Abstract  The complexity of microRNA (miRNA)-mediated pathway control has burgeoned since the discovery that miRNAs are found in the extracellular space and constitute a form of cell-cell communication. miRNAs have been found in plasma, urine, and saliva and have recently been shown to be carried on lipoproteins. This has led to the proposal that circulating miRNAs may be useful biomarkers of various diseases, including cardiovascular disease, diabetes, and other forms of dysregulated metabolism. Although our understanding of the cellular machinery responsible for the secretion of miRNA is incomplete, it has been demonstrated that miRNAs are packaged into exosomes, microvesicles, and apoptotic bodies by a broad range of cell types. Intriguingly, a large portion of extracellular miRNA is found outside of any lipid-containing vesicle, and instead is associated with RNA binding proteins like argonautes 1 and 2, which may aid in their protection from abundant nucleases in the extracellular space. The excitement for miRNAs as biomarkers is mounting as more and more evidence supports that these noncoding RNAs are actively secreted from diseased tissues, possibly before the onset of overt disease. While caution should be taken in these early days, there is little doubt that extracellular miRNAs will hold tremendous potential as both diagnostic and therapeutic agents.—Rayner, K. J., and E. J. Hennessy. Extracellular communication via microRNA: lipid particles have a new message. J. Lipid Res. 2013. 54: 1174–1181.

Supplementary key words  exosomes • secreted • biomarkers • lipid carriers • therapeutics

In the decade following the discovery that the mammalian genome contained functional microRNA (miRNA) sequences, there has been an explosion in our understanding of how these tiny powerhouse nucleic acids mediate such elegant control over gene function. This has been further exemplified by the recent ENCyclopedia Of DNA Elements (ENCODE) project revelation that over 90% of the human genome is comprised of functional noncoding RNA, which prompted a revisiting of this previously disregarded genetic information (1). miRNAs are a specific class of noncoding RNA (ncRNA), and are defined as small, 20–22 nucleotide RNA molecules that are processed from a much larger primary transcript. Once processed into their mature form, miRNAs generally bind to complementary sequences in the 3′ untranslated region (UTR) of specific genes but can also bind to other regions of the gene including the 5′ UTR and the coding region (2, 3). Via mRNA destabilization and/or protein translation inhibition, miRNAs mediate silencing of their bound targets. Recently, the importance of miRNAs in the extracellular space has been exemplified by a number of studies showing specific and regulated export of miRNA from the cell, and the uptake and functional consequences in recipient cells. Moreover, circulating miRNAs are emerging as attractive biomarkers in various disease states, including cancer, cardiovascular disease, and diabetes, owing to their ease of detection and inherent molecular stability. In this review, we will discuss the various routes of export of miRNAs into the extracellular space, what the consequences of this may be, and how miRNAs in the circulation may give us hints of the underlying biology of certain disease states.

THIS WAY OUT: miRNA EXPORT INTO THE EXTRACELLULAR SPACE

The first description of miRNAs in the extracellular space came in 2008, where it was proposed that these circulating miRNAs may serve as biomarkers of certain cancers
At first, this idea generated skepticism and the dismissal that these miRNAs were simply a result of passive release of cellular contents into the extracellular space as a result of cell death. Since these initial studies, our understanding of how miRNAs get released from cells in response to various stimuli and/or pathologies has broadened considerably. We now understand that the secretion of miRNAs is a controlled, active, and specific process. miRNAs can be packaged into lipid-based carriers such as exosomes, microparticles, or apoptotic bodies, and have been found on lipoproteins like high- and low-density lipoprotein (HDL and LDL, respectively). Additionally, a significant portion of extracellular miRNAs are found without a lipid carrier, and are protein-bound. While the mechanisms of the selectivity of miRNA packaging remain unclear, researchers are beginning to unravel some of the mysteries surrounding how these tiny RNA molecules make their way out of the cell (Fig. 1).

Exosomal structure and function

Since the identification of exosomes over three decades ago, these small vesicles have gained considerable attention. An exosome is officially classified as a secreted vesicle ranging in size from 30 to 100 nm. They are released from the cell after fusion of a multivesicular body (MVB) with the plasma membrane. This occurs when an endosomal vesicle forms an invagination (also known as an intraluminal vesicle, or ILV), carrying along with it specific cellular components, eventually fusing with the cellular plasma membrane and releasing the membrane-encapsulated exosomal contents into the extracellular space. The nature of the proteins, lipids, and RNAs packaged into an exosome is highly dependent on the cell type of origin, the trigger or stimulus for release, and the lipid content of the surrounding membranes (7). Proteins commonly found on exosomes include CD9, CD63, and CD81, all members of the tetraspanin family. Additionally, heat shock proteins (HSPs) like HSP70 and other cytosolic proteins like actin are often detected on exosomes as well as proteins from the plasma membrane and Golgi. While the specific function of each of these proteins within exosomes has yet to be elucidated, each is likely to have a specific and distinct role. For example, the tetraspanins likely bind other proteins such as integrins and major histocompatibility complex class II molecules and segregate them specifically into MVBs during the packaging of exosomal vesicles (8). The HSPs can bind immune receptors on recipient cells and signal an inflammatory event, which can be accomplished by the secretion of HSP-containing exosomes (9). Integrins and other cell adhesion molecules bind partnering proteins on recipient cells, allowing a specific interaction of the exosome with the target cell of interest (10). Finally, as will be discussed further below, ribosomal proteins and argonaute proteins (Ago1 and Ago2) binding partners have

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**Fig. 1.** MicroRNAs are secreted into the circulation and are biomarkers for various diseases. miRNAs are secreted by various lipid-containing vesicles, including exosomes, microvesicles, and apoptotic bodies, and can be found outside of vesicles but bound to RNA-binding proteins (left). Specific miRNAs that are being highlighted as biomarkers are indicated, as well as the tissue of origin, potentially where disease is occurring (i.e., adipose tissue, liver, heart, and aorta) (right).
also been associated with exosomes in the extracellular environment, suggesting that these exosomes have functional RNA modifying abilities (11). There is a plethora of proteins found on the surface of exosomes, and it is likely that each of them plays a distinct role in the biogenesis and/or delivery of exosomal contents to recipient cells.

The lipid content of exosomes appears to play an important role in both the packaging of exosomes and their delivery to downstream tissues. While the formation of exosomes was first described to be dependent upon the ESCRT (endosomal sorting complex required for transport) proteins, Trajkovic et al. (12) showed that the sphingolipid ceramide, which is found in high concentrations in the plasma membrane, was required for the appropriate sorting and secretion of exosomes in vitro, and inhibition of neutral sphingomyelinase (N-SMase) prevented this secretion by preventing the breakdown of sphingomyelin in the cell membrane and the release of ceramide. It was further demonstrated that exosomes are not only enriched in ceramide, but also in saturated phospholipids like phosphatidylcholine (PC) and phosphatidylethanolamine (PE) compared with the parental cell (13). The tetraspanin family of proteins has a high affinity for both sphingolipids and cholesterol, and while exosomes are being packaged prior to release, the tetraspanin proteins appear to preferentially bring these lipid molecules along with them (8, 12). Recently, the importance of ceramide in the secretion of miRNAs was confirmed by the observation that N-SMase2 was required for the exosomal secretion of miRNAs such as miR-150 and miR-143/145 are known to be secreted via the exosomal pathway.

Microvesicles and other lipid-based carriers

In addition to exosomes, other membrane-bound and lipid-containing vesicles transport miRNA in the extracellular space. Microvesicles are larger than exosomes, typically ranging from 100 nm to 1 μm in size, with most microvesicle preparations comprised of a heterogeneous mixture of particles (15). Microvesicles are secreted by a number of different cell types, including vascular cells, platelets, and inflammatory cells, and it has been speculated that almost all cell types are capable of secreting microvesicles under specific conditions (16). Unlike exosomes, microvesicles are formed from the outward blebbing of the plasma membrane upon an activation stimulus. Given that they originate from the cell surface, microvesicles contain very similar lipid content as the plasma membrane of the parental cell type, most often enriched with phosphatidylserine (PS) and PC (17). Similarly, the protein content of microvesicles is highly related to the originating cell, and there may be less selective cargo loading in these vesicles compared with exosomes. Nevertheless, microvesicles do carry functional protein, mRNA, and miRNA cargo, and delivery of these components to neighboring cells has significant effects on recipient cell function (18). A number of miRNAs have been shown to be secreted via microparticles under various conditions; including miR-15, miR-223, and miR-135 (see Table 1).

Apoprotic bodies are another variety of lipid-encapsulated vesicles known to carry miRNA. These types of vesicles are generally much larger (~500 nm to >2000 nm) and have a heterogeneous size distribution (19). As the name suggests, apoprotic bodies are released at the early stages

| Carrier | miRNA | Original Tissue Source | Target Tissue | Potential Target Gene | Functional Outcome | References |
|---------|-------|------------------------|---------------|-----------------------|--------------------|------------|
| Apoptotic bodies | miR-126 | ECs | SMCs | RGS16, VCAM-1, SPRED1, CXCL12 | Decrease in miR-126 is associated with loss of vascular integrity and angiogenesis, increased atherosclerosis, high LDL, Protects ApoE−/− mice from atherosclerosis | 21, 22, 48–52, 59–61 |
| Exosomes | miR-143/145 | ECs, upregulated during atheroprotective shear stress | SMCs | ELK1, KLF4, CAMK2d, SSH2, PHACTR4, CFL1 | Result of tissue damage due to AMI | 43–45 |
| Exosomes/microvesicles | miR-499, miR-133, miR-208 | Muscle specific, myocardium | Circulation | | Result of tissue damage associated with hypercholesterolemia, NAFLD and CAD Changes in expression detected before the onset of T2D | 46, 47 |
| Exosomes | miR-122 | Liver | Circulation | | Result of tissue damage | 31 |
| HDL/microvesicles | miR-223 | Monocytes | Circulation | RhoB | Changes in expression detected before the onset of T2D | 50 |
| Exosomes/microvesicles | miR-15a | Circulation | | | 38 |
| Exosomes | miR-150 | Monocytes and blood cells | ECs | c-Myb | Uptake by HMEC-1 cells altered expression of c-Myb to enhance their migration | 22 |

Examples of specific miRNAs known to be carried by the various lipid-based extracellular carriers are shown, as well as their tissue of origin and potential target genes/functional outcomes.
of apoptosis, and contain both a lipid bilayer derived from the plasma membrane and cytoplasmic contents that originate from the parent cell. Similar to microvesicles, apoptotic bodies contain PS on their surface, which signals to phagocytic cells like macrophages to engulf and clear the cellular debris. Although it was suggested many years ago that apoptotic bodies contain nucleic acids (20), it was not until a few years ago that it was noted that apoptotic bodies contain miRNA, when Zernecke et al. (21) showed that endothelial cells (ECs) release miR-126 in apoptotic bodies to alter chemokine responses in neighboring cells. Although the presence of miRNA within apoptotic bodies is of interest, this is one of the few studies demonstrating a miRNA-mediated effect in neighboring cells. Interestingly, miR-126 was not found to be enriched in shear flow-induced microvesicles from ECs, only in apoptotic bodies (22). It is interesting to speculate that this may be an example of a specific release of miRNA into a secreted pathway under certain conditions (i.e., to signal to neighboring cells that apoptosis has occurred) while remaining intracellular during others (i.e., vascular homeostasis during shear stress). It remains to be seen whether or not loading of miRNA into apoptotic bodies is specific and selective, or whether apoptotic cells nonspecifically release the contents of the cytoplasm as a response to a certain stimulus.

In addition to lipid-encapsulated organelles released from cells, lipoproteins have also been shown to associate with miRNA and are arguably the most important lipid carrier in the body. Lipoproteins are comprised of various proteins and lipids and are responsible for the delivery of cholesterol, triacylglycerols, steroids, and fat-soluble vitamins to peripheral tissues of the liver via LDL and removal from peripheral tissues to the liver/digestive system via HDL (23, 24). Due to their inherent solubility and their tendency to trap water-insoluble material in their core, lipoproteins have demonstrated an ability to carry nucleic acids and are often used as gene delivery agents, and it has been demonstrated in a recent study that different RNA structures display varying affinities for phospholipid membranes and this could affect the ability of an RNA molecule to bind to a lipoprotein (25, 26). Vickers et al. (27) were the first to demonstrate that human HDL and LDL have the capability to carry miRNAs in the core of the molecule hidden from the extracellular environment in serum, and most importantly these miRNAs can be transferred to recipient cells and alter target cell gene expression. Importantly, this study revealed, at least in the case of HDL, that the miRNAs carried in these lipoproteins differ between healthy subjects and those with atherosclerotic vessel disease. One of the most abundant miRNAs detected on HDL particles was miR-223 which, intriguingly, was found to be highly enriched in monocytes/macrophages (28). These data suggest that it may be peripheral cells like macrophages that may be off-loading their miRNA onto HDL particles, and not necessarily the cells responsible for HDL biogenesis (i.e., hepatocytes and intestinal cells). While these studies still require further exploration, the notion that lipoproteins not only regulate cellular cholesterol content but can also communicate to distal tissues will have an enormous pathological and therapeutic impact, and it will remain to be seen how modification of these particles (i.e., by oxidation) may modulate these communicative properties.

Nonlipid-associated miRNA transport

While there is no doubt that miRNAs are found encapsulated within lipid-containing membranous vesicles and are actively secreted by many cell types, it has recently been suggested that the majority of the miRNA found in the circulation is nonvesicle associated and is instead bound to protein complexes (29). Indeed, a number of studies have quantified the amount of circulating miRNA found within exosomes and microvesicles compared with that which is found outside of vesicles, and conclude that only a small minority (1–5%) of extracellular miRNA is contained in a lipid-based carrier (30–32). Interestingly, this appears to be a miRNA-specific phenomenon, as some miRNAs are found to be exclusively associated with vesicle carriers (i.e., let-7a) whereas others (i.e., miR-16 and miR-92a) were found outside any known membrane-containing structure (30).

As mentioned above, the argonaute proteins (Ago1/2), which act as effector molecules for miRNAs, are detected in cell supernatants and are not necessarily associated with vesicles of any kind (microvesicles or exosomes) (33). The binding of Ago2 to miRNA protects the miRNA from degradation by the abundant amount of RNases found in plasma, and it is believed that the Ago2 protein can assist in the functional transfer of the miRNAs that it carries (30). Furthermore, proteomic analysis of miRNA-containing supernatants from cells cultured in vitro revealed that a number of other RNA binding proteins [i.e., nucleophosmin-1 (NPM1), ribosomal protein L10a and L5] were found bound to miRNA outside of any vesicles (32). Overall, the physical interaction of miRNA with these protein complexes in the extracellular space serves to significantly protect them from extracellular nucleases and potentially enhances their functional properties once inside the host cell. In the coming years, as we understand more about how miRNA export is regulated, we will also discover that particular miRNAs associate with unique proteins in the extracellular space so that uptake and ultimately downstream functional consequences are highly specific.

GETTING THE INFORMATION INSIDE: UPTAKE OF miRNA AND ITS CONSEQUENCES

Following the discovery that miRNAs are actively exported from cells, it is perhaps not surprising that the first question that arose was whether or not these miRNAs can be taken up by nearby cells and alter gene expression. Early studies of microvesicles and exosomes had demonstrated that these particles could indeed transfer RNA and protein content, and alter recipient cell behavior (34–37). However, it was not until 2009 when miR-126 secreted in apoptotic bodies from ECs was shown to directly alter target gene (RGS16) expression in recipient cells, and subsequently alter chemokine receptor expression in the recipient cells (21). Soon thereafter, another group demonstrated that miR-150 secreted by monocytes and blood
 cells could alter target gene expression (c-Myb) in recipient ECs both in vitro and in vivo (38). Different miRNAs carried on healthy versus diseased (i.e., familial hypercholesterolemia) HDL were able to differentially alter gene expression in recipient cells. Vickers et al. (31) elegantly showed that after treatment with HDL, 87% of the downregulated genes in recipient cells contained putative target sites for the miRNA carried on the HDL particles. Among the most convincing evidence that extracellular miRNA can alter downstream cell behavior comes from Hergenreider et al. (22), who showed that miR-143/145 is upregulated during atheroprotective shear stress, is secreted from ECs, and is transferred to smooth muscle cells (SMCs) to alter target gene expression. Strikingly, this transfer of miR-143/145 from ECs to SMCs was able to protect Apo e−/− mice from developing atherosclerosis, indicating that this exogenous miRNA communication has important functional consequences. The exact mechanisms by which extracellular miRNAs are taken up into recipient cells are not known. It has been hypothesized that vesicles and other lipid carriers are engulfed by endocytosis, membrane fusion, and even through passive transfer of contents (7). It is likely that the exact mechanism of cellular uptake greatly depends on the phagocytic capacity of the recipient cell, as well as which proteins and lipids are present on the vesicle surface (i.e., tetraspanins or integrins). Perhaps nonvesicle associated miRNA is transferred to cells via a specific and as yet unidentified receptor that recognizes the proteins (i.e., Ago1 vs. Ago2) bound to the circulating miRNA.

The traditional and perhaps expected view is that extracellular miRNAs are transferred from a parent cell, either via lipid carriers or bound to protein, to communicate signals to nearby or even distal tissues within an organism. Surprisingly however, Zhang et al. (39) found that miRNAs from rice plants consumed by mammals can survive the digestive tract, get transferred to the blood stream, and get taken up by the liver. Indeed, rice-derived miR-168a is passed through the gastrointestinal tract after ingestion and can be detected in the serum of mice after feeding where it is then taken up by the liver, where it can directly repress the expression of the mammalian target gene LDLRAP1 and alter LDL uptake by the liver. These findings open up a new and exciting frontier in our understanding of how miRNAs and other ncRNAs may be mediating cell-cell and even cross-kingdom communication.

**UTILITY OF EXTRACELLULAR miRNA: THERAPEUTICS AND DIAGNOSTICS**

When RNA was first detected in the circulation in the early 1970s (40), it was immediately noted that despite the very high levels and activity of RNases present in human plasma, it was surprisingly resistant to nuclease degradation (41). This protection from nuclease degradation was also described for miRNAs in the circulation. At first, it was thought that perhaps miRNAs found in plasma had some inherent property that differed from cellular miRNA and prevented their RNase-mediated degradation. However, Arroyo et al. (30) showed that miRNA purified from plasma, yet free of any membrane vesicles or protein complexes, was easily degraded upon incubation with plasma. However, miRNAs that remained associated with their lipid carrier and/or protein complexes were indeed highly resistant to degradation (42). This garnered excitement for the possibility that circulating miRNAs may be used as biomarkers for various diseases and conditions, and since then over 200 publications report the use of one or more miRNAs as a disease biomarker.

There are likely two classes of miRNA biomarkers: 1) those miRNAs that are secreted passively due to tissue stress, injury, or necrosis, and therefore may not reflect the biology associated with disease pathogenesis; and 2) those miRNAs that are actively and/or chronically secreted during disease progression and perhaps contribute to the pathogenesis. An example of the former comes from the identification of myocardium-derived miRNAs in the circulation following a myocardial infarction. miR-208 and miR-499 are muscle-specific miRNAs and were the first to be identified in the circulation of patients following an acute myocardial infarction (AMI) (43). De Rosa et al. (44) went on to show that in conjunction with miR-208 and miR-499, miR-133 is also found in the serum of patients suffering from an AMI, and that these miRNAs are indeed derived from the myocardium. Much like cardiac troponin levels (cTnT and cTnI), which are reflective of cardiac muscle damage yet are a robust and sensitive measurement of an AMI, miR-133/208/499 are a result of tissue damage yet signal that a pathological event has taken place (45). Similarly, miR-122 is a highly abundant miRNA in the liver, and it was recently reported that levels of miR-122 in the circulation correlated with disease severity in patients suffering from chronic hepatitis C (46). And finally, hypercholesterolemia and nonalcoholic fatty liver disease (NAFLD) have both demonstrated unique circulating miRNA profiles, with both pathologies associated with elevated levels of the liver-specific miR-122, suggesting once again that this tissue-specific release may be a signal of injury rather than a mediator of pathology (46, 47). It remains to be seen whether circulating miRNAs in AMI and liver diseases will provide improved diagnostic power compared with the tests that are already in place, and/or if they will provide any hints toward the etiology of these diseases.

In more chronic conditions, such as type 2 diabetes (T2D), atherosclerosis, and hypercholesterolemia, miRNAs have also been used as biomarkers, and may reflect an involvement in disease pathogenesis. An early report from miRNA profiling of plasma from T2D patients and matched controls showed a number of significant alterations in circulating miRNAs between the two groups, yet the most notable among them was miR-126, which has been reported as an important miRNA for maintaining EC homeostasis (48). As discussed above, miR-126 is released in apoptotic bodies into the circulation (21), and a decrease in miR-126 expression in the circulation results in loss of vascular integrity and impaired angiogenesis (49, 50). Although miR-126 was found as a biomarker for T2D, it is not among the miRNAs most highly expressed in adipose tissue, a key
tissue involved in the pathogenesis of T2D. Furthermore, miR-126 in the circulation is also reduced in patients with coronary atherosclerosis (51) and is inversely correlated with patients with high LDL levels (52), underscoring its potential importance in maintaining vascular homeostasis across multiple tissues. Intriguingly, in patients with overt diabetes, there were measurable alterations in miRNA expression in the circulation before the onset of disease, namely in miR-126, miR-223, and miR-15a (50). This argues that these miRNAs may actually be causally involved in the development of diabetes, and are not simply a result of tissue injury/stress after disease onset. Of note, miR-223 was among the most highly expressed miRNAs found on HDL particles (31), which may signal that HDL-contained miRNAs are playing a role in the pathogenesis of T2D.

Besides their potential predictive powers, the presence of miRNAs in the circulation, especially within lipid-derived particles, provoked the idea that miRNAs can be encapsulated in vesicles as a drug delivery system. In addition to their sensitivity to plasma nucleases, unbound double-stranded miRNAs also have potent immune-stimulating activity via toll-like receptor activation, making them undesirable as therapeutics. Consequently, the concept of using lipid-encapsulated miRNA is being explored as a drug delivery tool to deliver miRNAs in an otherwise hostile environment. Wolfrum et al. (53) were among the first to exploit the properties of lipoprotein particles, especially HDL, to deliver small interfering RNA (siRNA) in vivo to mediate target gene silencing. They have since described both apoA1- and apoE-conjugated mimetic lipoprotein particles as efficient siRNA delivery molecules (54). Interestingly, Vickers et al. (31) cited this work as inspiration for their hypothesis that HDL carries endogenous miRNAs in the circulation. The efficient delivery of miRNAs in vivo, whether via nanoparticles, exosomes, or lipoproteins (54–56), remains the single biggest challenge to this novel class of therapeutics. While strides are certainly being made in improving the efficacy of in vivo delivery, this technology lags behind significantly compared with miRNA inhibition strategies. There has yet to be miRNA overexpression therapy to advance into the clinic, but this continues to be a focus of many pharmaceutical companies looking to capitalize on this exciting frontier (57).

LOOKING FORWARD: THE HIGHS AND LOWS OF EXTRACELLULAR miRNA

It is clear that the potential of extracellular miRNA for use as both a diagnostic and a therapeutic tool is tremendous, and in the span of a few years, the field has turned from skepticism to acceptance that these tiny nucleic acids might be playing a role in the extracellular space (Fig. 1). However, some of the details regarding exactly how miRNAs are packaged for cellular export, and most importantly, which miRNAs are packaged when, await further mechanistic insight. Additionally, is it unknown whether miRNAs “lost” to the secretory pathway can alter the function of the cell of origin in addition to the recipient cell. Moreover, our understanding of exactly how each extracellular miRNA is taken up by recipient cells, and whether they are functional once inside, lags even further behind.

There are a number of important factors to consider when miRNAs are being touted as potential biomarkers. First, are the miRNAs detected in plasma of certain groups of patients simply a result of blood cell lysis? Some groups have suggested this is indeed the case, and caution should be taken when interpreting these data (58). Second, is the specific miRNA in question involved in any other disease processes/pathologies that may confound its use as a clinical diagnostic? As discussed above, miR-126 has been shown to be dysregulated in patients with diabetes, coronary artery disease, and/or AMI (48, 51, 52). Moreover, circulating miR-126 is a suggested biomarker for a variety of malignancies arising from a variety of tissues, including lung, kidney, and T-cell carcinomas, among others (59–61), which dampens the enthusiasm for this particular miRNA as a specific disease biomarker. As discussed above, one of the most exciting recent findings regarding miRNAs in the extracellular space came from the identification that circulating miRNA signatures became dysregulated before the onset of disease (in this case T2D), giving credence to the notion that extracellular miRNA is likely contributing to disease pathogenesis, and identification of these dysregulated miRNAs could realistically lead to a viable therapy.

The field of miRNAs as biomarkers is in its infancy, and as such, many of these outstanding issues are slowly being resolved. Investigators are now going to greater lengths when preparing samples and analyzing miRNA content in plasma, and streamlined protocols and procedures are assisting in the comparison across data sets. The recent advent of the miRandola database (62) (http://atlas.dmi.unict.it/mirandola/index.html) will enable researchers to curate their findings into one location and compare and contrast findings from other studies, which will make the identification of miRNA-based biomarkers more robust. Undoubtedly an improved understanding of miRNA export and uptake will aid in the therapeutic arena, and will greatly assist the tailoring of individual miRNA therapies for specific diseases and tissues and hopefully aid in the eradication of possibly preventable diseases like atherosclerosis and T2D. 

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