Opinion

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Secreted microRNAs: a new form of intercellular communication

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In multicellular organisms, cell-to-cell communication is of particular importance for the proper development and function of the organism as a whole. Intensive studies over the past three years suggesting horizontal transfer of secreted microRNAs (miRNAs) between cells point to a potentially novel role for these molecules in intercellular communication. Using a microvesicle-dependent, or RNA-binding protein-associated, active trafficking system, secreted miRNAs can be delivered into recipient cells where they function as endogenous miRNAs, simultaneously regulating multiple target genes or signaling events. In this Opinion, we summarize recent literature on the biogenesis and uptake of secreted miRNAs, propose a possible working model for how secreted miRNAs might be sorted and transferred between cells and speculate on their biological significance.

Secreted miRNAs as an emerging new form of intercellular communication

The ability of a cell to communicate with neighboring cells and to sense their local microenvironment forms the basis for coordinated cellular activity in multicellular organisms. Classic cell-to-cell communication is mediated by several methods, including cell junctions, adhesion contacts or soluble messengers (Figure 1) [1–4]. Findings over the past several years, however, suggest the existence of an additional form of intercellular communication: horizontal transfer of secreted miRNAs (Figure 1).

Importantly, secreted miRNAs represent a newly discovered mechanism by which donor cells can influence the gene expression of recipient cells. The secretory mechanism remains essentially unknown, however, and the biological impact of these molecules in multicellular organisms is unclear. In this Opinion, we summarize the known characteristics of secreted miRNAs and propose a mechanism for their secretion and incorporation into cells. We also discuss recent reports that suggest regulatory roles for secreted miRNAs in physiological and pathological processes.

Definition of secreted miRNAs

Numerous studies have documented the importance of miRNAs, which are a class of endogenous noncoding RNAs consisting of 19 to 24 nucleotides that regulate the post-transcriptional silencing of protein-coding genes in eukaryotes (Box 1) [5–7]. Once thought to be unstable molecules, miRNAs were recently demonstrated to circulate in a highly stable, cell-free form in various body fluids, including serum [8–10], plasma [11], saliva [12], urine [13] and milk [14,15]. Furthermore, circulating miRNAs can be significantly altered in a wide range of pathological conditions, including cancers [8,9,11–13], diabetes [9] and tissue injuries [16–18]. The source of such extracellular miRNAs is not known but three different pathways have been suggested (Figure 2): (i) passive leakage from broken cells due to tissue injury, chronic inflammation, cell apoptosis or necrosis, or from cells with a short half-life, such as platelets. (ii) Active secretion via microvesicles, including exosomes and shedding vesicles, which are membrane-enclosed cell fragments released by almost all cell types under both normal and pathological conditions (Box 2) [19–23]. Recent studies have suggested that not only are microvesicles specifically targeted to recipient cells to exchange proteins, mRNA and lipids [24,25] but also they can deliver miRNAs to trigger downstream signaling events [26–30]. (iii) Active secretion using a microvesicle-free, RNA-binding protein-dependent pathway. A recent study indicated that high-density lipoprotein (HDL) can readily associate with exogenous miRNAs and deliver them to recipient cells [31]. Moreover, studies have shown that a significant portion of circulating miRNAs in plasma is not encapsulated in microvesicles but is associated with Argonaute2 (AGO2) [32,33]. Another RNA-binding protein, nucleophosmin 1 (NPM1), was also found to bind circulating miRNAs [34]. However, there is no direct evidence that microvesicle-free, AGO2- or NPM1-associated miRNAs are actively released from cells, nor is there evidence of their uptake into recipient cells. Whether these protein-bound extracellular miRNAs originate from cell lysis or broken vesicles, or are secreted from cells in a vesicle-independent way, requires further study.

In contrast to passive leakage from broken cells, secretion of miRNAs via microvesicles and HDL is active and energy dependent, and this is the key characteristic that distinguishes secreted miRNAs from other types of circulating miRNAs. The physiological and functional differences between microvesicle-enclosed and RNA-binding protein-associated secreted miRNAs are largely unknown, although these processes may use different secretion mechanisms.

Molecular basis for the stability of secreted miRNAs

A crucial question surrounding secreted miRNAs concerns their stability in the circulation despite the presence of ubiquitous ribonucleases (RNases). Two possible

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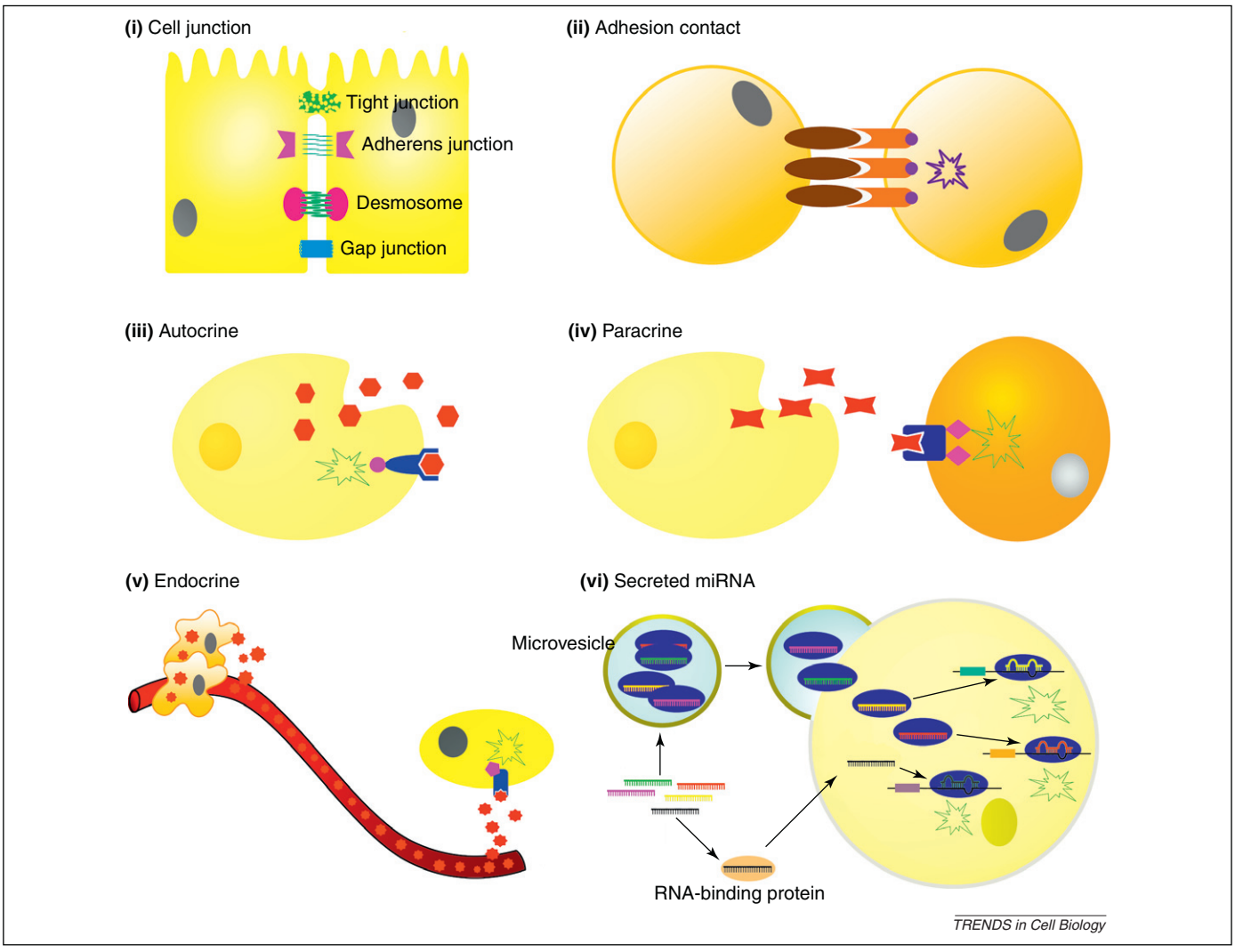

Figure 1. A secreted microRNA (miRNA)-mediated gene regulatory network as a novel form of intercellular communication. Cells can communicate by several means: adjacent cells can communicate through **(i)** specific junctions that allow the exchange of small intracellular signaling molecules or **(ii)** direct adhesion contacts between a membrane-bound signaling molecule on one cell and a receptor on the surface of another cell. Cells also can communicate via soluble messengers, such as hormones, cytokines and chemokines, which may act **(iii)** on the original cells (autocrine action) or **(iv)** on adjacent cells (paracrine action) or **(v)** travel long distances through intercellular nanotubes to affect target cells (endocrine action). In addition to these methods, **(vi)** secreted miRNA-mediated gene regulatory networks represent another type of intercellular communication in which a group of specific miRNAs can be transferred to target cells via microvesicles or RNA-binding proteins. These exogenous miRNAs can then activate myriad signaling events in the recipient cells by modulating expression of their target genes.

mechanisms have been suggested: (i) protection of secreted miRNAs by the membrane structures of microvesicles [26–28,30]; and (ii) stabilization of secreted miRNAs by their association with RNA-binding proteins, such as AGO2 and NPM1 [32–34]. Both of these mechanisms – microvesicle membrane enclosure and protein conjunction – could provide a protected and controlled internal microenvironment for secreted miRNAs, allowing them to travel long distances without degradation.

Molecular mechanism for packaging and secretion of miRNAs

Evidence suggests that miRNA packaging occurs non-randomly and that specific miRNA populations are preferentially sorted into microvesicles. We have found direct evidence that blood cells and cultured THP-1 cells actively and selectively package miRNAs into microvesicles and secrete them into the circulation or culture medium in response to various stimuli [27]. Other studies have demonstrated that certain miRNAs are present dominantly or at higher levels in exosomes than in the donor cells when normalized to the total RNA or total protein content [26,28,29].

One study has demonstrated that a ceramide-dependent secretory mechanism, which can induce endosome sorting into the exocytic multivesicular bodies (MVBs) [35], actively regulated the release of exosomal miRNAs from HEK293 cells [30]. Neutral sphingomyelinase 2 (nSMase2), which controls ceramide biosynthesis, regulates the secretion of exosomal miRNAs [30]. However, the endosomal sorting complex required for transport (ESCRT) system, which has a central role in the accumulation of exosomes to be targeted to lysosomes [36,37], is unnecessary for the release of miRNAs [30]. These results suggest a ceramide-triggered, ESCRT-independent mechanism for exosomal miRNA secretion. The increase in miRNA secretion mediated by nSMase2 might be associated with an increase in the number of exosomes released

Box 1. Biogenesis and function of miRNAs

miRNAs are naturally occurring small non-coding RNAs of about 22 nucleotides in length [5–7]. In the nucleus, miRNA is transcribed by RNA polymerase II (Pol II) to generate a long transcript called primary miRNA (pri-miRNA). pri-miRNA is subsequently recognized and cleaved by a complex formed by the RNase III enzyme Drosha and its binding partner DGCR8 (DiGeorge syndrome critical region 8, or Pasha), yielding precursor miRNA (pre-miRNA). pre-miRNA is 70–90 nucleotides in length and has an imperfect stem loop hairpin structure. It is exported to the cytoplasm by Exportin 5 in a Ran-GTP-dependent mechanism. Upon entering the cytoplasm, the pre-miRNA is cleaved by a complex formed by the RNase III enzyme Dicer and its binding partner transactivation-responsive RNA-binding protein (TRBP), resulting in a small double-stranded RNA duplex that contains both the mature miRNA strand and its complementary strand [5–7]. The miRNA duplex is subsequently unwound by RNA helicase, and the single-stranded mature miRNA is incorporated into the RNA-induced silencing complex (RISC). The miRNA then guides RISC to complementary sites (usually 3′-UTR) within target mRNA and inhibits the function of mRNA by two different mechanisms, depending upon the complementarity between the miRNA and its target mRNA: perfect base-paring results in mRNA degradation whereas imperfect binding represses protein translation [5–7]. miRNAs modulate various crucial biological processes, including cell proliferation, differentiation, apoptosis, tumorigenesis, immune response and viral infection, and their altered expression contributes to the pathogenesis of many human malignancies [5–7].

Box 2. Microvesicles, exosomes and shedding vesicles

Microvesicles, exosomes and shedding vesicles are small membranous vesicles released by almost all cell types under both normal and pathological conditions. They are important mediators of intercellular communication, although they were previously thought to be inert cell debris. The nomenclature of these subcellular vesicles is controversial, and it is unclear to what extent they are normally present [19–23]. Therefore, the introduction of a consensus non-overlapping terminology is needed. In general, they can be distinguished depending on their mechanism of biogenesis and release. Microvesicles are mixed vesicle populations containing both shedding vesicles and exosomes, which are heterogeneous in size ranging from 30 to 1000 nm in diameter [19,21].

Exosomes are small secreted vesicles with a diameter between 30 and 100 nm [20–23]. Exosome generation is initiated when cell membrane proteins transfer to early endosomes by inward budding. Intraluminal vesicles are then generated through invagination of endosomal membranes, thus forming MVBs. The molecules within the intraluminal vesicles (or exosomes) can either be sorted for degradation by fusion of the MVB with lysosomes or secreted by fusion of the MVB with the plasma membrane. ESCRT, a well-characterized multiprotein complex, plays a central role in the accumulation of vesicles destined for lysosomes. Another process independent of the ESCRT machinery but triggered by the ceramide pathway can induce vesicle sorting into the exocytic MVBs [20–23].

Shedding vesicles are a heterogeneous population of larger vesicles with a diameter between 100 and 1000 nm that are released into the extracellular space by outward budding and fission of the plasma membrane [19].

Microvesicles contain a variable spectrum of parental cell molecules, including RNA, proteins and lipids, either enclosed inside the vesicles or located on the surface, but they lack cellular organelles, such as mitochondria, lysosomes and nuclei [19–23]. Once shed, microvesicles can be transferred to target cells and are functionally active at their new location, thus facilitating the horizontal delivery of bioactive molecules from cell to cell or activating receptors on the plasma membrane of the recipient cells with surface ligands. Microvesicle release has been described from reticulocytes, dendritic cells, B cells, T cells, epithelial cells, platelets, adipocytes, mast cells, macrophages, monocytes, mesenchymal stem cells, embryonic stem cells, hematopoietic stem cells, neurons and tumor cells. In addition, microvesicles exist in a variety of biological fluids, including plasma, urine, breast milk, saliva, semen, amniotic fluid and malignant effusions. They are thought to play pivotal roles in various physiological and pathological processes, such as tumor progression, immune surveillance and pregnancy [19–23].

by cells, or with an increase in the amount of miRNAs packaged into exosomes. It is currently unclear which mechanism accounts for the increased extracellular levels of miRNAs. In addition, the biogenesis of shedding vesicle-carried miRNAs is not understood. It is therefore essential to uncover the molecular machinery that directs specific miRNA molecules to the sites of vesicle shedding at the plasma membrane.

There is evidence that HDL transports endogenous miRNAs to recipient cells [31]. Strikingly, nSMase2 and also probably the ceramide pathway were shown to repress the cellular export of miRNAs to HDL [31]. Because overexpression of nSMase2 and activation of the ceramide pathway have previously been shown to induce exosome release from cells and trigger cellular export of miRNAs [30], the export of specific miRNAs through the exosomal pathway and the HDL pathway may be distinct and possibly opposing mechanisms.

Evidence has been provided that the loading of miRNAs into exosomes may not be a random event but instead is controlled by specific proteins of the RNA-induced silencing complex (RISC) [38]. GW182, which is required for miRNA function through its association with AGO2, is dramatically enriched in exosomes [38]. In agreement with this, the AGO2 protein has been observed in exosomes derived from cultured THP-1 cells [27]. These findings suggest that exosomes not only transfer miRNAs but also deliver cellular components of the RISC to enhance miRNA function.

Several mechanisms have been suggested to control the biogenesis, sorting and release of secreted miRNAs (Figure 3a). Although the underlying mechanisms remain unclear, the packaging of specific miRNA populations into microvesicles appears to be a selective process. Because components of RISC, such as AGO2 and GW182, have been detected in exosomes, it is possible that RISC may be involved in the packaging of miRNAs into exosomes. Furthermore, a ceramide-dependent pathway controls the incorporation of miRNAs into exosomes, but it represses miRNA packaging into HDL particles. Thus, nSMase2 might be a key factor in determining miRNA sorting to exosomes or RNA-binding proteins. Further studies are necessary to unveil how miRNAs are sorted into exosomes, shedding vesicles and HDL particles, and whether there is regulated specificity in the process.

Uptake of secreted miRNAs by recipient cells

Another important point that requires future study is how secreted miRNAs execute their functions in recipient cells. THP-1 cell-derived microvesicles containing abundant miR-150 can deliver miR-150 into the human microvascular endothelial cell line (HMEC-1), resulting in suppression of *c-Myb* (a known target gene for miR-150 [39]) in recipient cells and enhanced migration capacity [27]. Secreted miR-146a suppresses expression of its known target gene *ROCK1* [40] in recipient prostate cancer PC-3 M cells [30]. In addition, intratumoral injection of exosomes enriched with

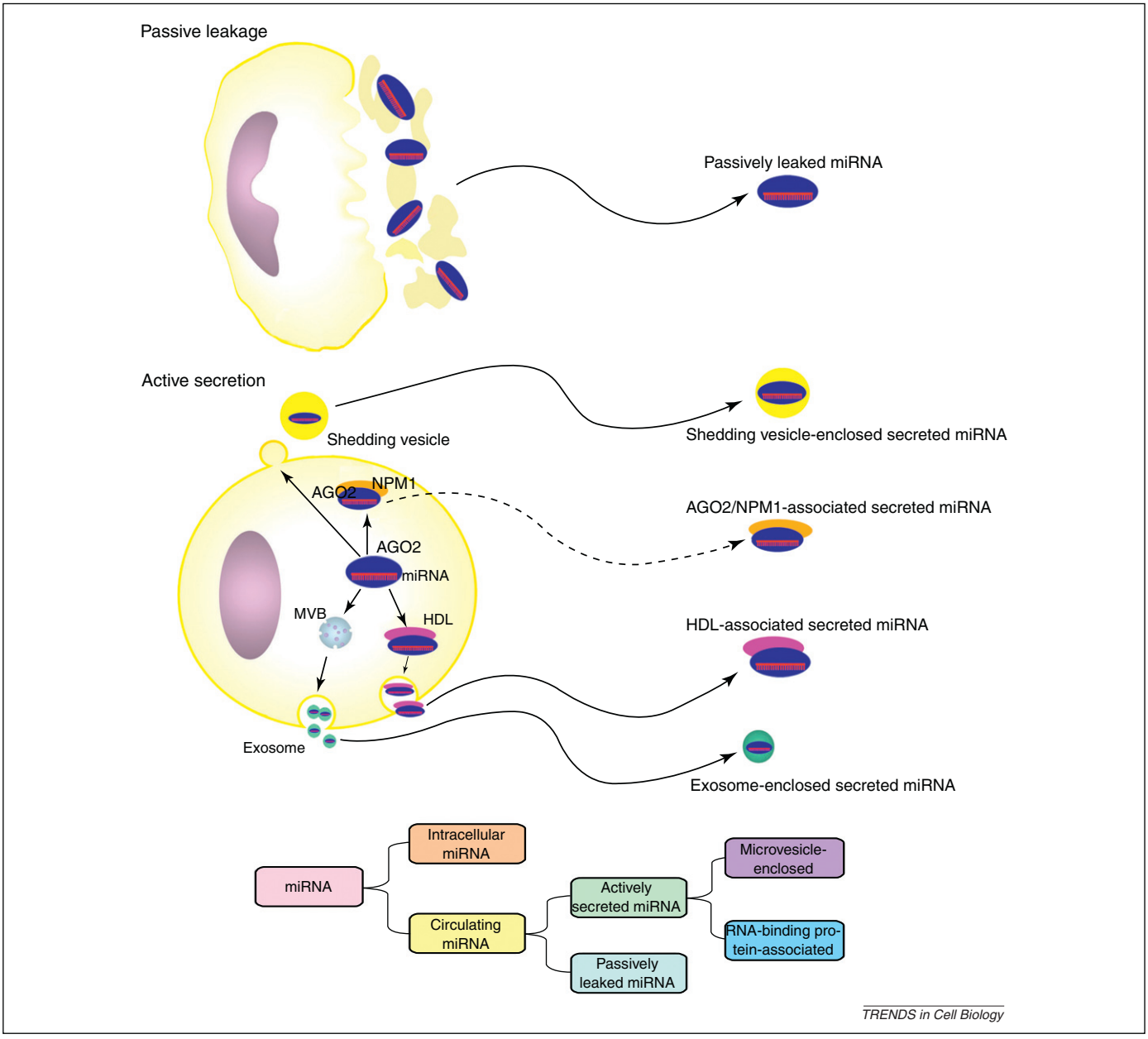

Figure 2. The relationship between circulating microRNAs (miRNAs) and secreted miRNAs. miRNAs can enter the circulation through three pathways: **(i)** passive leakage from broken cells; **(ii)** active secretion via microvesicles, including exosomes and shedding vesicles; and **(iii)** active secretion in conjunction with the RNA-binding protein high-density lipoprotein (HDL). Other RNA-binding proteins, including Argonaute2 (AGO2) and nucleophosmin 1 (NPM1), are found to bind circulating miRNAs; however, whether AGO2- or NPM1-bound miRNAs are actively released from cells and can be taken up by recipient cells is currently unclear. miRNA secretion via microvesicles and HDL is active and energy dependent, and this is the key characteristic that distinguishes secreted miRNAs from passively leaked miRNAs.

miR-16 into nude mice implanted with prostate cancer PC-3 M cells results in the transfer of secreted miR-16 into cancer cells and suppression of a luciferase reporter containing the 3′-untranslated region (3′-UTR) of BCL2 (a direct target of miR-16) [41]. Taken together, these results demonstrate that secreted miRNAs packaged in microvesicles can be delivered into recipient cells and act as physiologically functional molecules to exert gene silencing through the same mechanism as endogenous miRNAs.

It has also been demonstrated that HDL delivers endogenous miRNAs to recipient cells with functional targeting capabilities, leading to altered gene expression [31]. HDL-mediated delivery of miRNAs appears to be dependent on scavenger receptor class B type I (SR-BI), which is a cell surface HDL receptor that mediates selective uptake of the lipid cargo of HDL [31]. SR-BI-mediated transfer of HDL-miRNAs may prevent the delivery of miRNAs into the lysosomal pathway and instead divert them into the cytoplasm where they would be expected to be more stable, have increased functional integrity and potentially alter gene expression [31].

One of the least understood issues in this field is how secreted miRNAs are taken up into recipient cells and whether specific cells are targeted. The mode of uptake and its specificity will influence the functional role of secreted miRNAs. We postulate that microvesicle-enclosed secreted miRNAs can be taken up when microvesicles are internalized by endocytosis, phagocytosis or direct fusion with the plasma membranes. RNA-binding protein-associated secreted miRNAs could be taken up via specific recep-

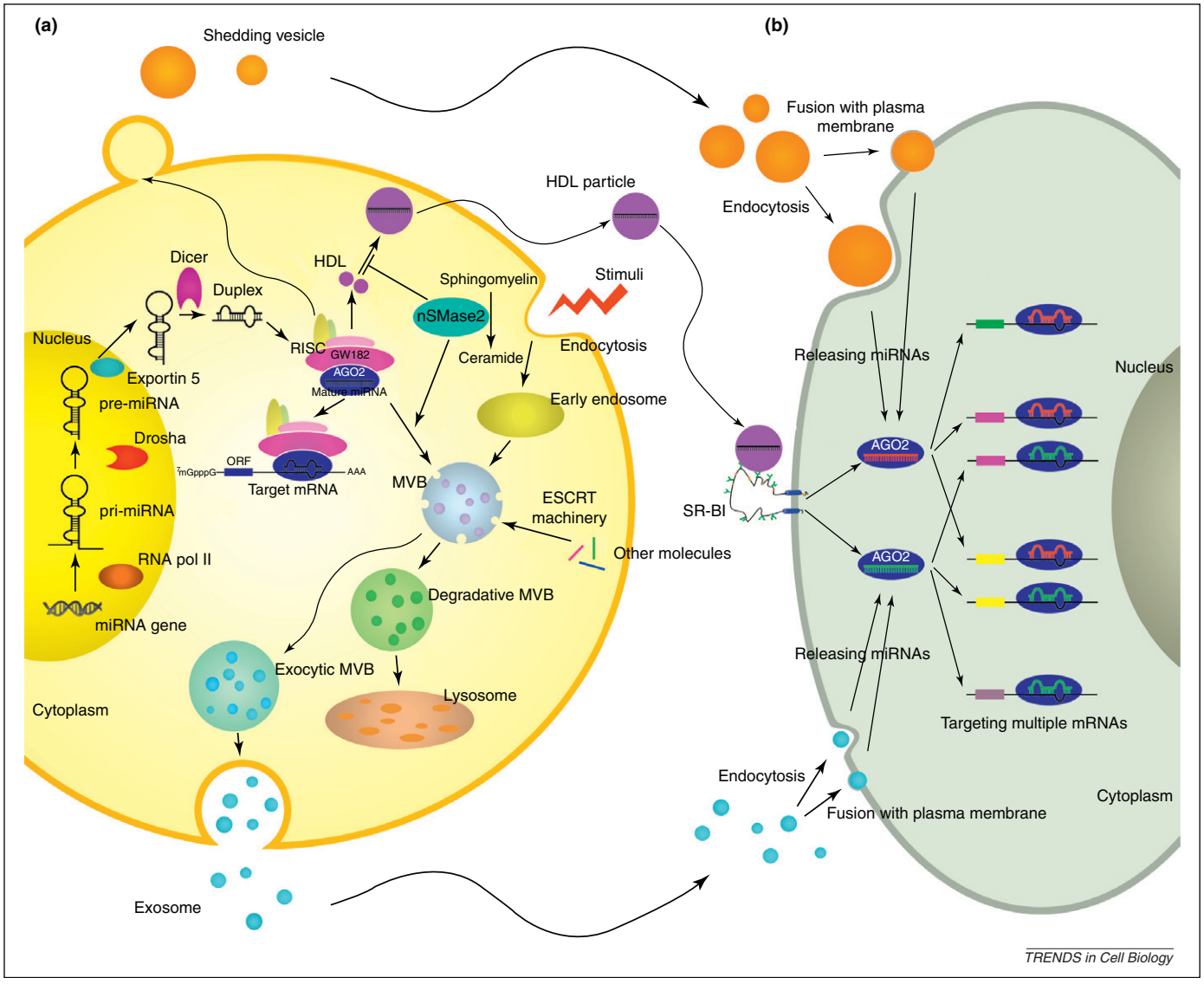

Figure 3. The biogenesis and proposed working model of secreted microRNAs (miRNAs). **(a)** Schematic description of the sorting and release of secreted miRNAs. After being transcribed in the nucleus, exported to the cytoplasm and processed into a mature form, miRNAs can bind to complementary sequences on target mRNAs to repress translation or trigger mRNA cleavage. They can also be packaged and transported to the extracellular environment via three different pathways. **(i)** The generation of exosomal miRNAs requires ceramide production on the cytosolic side by neutral sphingomyelinase 2 (nSMase2), and other molecules that are targeted to lysosomes depend on the endosomal sorting complex required for transport (ESCRT) machinery. Thus, a ceramide-dependent, ESCRT-independent pathway may control the incorporation of miRNAs into exosomes. Furthermore, exosomes may deliver cellular components of the RNA-induced silencing complex (RISC), such as GW182 and Argonaute2 (AGO2), to enhance the biological function of the secreted miRNAs. After fusion of multivesicular bodies (MVBs) with the plasma membrane, exosomal miRNAs are released into the circulation accompanying the release of exosomes. **(ii)** Shedding vesicles are formed by the process of blebbing or shedding from the plasma membrane. However, it is currently unknown how miRNAs are shed at the cell surface. **(iii)** miRNA inside the donor cell can be stably exported in conjunction with RNA-binding proteins, such as high-density lipoprotein (HDL). nSMase2 represses cellular export of miRNAs to HDL. **(b)** Schematic description of the uptake of secreted miRNAs in recipient cells. Exosomes and shedding vesicles can donate their miRNAs to recipient cells by the process of endocytosis, phagocytosis or direct fusion with the plasma membrane. HDL-associated miRNAs are taken up by recipient cells through binding to scavenger receptor class B type I (SR-BI) receptors present at the recipient cellular membrane. Because one miRNA can target numerous mRNAs and numerous miRNAs can target one mRNA simultaneously, secreted miRNAs may function in networks that form a complex system regulating myriad signaling events in the target cells.

tors on the cell surface. Cell recognition molecules on microvesicle surfaces or specific receptors on recipient cell membranes could allow specificity in uptake. We propose a possible working model by which secreted miRNAs might function in recipient cells (Figure 3b). As shown, numerous miRNAs could be incorporated at once, thus regulating multiple target genes simultaneously.

Role of secreted miRNAs in regulating physiological and pathological processes

miRNAs secreted by immune cells, stem cells, adipocytes and blood cells have recently been identified (Table 1). There is evidence for antigen-driven unidirectional transfer of miRNAs, such as miR-335, from T cells to antigen-presenting cells (APCs) via $CD63^+$ exosomes during T cell–APC cognate immune interactions [29]. Furthermore, upon co-culture of T cells with APCs, a luciferase reporter containing the 3′-UTR of *SOX4* (a direct target of miR-335) was significantly reduced, presumably via exosomal miR-335 [29]. The authors proposed that this miRNA transfer can fine-tune gene expression during generation of the immune response and increase the complexity of communication between immune cells. Microvesicles derived from embryonic stem cells contain

Table 1. The potential roles of secreted miRNAs in various physiological and pathological processes

Secreted miRNA	Transport system	Donor cell	Target cell	Target gene	Biological function of secreted miRNA	References
let-7, miR-1, miR-15, miR-16, miR-181 and miR-375	Exosome	Mast cell line (MC/9 and HMC-1), primary bone marrow-derived mast cell				[26]
miR-150	Microvesicle	Human monocytic cell line (THP-1)	Human microvascular endothelial cell line (HMEC-1)	*c-Myb*	Promote cell migration	[27]
miR-335	Exosome	Human Jurkat-derived T-cell line (J77 cell expressing miR-335)	Human lymphoblastoid B cell line (Raji)	*SOX4*	Regulate immune synapsis	[29]
miR-16, miR-21, miR-143, miR-146a and miR-155	Exosome	Human embryonic kidney cell line (HEK293)				[30]
miR-290, miR-291-3p, miR-292-3p, miR-294 and miR-295	Microvesicle	Mouse embryonic stem cell	Mouse embryonic fibroblast			[42]
miR-21, miR-99a, miR-100 and miR-223	Microvesicle	Human bone marrow derived mesenchymal stem cell, liver resident stem cell	Murine tubular epithelial cell	*PTEN*, *cyclin D1* and *Bcl-2*		[43]
miR-223, miR-484, miR-191, miR-146a, miR-16, miR-26a, miR-222, miR-24, miR-126 and miR-32	Microvesicle	Peripheral blood cell				[44]
let-7b, miR-103, miR-148a, miR-16, miR-27a, miR-146b and miR-222	Microvesicle	Mouse preadipocyte cell line (3T3-L1), primary rat adipocyte				[45,46]
miR-21	Microvesicle	Primary human glioblastoma cell	Human brain microvascular endothelial cell (HBMVEC)			[28]
EBV-miRNA	Exosome	EBV-transformed B lymphoblastoid cell	Monocyte-derived dendritic cell	*CXCL11* and *LMP1*	Repress immunostimulatory gene	[52]
EBV-miRNA	Exosome	EBV-positive nasopharyngeal carcinoma cell line (C666)	Human umbilical vein endothelial cell (HUVEC)			[54]
miR-517a	Exosome	Human chorionic villi (especially syncytiotrophoblast), human trophoblast cell line (BeWo)				[57]
miR-101, miR-125b, miR-150, miR-223, miR-24 and miR-93	Microvesicle	Bovine milk				[58]
miR-223.	HDL particle	Human plasma	Hepatocyte (Huh7)	*RhoB* and *EFNA1*		[31]

abundant miRNAs that can be transferred to mouse embryonic fibroblasts *in vitro* [42]. Moreover, miRNAs from mesenchymal stem cells are transferred to recipient murine tubular epithelial cells after microvesicle incorporation [43]. In other studies, miRNAs were detected in microvesicles from peripheral blood [44] and adipocytes [45,46]. In these reports, the existence of secreted miRNAs from immune cells, stem cells, adipocytes and blood cells is clear, but the secretory mechanism and the biological function of intercellular transfer of miRNAs from donor cells to recipient cells remain largely unclear. These findings open the intriguing possibility that, at least for these cell types, a cell can alter the expression of genes in neighboring cells by transferring miRNAs contained in microvesicles. Further investigations are required to assess the role and biological significance of secreted miRNAs in the regulation of communication among these cells.

The role of secreted miRNAs in tumor progression and viral infection was recently explored (Table 1). Many tumors have a remarkable ability to manipulate their stromal environment to their own advantage. Studies have shown the importance of communication between cancer cells and their surroundings through microvesicles [47]. Brain microvascular endothelial cells were shown to take up exosomes that contained mRNA, miRNA and angiogenic proteins released by glioblastoma cells [28]. Moreover, miR-21, which is known to be overexpressed in glioblastoma tumors, was higher in serum microvesicles from glioblastoma patients than in healthy controls [28]. Interestingly, two other studies also demonstrated the existence of abundant miRNAs in tumor-derived exosomes

and showed that the miRNA signature in secreted exosomes parallels that of the originating tumor cells [48,49]. These results suggest that the miRNA composition in secreted microvesicles might reflect molecular changes in cells from which they are derived and, therefore, may provide diagnostic information and aid in therapeutic decisions for cancer patients. However, there is no direct evidence demonstrating that exosomes of cancer cells can contribute to the horizontal transfer of oncogenic miRNAs to normal cells. Whether the secreted miRNAs released from tumor cells have a fundamental role in tumorigenesis, such as suppressing the immune response, accelerating metastasis or facilitating the formation of a tumor microenvironment, requires further study. Epstein–Barr virus (EBV) is the first known virus to encode miRNAs [50]. EBV-miRNAs are abundantly expressed in EBV-associated tumors, such as nasopharyngeal carcinoma (NPC) [51]. Viral miRNAs secreted by EBV-infected cells are transferred to, and act in, uninfected recipient cells through exosomes [52]. These EBV-miRNAs also repressed the confirmed target gene chemokine (C-X-C motif) ligand 11 (CXCL11) [52], a small chemokine belonging to the CXC chemokine family that elicits effects on target cells by interacting with the cell surface chemokine receptor CXCR3 and inducing a chemotactic response in activated T cells [53]. Two other studies also reported that exosomes released from EBV-positive NPC cell lines contain viral miRNAs [54,55]. These studies demonstrate that miRNAs secreted by EBV-infected cells can be transferred to uninfected recipient cells where viral miRNAs hijack the exosome of host cells and silence immunoregulatory genes in the recipient cells.

Given the emerging nature of this field, it is important to note that there are more questions than answers at this point in terms of the biological roles for secreted miRNAs. Because many investigations only test reporter expression and do not show that endogenous mRNA or protein targets are affected by secreted miRNAs, it is difficult to determine the functional consequences of miRNA transfer in recipient cells. To better understand the crosstalk between donor and target cells, further studies are needed to more precisely elucidate the physiological relevance of these miRNAs.

Secreted miRNAs as a new tool for fetal–maternal crosstalk

Recent studies have demonstrated that placenta-derived exosomes function as immune regulators in fetal–maternal crosstalk, thus improving maternal adaptation to the ongoing pregnancy and promoting fetal allograft survival [56]. Placenta-specific miRNAs have been observed in exosomes from villous trophoblasts [57]. More investigation is needed to elucidate the role of these miRNAs during pregnancy, however, and to test if they have the capacity to enter and reprogram maternal cells in favor of fetal survival. Some immune-related miRNAs are present in bovine milk-derived microvesicles at considerable levels [58]. Notably, this was confirmed by two other papers providing evidence for the presence of abundant immune-related miRNAs in milk, especially in colostrum [14,15]. However, the molecular basis of this phenomenon remains unknown. Further studies are required to test whether miRNAs from breast milk are biologically functional and contribute to the development of the immune system of infants.

Concluding remarks

It has traditionally been thought that 'classic' cell-to-cell communication is mediated by cell junctions, adhesion contacts or secreted signals, such as hormones (Figure 1). However, an additional mechanism of intercellular communication mediated by secreted miRNA has recently been uncovered (Figure 1). As a new type of signaling molecule, secreted miRNAs have some important features: (i) they appear to be a common phenomenon affecting many cell types; (ii) they can have biological effects close by or at a distance; (iii) they can be delivered independent of cell contact or adhesion; and (iv) they can deliver multiple messages at once and regulate numerous target genes simultaneously, allowing immediate control over target cells.

The discovery of secreted miRNAs has triggered an explosion of research activity but left many unanswered questions. For example, it is important to elucidate the mechanisms governing the biogenesis, sorting, release and uptake of secreted miRNAs. Other questions that need to be addressed are whether miRNAs convey physiologically important information for cells and whether secretion is a selective process. Moreover, it is essential to elucidate the role of secreted miRNAs in regulating physiological and pathological processes and to determine the physiologically relevant amounts of secreted miRNAs required for cell-to-cell signaling. Whether the secretion and incorporation of miRNAs are generally conserved phenomena remains to be described. Nevertheless, this new form of intercellular communication may herald a new era in our understanding of signal and molecule transfer between cells. The elucidation of this novel information transfer system will be important in understanding many biological processes including development, immune response and tumorigenesis.

Acknowledgments

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