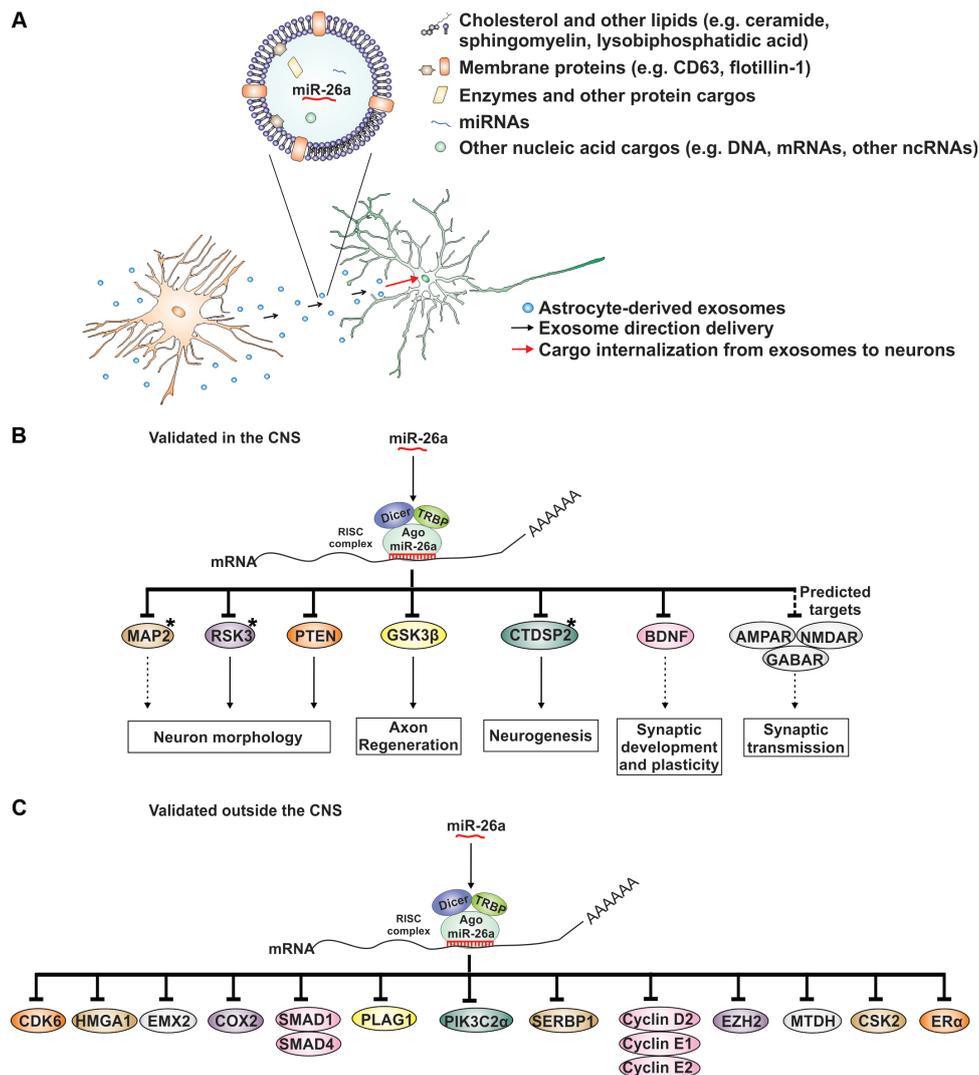


Figure 1. Exosomes are released by astrocytes and their cargo internalized by neurons, resulting in the regulation of neuronal function according to the cargo identity. **(A)** A simplified diagram of an exosome and its principal components. miR-26a is highlighted as an example of an miRNA that is highly expressed in astrocytes and transported by exosomes. **(B)** Targets of miR-26a in the central nervous system. miR-26a is incorporated into the RNA-induced silencing complex, where it can recognize a mRNA sequence complementary to its seed region, leading to RNA silencing. Those mRNA targets of miR-26a that have not been validated by luciferase assays are marked with an asterisk (*). Included in the diagram are targets found in silico that need further validation. Solid arrows show the reported impact on neuronal physiology. Dashed arrows show a possible impact on neuronal physiology that needs to be corroborated by experimental evidence after modulation of miR-26a levels. Protein names: MAP2, microtubule associated protein 2; RSK3, ribosomal protein S6 kinase; PTEN, tumor suppressor phosphatase and tensin homolog; GSK-3 β , glycogen synthase kinase-3 β ; CTDSP2, C-terminal domain of small phosphatases 2; BDNF, brain-derived neurotrophic factor; NMDAR, *N*-methyl-D-aspartate receptor subunits; AMPAR, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor subunits; GABA, (ionotropic) gamma-aminobutyric acid receptor subunits. **(C)** MiR-26a targets outside the central nervous system. Their possible regulation in the brain needs to be explored experimentally.

Abbreviations: CDK6, cyclin-dependent kinase 6; HMGA1, high mobility group AT-hook 1; EMX2, empty spiracles homeobox 2; COX-2, cyclooxygenase 2; SMAD1, mothers against decapentaplegic homolog 1 and 4; PLAG1, pleiomorphic adenoma gene 1; PIK3C2 α , phosphatidylinositol-4-phosphate 3-kinase C2 domain-containing alpha polypeptide; SERBP1, plasminogen activator inhibitor 1 RNA-binding protein; EZH2, enhancer of zeste homolog 2; MTDH, metadherin; CSK2, cyclin-dependent kinases regulatory subunit 2; ER α , estrogen receptor alpha.

(PIK3C2 α),¹³⁴ and mothers against decapentaplegic homolog 1 and 4 (Smad1 and Smad4).^{135–137} Cyclooxygenase-2 (COX-2),¹³⁸ metadherin (MTDH),^{139,140} cyclin-dependent kinases regulatory subunit 2 (CSK2),¹⁴¹ enhancer of zeste homolog 2 (EZH2),^{139,142,143} estrogen receptor alpha (ER α),¹⁴⁴ pleiomorphic adenoma gene 1 (PLAG1),¹⁴⁵ empty spiracles homeobox 2 (emx2),¹⁴⁶ high mobility group AT-hook

1 (HMGA1),¹⁴⁷ cyclin-dependent kinase 6 (CDK6), cyclin E1,¹⁴⁸ and cyclins D2 and E2.¹⁴⁹ Further experiments will be needed to study if miR-26a is also regulating some of these targets in the CNS. Last, it is worth mentioning that in silico studies of predicted targets for miR-26a include mRNAs that express subunits for receptors involved in synaptic transmission, such as GABAR, AMPAR, and NMDAR. These need



to be validated in the CNS and the impact of this modulation remains to be assessed.

Conclusion

The role of exosomes derived from astrocytes on neuronal physiology promises to be an exciting area of study. Such a mechanism of communication suggests a new level of complexity in the processing of information in the CNS. MiRNAs stand out as key regulators of cellular processes, due to their capacity to inhibit hundreds of different mRNA targets. We have focused on miR-26a in this review, as it targets mRNAs that may impact neuronal function and morphology, and its dysregulation has been implicated in many neurological disorders, such as depression and AD. As such, it may be an interesting therapeutic target and biomarker for pathologies of the CNS. This and other miRNAs packaged in exosomes of astrocytic origin may open the doors to a better understanding of how astrocytes impact on neuronal functions, and it may also provide us with new tools to compensate for cellular malfunctions under pathological conditions.

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Author Contributions

Wrote the first draft of the manuscript: JPR, CL, and AL. Developed the structure and arguments of the paper: CL, AL, and UW. Produced figures and tables: JPR, AF, CL, AL, and UW. Agreed with manuscript proposal and conclusions: CL, JPR, AL, AF, and UW. Wrote the final version of the manuscript: UW. Made critical revisions: AL. All the authors reviewed and approved the final manuscript.

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