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Lipid-based carriers of microRNAs and intercellular communication

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Abstract

Purpose of review—Extracellular microRNAs (miRNAs) are uniquely stable in plasma, and the levels of specific circulating miRNAs can differ with disease. Extracellular miRNAs are associated with lipid-based carriers and lipid-free proteins. miRNAs can be transferred from cell-to-cell by lipid-based carriers and affect gene expression. This review summarizes recent studies that demonstrate the transfer of miRNA between cells and their potential role in intercellular communication.

Recent findings—Microvesicles, exosomes, apoptotic bodies, lipoproteins, and large microparticles contain miRNAs. Recent studies have demonstrated that miRNAs are transferred between dendritic cells, hepatocellular carcinoma cells, and adipocytes in lipid-based carriers. miRNAs are also transferred from T cells to antigen-presenting cells, from stem cells to endothelial cells and fibroblasts, from macrophages to breast cancer cells, and from epithelial cells to hepatocytes in lipid-based carriers. The cellular export of miRNAs in lipid-based carriers is regulated by the ceramide pathway, and the delivery of lipid-associated miRNAs to recipient cells is achieved by various routes, including endocytotic uptake, membrane-fusion, and scavenger receptors.

Summary—Cellular miRNAs are exported in and to lipid-based carriers (vesicles and lipoprotein particles) and transferred to recipient cells with gene expression changes as intercellular communication.

Keywords

intercellular communication; lipids; microRNAs

INTRODUCTION

microRNAs (miRNAs) are short noncoding RNAs that post-transcriptionally regulate gene expression by targeting coding and untranslated regions (5′ and 3′ untranslated regions) of messenger RNA (mRNA) [1–3]. Over 1500 human miRNAs have been curated in miRBase (miRBase.org), and each cell type typically contains a specific subset of approximately 150–

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300 miRNAs. One miRNA can target and regulate potentially hundreds of genes (mRNAs) through seed-based targeting, and one gene (mRNA) can be under negative repression of more than one miRNA. The nature of miRNA gene repression is, therefore, complex and highly interconnected with both direct and indirect effects. Nonetheless, miRNA networks modulate many aspects of cellular homeostasis and physiology and thus are a promising new area of investigation.

The role of miRNAs in cholesterol homeostasis and lipid metabolism has been the focus of multiple recent studies [4–6]. miR-33, an intronic miRNA cotranscribed with the sterol regulatory element binding transcription factor 2, has been demonstrated to modulate cholesterol metabolism through regulation of ATP-binding cassette transporter A1 [5,6] and overall lipid homeostasis through multiple targets in the liver [7]. Although miRNAs likely play a role in most homeostatic pathways, they also appear to be powerful regulators in the development of various diseases. Dysregulation or altered miRNA expression/function has been implicated in diabetes [8■], cardiovascular disease [9–12,13■], and numerous types of cancers. The role of miRNAs as biomarkers has also gained significant attention in both academic research labs and in-vitro diagnostic test companies. miRNAs are stable in plasma and differential plasma miRNA profiles have been described for many diseases, including fatty liver [14], atherosclerosis [15–17], and cancer [18–22]. Circulating miRNAs have enormous potential as novel disease biomarkers; however, circulating extracellular miRNAs may also be biologically active. Extracellular miRNAs have been demonstrated *in vitro* to be taken up by recipient cells and to alter gene expression [23,24]. miRNAs could, therefore, be considered like hormones as a possible form of intercellular communication; however, their physiological function and in-vivo role, in this regard, remains to be definitively established. Many of the steps, however, that would be necessary for miRNAs to act as messengers in intercellular communication, such as cellular export, plasma transport and cellular uptake have been described. Here, we highlight recent studies describing the interaction of miRNA with various lipid-based carriers and how they participate in the transport and cellular delivery of miRNAs in the modulation of gene expression.

DIVERSITY OF LIPID-BASED CARRIERS OF miRNAs

Given the lability of most RNAs, it is perhaps surprising that circulating miRNAs in plasma are relatively stable and appear to be resistant to plasma ribonucleases (RNase) [25]. This protection has been shown to be conferred by their lipid-based carriers that shield the small RNAs from plasma RNases [24–27]. Circulating miRNAs themselves are not inherently resistant to degradation [28■■]. Once their lipid-based carriers are disrupted, either by the addition of detergents [23] or by sonication [29■■], they are sensitive to RNase degradation. The most widely studied lipid-based miRNA carriers are exosomes, which are membrane-derived vesicles that originate from endosomal multivesicular bodies [24,30,31]. Exosomes, which are derived from intracellular vesicles of approximately 100 nm in diameter, have a similar size range of 40–100 nm when released into the interstitial fluid [32]. The external release of exosomes by the fusion of multivesicular endosomes with the plasma membrane is a distinct process that is not shared by other membrane-derived vesicles. In addition to their outer shell of membrane lipids, exosomes also contain apparently nonrandom sets of proteins (cytoplasmic and transmembrane), mRNAs, and

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small RNAs as their cargo, which are either trapped in the aqueous core or associated with the lipid shell [31,33]. Exosomes are secreted from most cell types, including inflammatory cells, tumor, stem cells, muscle, neurons, and epithelial cells [21,34■,35■,36,37]. Exosomes were first shown to participate as vehicles for intercellular communication in studies that demonstrated the transfer of antigens to recipient immune cells [38]. In 2007, it was first shown that exosomes contain miRNAs and can deliver RNA to recipient cells [37]. With the many recent advances in the experimental tools for measuring miRNAs, many studies have now profiled the exosomal miRNA content in different diseases [18,20,22,30].

In addition to exosomes, there are various other membrane-derived vesicles (~50–4000 nm diameter) in the circulation, and many of these have also been described to contain miRNAs [32]. Vesicles can also originate from the budding of the plasma membrane or blebbing under various pathophysiologic states. Although various nomenclatures have been proposed for these other types of membrane vesicles, ‘microvesicles’ are the most widely used. It is important to note, however, that many of the commonly used isolation approaches separate (pellet) both exosomes and microvesicles; thus, many of the reported vesicle-miRNA signatures cannot be definitively assigned to one or the other lipid-based carrier. However, if one classifies microvesicles by size greater than exosomes (>100 nm diameter), sometimes referred to as microparticles, then ultracentrifugation techniques can be used to generally compare the two carriers [32,39]. Multiple studies have characterized microvesicle-miRNA signatures and have shown cell-to-cell transfer of their miRNA cargo [40–43]. Apoptotic bodies have also been demonstrated to contain miRNAs and deliver them to cells. For example, miR-126 in apoptotic bodies derived from endothelial cells is taken up by other cells in the vessel wall and can stimulate cytokine production [19,32,44,45]. Lipoproteins, of course, are the other major lipid-based particle in the plasma and we have recently demonstrated that HDLs and LDLs also contain miRNAs [46■■]. Highly pure fractions of HDL were found to contain distinct miRNA signatures, and the HDL-miRNA profiles were altered with cardiovascular disease in humans and mice. Furthermore, HDL was demonstrated to deliver miRNAs to human hepatocellular carcinoma cells (Huh7) and scavenger receptor class B, type 1 (SR-BI) overexpressing baby hamster kidney cells [46■■].

Recent findings suggest that many, if not the majority, of circulating miRNAs in plasma may not be associated with or confined within exosomes or microvesicles [26■■,28■■,39]. Multiple studies agree that the majority of circulating miRNAs remain in the supernatant fraction after ultracentrifugation to isolate (pellet) exosomes/microvesicles [26■■,39]. Furthermore, evidence suggests that a significant fraction of nonvesicle extracellular miRNAs may be associated with protein complexes, with a molecular weight between 50 and 300 kDa [26■■]. Argonaute 2 (AGO2), an intracellular structure–function protein found within the RNA-induced silencing complex, has been observed in the circulation associated with microvesicles [40], but also independent of vesicles [26■■,28■■]. Extracellular AGO2 (~96 kDa) has been demonstrated to be associated with miRNA and has been observed to be retained by 100 kDa ultrafiltration of plasma after the ultracentrifugation removal of vesicles [26■■,28■■]; however, AGO2-independent miRNA was also observed [26■■]. Using mass spectrometry, we did not observe AGO2 on human HDL [46■■]. Recently, a comprehensive study into circulating ribonucleoprotein

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complexes confirmed that circulating miRNAs are not confined to exosomes or microvesicles, and that a significant fraction of circulating miRNAs are likely to be associated with proteins [28■■]. In this study, many extracellular miRNAs were found by size-exclusion chromatography to coelute with AGO2 [28■■]. Although these findings do not negate the relevance of the transport and cell delivery of miRNAs by lipid-based microcarriers, they do raise intriguing questions about whether these miRNAs have a different biologic function or source. It has been proposed that extracellular AGO2 bound to miRNAs are likely to be released into the circulation as a consequence of cell lysis/necrosis and may not participate in intercellular communication; however, this hypothesis has yet to be carefully tested.

CELLULAR MICRORNA EXPORT

Many different proteins and pathways have been linked to exosome export, including the ceramide pathway. Ceramide has been shown to facilitate the formation of endosomal vesicles [47], and the export of specific miRNAs to exosomes [39]. Knockdown of neutral sphingomyelinase 2 (nSMase2), the key enzyme in ceramide synthesis, with siRNAs leads to a decrease in the cellular export of miR-16 and miR-146a [24]. Furthermore, chemical inhibition of nSMase2 with manumycin-A [48■■] or GW4869 [24,49■■] attenuated the release of exosomes and exosomal-miRNA export, including miR-16. Conversely, we observed that inhibition of nSMase2 with GW4869 resulted in a significant increase of cellular miRNA export to HDL [46■■]. The substrate of nSMase2 is the bioactive lipid sphingomyelin, which is transported on lipoproteins and is found in cellular membranes. Furthermore, nSMase2 is described to be predominantly localized to the inner leaflet of the plasma membrane [50–52]. Future studies are required, however, to fully understand the miRNA export process and their packaging into lipid-based carriers.

CELLULAR miRNA UPTAKE FROM LIPID-BASED miRNA CARRIERS

Less is even known about the cellular uptake of miRNAs than their export, but there are two main hypotheses. For vesicles (microvesicle/exosomes) containing miRNAs, it has been proposed that they are delivered to cells either by a process involving endocytosis [53,54] or by membrane fusion [55■■,56]. Surface or transmembrane proteins on vesicles and/or cell surface receptors likely contribute to the initial recognition of target cells; however, the identity of these cell surface receptors or their ligands is unknown. Once bound, the vesicles are likely to be internalized by an endocytotic pathway [54] or by macropinocytosis [57]. It has also been shown that exosomes can be taken up by phagocytosis [58]. Pathways involving membrane fusion and the dumping of vesicle lumen contents directly into the cytoplasm may preserve the functional integrity of transferred miRNAs as opposed to the endocytotic pathway, which may lead to the degradation of miRNAs. We have observed that SR-BI mediates the uptake of miRNAs associated with HDL [46■■]. Because the selective core uptake of lipids on HDL by SR-BI also bypasses the endosomal–lysosomal pathway [59,60], delivery of miRNAs by this pathway may also lead to less degradation of miRNAs.

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INTERCELLULAR COMMUNICATION BY miRNAs

We are only beginning to understand the physiologic impact of miRNA intercellular communication, but many studies have now shown that this process leads to gene expression changes in recipient cells (Table 1) [61■■–63■■]. Dendritic cells have been shown to communicate through exosomal miRNA export and delivery to recipient dendritic cells through membrane fusion, leading to the release of miRNAs into the cytosol of recipient cells [55■■]. The miRNA content of dendritic cell-released exosomes was found to be dependent upon cell maturity and exosome-delivered miRNAs were shown to be functional in recipient dendritic cells, as determined by gene reporter (luciferase) assays [55■■]. Exosomes derived from T cells, B Cells, and dendritic cells all have distinct miRNA signatures from their parent-cell type. Recently, functional exosomal miRNA (miR-335) was found to be transferred to antigen presenting cells (APC) from T cells during immune synapsis, as determined by miR-335 target sex determining region Y-related high mobility group (HMG) box 4 (luciferase) assays. Communication between T cells and APCs were dependent upon nSMase2 and Brefeldin A-inhibited guanine nucleotide-exchange protein 2 activities [48■■].

Multiple studies have found that tumor cells secrete microvesicles/exosomes containing specific miRNAs [22,37,43,64]. A recent report demonstrated that microvesicles secreted from renal cell carcinoma $CD105^+$ stem cells promote angiogenesis [61■■]. One proposed mechanism of increased angiogenesis is the delivery of proangiogenic miRNAs to endothelial cells by microvesicles. $CD105^+$ microvesicles-induced capillary formation in Matrigel, increased endothelial invasion, and increased tumor cell adherence in human umbilical vein endothelial cells (HUVECs) *in vitro*. These effects were blunted by pretreatment of microvesicles with RNase prior to delivery. Furthermore, HUVECs pretreated with $CD105^+$ microvesicles proliferated into small vessels within Matrigel plugs *in vivo* [severe combined immunodeficiency (SCD) mice], whereas HUVECS pretreated with RNAse-treated $CD105^+$ microvesicles or $CD105^-$ microvesicles did not. Both in-vitro and in-vivo results suggest a role for microvesicle genetic exchange in mediating the angiogenic response; however, the transfer and functionality of delivered miRNAs was not fully tested [61■■].

Hepatocellular carcinoma tumor cells also likely to communicate through the export and transfer of specific miRNAs [49■■]. Exosomes secreted by hepatocarcinoma cells (Hep3B) were found to transfer genetic material (luciferase transgene) between hepatoma cells. Over 100 unique miRNAs were observed in Hep3B-secreted exosomes. In-silico prediction studies identified putative mRNA targets for eleven miRNAs highly-enriched in Hep3B-derived exosomes, and found that transforming growth factor β activated kinase-1 (TAK1) and members of its pathway were likely target genes. Incubation of Hep3B cells with Hep3B-derived exosomes significantly reduced TAK1 protein expression after 72 h, which likely altered downstream TAK1 signaling and carcinogenesis [49■■,65]. These results suggest that exosomes transfer functional miRNAs between hepatoma cells and can influence tumorigenesis. Tumor-associated macrophages (TAM, interleukin 4 stimulated) have been shown to release exosomes containing miR-223 [63■■]. TAM-derived exosomes were found to increase breast cancer cell invasion *in vitro*. This effect is likely due to the

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delivery of miR-223, which was elevated in cocultured breast cancer cells, and is likely to be mediated by an altered Mef2C-β-catenin pathway as Mef2C is a validated miR-223 target [63■■,66].

Microvesicles released from THP-1 cells, a macrophage-like cell line, were found to deliver miR-150 to human microvascular endothelial cells (HMEC-1), with functional targeting and gene expression changes. THP-1-derived microvesicle delivery resulted in a 12-fold increase in cellular miR-150 levels and a significant reduction of the validated miR-150 target c-Myb (protein) in recipient endothelial cells [23,67]. c-Myb, a master transcription factor in proliferation, likely regulates cell migration and treatment of HMEC-1 cells with THP-1-derived microvesicles resulted in a significant increase in endothelial cell migration [23,67,68]. Furthermore, intravenous injections of THP-1-derived microvesicles increased miR-150 levels in mouse vasculature [23]. Adipocytes have also been shown to export miR-16, miR-27a, miR-222, and miR-146b in microvesicles [29■■]. Microvesicles from large adipocytes (Gce1-harboring/CD73-harboring) induced lipogenesis and esterification of palmitate in small adipocytes [29■■]. The induced esterification effects were dependent upon microvesicle-RNA integrity as sonication with RNase-treatment attenuated the microvesicle effect [29■■]. In addition to potential transfer of endogenous miRNAs, several studies have shown that exosomes and microvesicles can also transfer and deliver viral miRNAs to recipient cells [69–72].

Recently, plant miRNAs abundant in food (rice) were detected in human and animal sera and tissues (liver) of animals [62■■]. In this study, microvesicles secreted from small intestinal epithelial cells (Caco-2, transfected with MIR168a) contained specific miRNAs (plant MIR168a) that could be transferred to human hepatocytes (HepG2). This transfer resulted in a 100-fold increase in HepG2 MIR168a levels and a significant loss of MIR168a target protein [62■■]. This study suggests that exogenous miRNA from our diet can potentially affect gene expression in tissues. Finally, miRNA transport and delivery may also be important in development. Embryonic stem cells have been shown to secrete microvesicles with miRNAs, which can then deliver miRNA to embryonic fibroblasts [73].

CONCLUSION

Circulating miRNAs can be associated with a variety of lipid-based vesicles and lipoproteins, as well as with lipid-free protein complexes. The physiological relevance of miRNA intercellular communication to wellness or disease remains to be determined; however, circulating miRNAs show great promise as novel disease biomarkers and may lead to new diagnostic tests. Much remains to be learned, however, about the cellular export and import of miRNAs, but one possible consequence of this process is the generation of mobile carriers for intercellular communication. If the early in-vitro findings of circulating miRNAs affecting gene expression are confirmed in animal models, circulating miRNAs may also represent a new way to intervene therapeutically in treating a wide variety of diseases and in better understanding gene regulation.

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■ of special interest

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 159–160).

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KEY POINTS

- Lipid-based vesicles and lipoprotein particles transport miRNAs.
- Cellular miRNA export is mediated by the ceramide pathway.
- Lipid-based carriers deliver miRNAs to recipients cells and alter gene expression.
- Lipid-based miRNA carriers participate in intercellular communication.

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Table 1

MicroRNA intercellular communication

Mediator	Originating cell	Recipient cell	miRNA	Reference
Microvesicles (THP-1)	Monocytic leukemia cells (THP-1)	HMEC-1	miR-150	[23]
Microvesicles (Gce1-harboring/CD73-harboring)	Large adipocytes	Small adipocytes	miR-16; miR-27a; miR-222; miR-146b	[29■■]
Exosomes (J77 T-cell)	Jurkat-derived T-cells (J77cl20)	Antigen-presenting cells (Raji)	miR-335; miR-92a	[48■■]
Exosomes (Hep3B)	Hepatocellular carcinoma cells (Hep3B)	Hepatocellular carcinoma cells (Hep3B)	multiple	[49■■]
Exosomes (BMDC)	DC (BMDC)	DC2.4	miR-451; miR-148a	[55■■]
Microvesicles ($CD105^+$)	Renal cell tumor $CD105^+$ stem cells	Endothelial cells (HUVEC)	n/a	[61■■]
Microvesicles (Caco-2)	Small intestinal epithelial cells (Caco-2)	Human hepatoma cells (HepG2)	Plant MIR168a	[62■■]
Exosomes (TAM)	TAM	Breast cancer cells (SKBR3; MDA-MB-231)	miR-223	[63■■]

BMDC, bone marrow-derived dendritic cell; DC, dendritic cells; HMEC, human microvasculature endothelial cell; HUVEC, human umbilical vein endothelial cells; TAM, tumor-associated macrophages.