Chapter 2
The Role of Exosomal Shuttle RNA (esRNA) in Cell-to-Cell Communication

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Abstract Cell-to-cell communication can occur in several ways, with or without cell contact. Exosomes play a role in one of the most recently discovered and versatile cell-to-cell communications, which do not require cell contact and that can act over long distances. The RNA content, mRNA and microRNA, is protected by the exosomes rigid membranes, which makes it possible for cells to communicate long-distance RNA messages via the circulation system. Their mRNA content differs substantially from their mother cell mRNA content, whereas their microRNA content seems to reflect their cellular origin more. This chapter reviews the role of exosomes in cell-to-cell communication and in particular the role of exosomal shuttle RNA (esRNA). This is a new and rapidly expanding field of research that has given cell-to-cell communication an increased complexity and that has great potential within both diagnostic and therapeutic applications.

2.1 Introduction

Exosomes were discovered in the early 1980s and were then mainly studied for their role in discarding unwanted proteins during the maturation of reticulocytes [1, 2]. In the late 1990s, exosomes were shown to have immune regulatory effects [3, 4], which introduced exosomes as an important factor in cell-to-cell communication. In 2007, mast cell-derived exosomes were shown to not only contain proteins, but also to contain functional microRNA (miRNA) and messenger RNA (mRNA) that could be transferred to recipient cells [5]. This RNA was named exosomal shuttle RNA (esRNA) [5].

All cells communicate with their surrounding environment through many different pathways, including growth factors, cytokines, hormones, chemokines, and surface-to-surface communication via membrane-bound proteins and lipids. The discovery of transferrable RNA in exosomes further increases the complexity of cell-to-cell communication, especially in relation to the presence of miRNA in exosomes, which can induce RNA interference (RNAi). RNAi is a natural process within living cells that participates in the control of gene activity. Two types of small RNA molecules

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are important for RNAi, including miRNA and small interfering RNA (siRNA). These small RNAs (20–25 nucleotides) can bind to specific mRNAs and decrease their activity by preventing the mRNA from producing its corresponding protein.

Not only exosomes, but also other larger extracellular vesicles such as microvesicles [6] and apoptotic bodies [7] also contain RNA. Furthermore, both high-density lipoproteins [8] and Argonaute 2 [9], an RNA-binding protein complex, have been suggested to carry and protect extracellular RNA. However, most of the reports that show functional effects of extracellular RNA have studied exosomes.

2.2 Presence of Exosomal Shuttle RNA (esRNA)

The field of esRNA is seeing an explosive growth over the last few years, after the initial report in 2007. In the first year after the discovery of esRNA, only three studies were published supporting the finding that exosomes contained RNA [10–12]. Two of these studies were investigating the RNA content of exosomes circulating in the plasma of cancer patient, suggesting a role for esRNA to function as biomarkers for ovarian and glioblastoma cancer [10, 12]. The third study reported the presence of esRNA in a prostate cancer cell line. Together, these studies highlight the importance of esRNA in the field of malignant disease, which has further boosted the field. In 2009, an additional five studies on esRNA were published [13–17]. Two of these reports supported the previous findings that esRNA could be used as a biomarker [14, 15]. Interestingly, the possible importance of esRNA in the fields' innate immunity [13] and reproduction [16] was also suggested, supporting the importance of esRNA in a wider range of cell-to-cell communication, beyond malignant disease.

Over the last 5 years, approximately 40 different publications have documented the presence of RNA in exosomes and cover diverse fields, including cell biology, immunology, cancer, neurology, stem cell function, and beyond. There is, however, a confounding in the literature, as the nomenclature in the field of exosomes and microvesicles is not homogenous and sometimes outright confusing. Exosomes are sometimes referred to as microvesicles and vice versa, and as many different isolation methods are used. Some investigators will have a mixture of both exosomes (40–100 nm) and larger microvesicles in their studies of extracellular RNA. The authors of this chapter suggest that the name "microvesicles" should only be used for vesicles larger than 100 nm that are of nonendosomal origin, and protocols that lead to mixtures of exosomes and microvesicles should be referred to as "extracellular vesicles." Depending on how stringent the classification of "exosomes" in each publication is, different number of publications can be identified. All extracellular vesicles seem to contain RNA, but the differences in their biogenesis, loading of RNA, and purification methods will most probably influence the specific RNA profiles identified. In this chapter, we are focusing on RNA in exosomes, with exosomes defined as vesicles smaller than 100 nm, and with evidence of having been produced in the endosomal pathway.

Table 2.1 microRNA (miRNA)

Exosomal source	Species	Number of miRNA	Method	Reference
The mast cell line, MC/9	Mouse	121	miRCURY LNA array	[5]
Plasma from patients with ovarian cancer	Human	218	Microarray	[10]
The Jurkat-derived J77 T cell line	Human	236	Agilent micro-RNA microarray	[18]
The Raji B cell line	Human	67	Agilent micro-RNA microarray	[18]
Peripheral blood monocyte-derived dendritic cells	Human	148	Agilent micro-RNA microarray	[18]
Bone marrow-derived immature dendritic cells	Mouse	144	Illumina miRNA expression array	[19]
Bone marrow-derived mature dendritic cells	Mouse	197	Illumina miRNA expression array	[19]
The hepatocellular carcinoma cell line, Hep3B	Human	134	qRT-PCR	[20]
The hepatocellular carcinoma cell line, PLC/PRF/5	Human	144	qRT-PCR	[20]

The Internet-based database, ExoCarta (http://www.exocarta.org/), lists at this time 764 miRNA as found in exosomes. This database is mainly based on the findings in exosomes derived from mouse mast cells (2007) [5], plasma of ovarian cancer patients (2008) [10], human dendritic cells, and B and T lymphocytes (2011) [18]. However, many miRNA have been identified in several of these studies and approximately 450 unique miRNA have been identified in exosomes. Recently, Montecalvo et al. [19] published miRNA data identifying 202 miRNA in exosomes from immature and mature dendritic cells and Kogure et al. [20] have identified 134 and 144 miRNA, respectively, in two different hepatocellular carcinoma cell lines, although these two datasets are not yet in ExoCarta. All studies reporting compressive lists of miRNA in exosomes till date are summarized in Table 2.1, but, of course, other studies have also reported the presence of individual miRNAs.

The presence of different mRNAs in exosomes has been clearly documented in many studies and is also reported in ExoCarta. The presence of mRNA in exosomes was earlier shown to be fundamentally different form the exosome-producing cell, and is not a random sample of the cell's cytoplasmatic mRNA, arguing for specific packaging mechanisms of RNA into exosomes. The total number of reported mRNAs in exosomes exceeds 2,300, however, these data are based on two studies only, Valadi et al. 2007 [5] and Nazarenko et al. 2010 [21]. Table 2.2 shows these 2 studies, but furthermore, it highlights that other studies have also detected large amounts of mRNAs in exosomes, which are not presented in ExoCarta.

Table 2.2 Messenger RNA (mRNA)

Exosomal source	Species	Number of mRNA	Method	Reference
The mast cell line, MC/9	Mouse	1,300	Affymetrix microarray	[5]
Culture of primary cells from glioblastoma tumor	Human	27,000	Agilent 44 K whole genome microarray	[12]
The colorectal adenocarcinoma cells, SW480	Human	11,327	Illumina Human-6 v2 expression	[28]
Saliva	Human	509	Affymetrix microarray	[24]
BDX-derived pancreatic adenocarcinoma	Rat	1,500	Illumina RatRef-12 chip	[21]
The mast cell line, MC/9	Mouse	?	Affymetrix microarray	[32]

2.3 Exosomal Shuttle RNA (esRNA)

2.3.1 Profile of the esRNA

When RNA was first discovered in exosomes, it was clearly shown that the exosomal mRNA content differs substantially from the RNA profile of the donor cells [5]. Also, the donor cells and exosomes' total RNA profile differed, where the exosomal RNA profile is lacking the 18S and 28S ribosomal RNA (rRNA) peaks that are highly prominent in the donor cells. Exosomes are instead enriched with smaller RNA such as mRNA and miRNA [5]. Since then, most studies have reported the same RNA profile with no or little rRNA in exosomes from plasma [10, 12, 22], amniotic fluid [23], saliva [22, 24], hepatocellular carcinoma cell line [20], embryonic kidney cell line [25], breast milk [22], and nasal lavage fluid [26]. However, there are studies reporting RNA profiles with more prominent rRNA peaks in samples collected from urine [23, 27] and colorectal cancer cell lines [28]. Current research seems to suggest that the RNA profiles may differ in exosomes and larger extracellular vesicles, where vesicles with a diameter >100 nm seem to contain a greater fraction of rRNA. However, the RNA profile for other nonexosomes vesicles have to be further investigated before this can be conclusively stated.

2.3.2 Horizontal Transfer of esRNA

In the first study showing the presence of RNA in exosomes, Valadi et al. [5] also showed that exosomes are able to transfer their RNA to recipient cells. This was shown by culturing mouse mast cells with radioactive uridine, prior to exosome isolation. The exosomes isolated from these cells, containing radiaoactive RNA, were then cocultured with recipient cells. The radioactive RNA was then quantified in the recipient cells, which showed that mouse mast cell-derived exosomes can transfer their RNA to other mast cells.

Subsequently, several studies have demonstrated that exosomes from many different cellular sources can be taken up by a variety of recipient cells [12, 20, 22, 29], commonly by labeling them with a fluorescent membrane dye, for example, PHK67. For the RNA to be functional in the recipient cell, exosomes cannot only be internalized by the recipient cell, but the RNA also needs to be transported to the cytosol. Montecalvo et al. [19] showed that the membrane of the exosomes can fuse with the plasma membrane and that the content of the exosomes can be released into the cytosol of the recipient cell. The second step, showing that the exosomes are able to transfer their luminal cargo to a recipient cell, was important to prove as adherence or hemifusion would not result in delivery of the RNA into the recipient cell's cytoplasm. To prove that the fusion was complete, luciferin, which is not able to cross lipid membranes, was captured inside the exosomes. Activity could be measured in the recipient transgenic luciferase cells already after 8 min, which indicates the delivery of the luminal cargo of the exosomes to the recipient cell, including miRNA and mRNA [19].

2.3.3 Functionality of Horizontally Transferred esRNA

It has also been important to show that the transfer of RNA from one cell to another via exosomes leads to functional RNA-related biological effects in the recipient cell. When exosomal RNA was first identified, the functionality of the exosomal mRNA was proven by using an in vitro translation assay, as exosomes do not themselves have the complete machinery to produce proteins. Thus, the exosomal mRNAs isolated from mouse mast cells were used as templates in an in vitro translation assay, where it was shown that it could be translated into proteins. Furthermore, both the mRNA and the protein content of the mouse mast cell-derived exosomes were analyzed before these exosomes were added to human mast cells. Newly produced mouse proteins could subsequently be identified in the human cells that were only present as mRNA in the donor mouse exosomes, and not as proteins. The combination of these data thus shows that the exosomal mRNA is functional as it can be translated into proteins in the recipient cells [5].

More recently, it has been further proven that mRNA in exosomes are functional as two independent studies have shown that an mRNA coding for a luciferase reporter gene can be transferred via exosomes and lead to luciferase activity in a recipient cell. Interestingly, the luciferase activity in the recipient cell was dependent on the amount of exosomes that were added [20] and increased over time [12].

It has also been shown that not only mRNA can be transferred to recipient cells via exosomes, but also that miRNAs can be transferred to and be functional in recipient cells. Thus, Epstein-Barr virus (EBV) transformed B lymphocytes release exosomes containing mature EBV-miRNAs that can be delivered and internalized by recipient cells [30]. Interestingly, when incubating the EVB-miRNA containing B lymphocyte exosomes with cells transfected with a luciferase vector carrying an EBV-miRNA-regulated sequence they showed an 80 % reduction in luciferase activity in

the transfected recipient cells. Cells expressing disrupted EBV-miRNA-binding sites were shown to have significantly less luciferase activity reduction, indicating that exosomes can regulate miRNA-mediated functional gene repression of specific target mRNAs in recipient cells [30].

Subsequent to this Montecalvo et al. [19] have further confirmed the delivery of functional miRNA via exosomes. This was also shown by transfecting the recipient cells with a luciferase reporter gene, with copies of the complementary target sequence for a specific miRNA, which resulted in an inhibited luciferase activity when incubated with exosomes containing the specific miRNA. The functionality of exosomal miRNA has also been shown by transfecting cells with lentiviral vector short hairpin RNA (LV-sh). Exosomes isolated from cells transfecting with LV-shCD81 were shown to induce a downregulation of the surface expression of CD81 in the recipient cells [31].

In conclusion, these studies demonstrate that the miRNA encapsulated and transferred via exosomes is functional in the recipient cells and can exhibit its regulatory effect on a targeted mRNA, which leads to a changed cellular phenotype.

2.4 Specific Loading of RNA into Exosomes

Several papers have shown that the levels of mRNA [5, 12, 32] and miRNA [5, 18] in exosomes and their donor cells correlate poorly, suggesting that the cell sorts specific RNA species into the lumen of exosomes. Thus, the RNA in exosomes is not related to just an engulfment of arbitrary RNA from the cytoplasm, which, however, could be true for larger vesicles with different production process. The mechanism behind this sorting is not yet known, but complementary papers in the same issue of *Nature Cell Biology* in 2009 possibly shed some light into these questions [17, 33]. Gibbings et al. [17] showed that miRNA were mainly detected together with RNA-inducing silencing complex (RISC) proteins, GW182 and Argonaute 2 (AGO2), in compartments assembled with endosomes and multivesicular bodies (MVB). Interestingly, GW182 and AGO2 are two major components of RISC and are required for effective gene silencing [34, 35]. In addition, these were not associated with lysosomes, endoplasmic reticulum (ER), or P-bodies. Furthermore, these investigators showed that the miRNA-silencing capacity and the sorting of GW182 into the MVB, was dependent on MVB-associated endosomal sorting complex required for transport (ESCRT) proteins, such as Alix. The authors therefore suggest that the MVB is the location for assembling and loading of RISC. These observations may be important findings for the understanding of the loading of RNA into exosomes as the exosomes are formed in the MVB and also contain high levels of GW182 [17].

In addition, another study also showed that inhibition of Alix led to loss of the suppressive effect of miRNA [25], which supports the findings above. However, the inhibition of Alix did not affect the secretion of miRNA. Instead, the authors demonstrated that inhibition of neutral sphingomylinases (nSMase) that did not have an effect on the activity of miRNA, but rather had an effect on the secretion of miRNA [25].

nSMase is an enzyme involved in the hydrolysis of sphingomyelin to form ceramide and has previously been shown that nSMase is also important for the budding of intracellular vesicles into the MVB [36]. Inhibition of nSMase left the intracellular levels of miRNA unchanged, but reduced secretion of not only miRNA, but also exosomal proteins. Furthermore, overexpression of nSMase induced secretion of miRNA, without affecting the intracellular miRNA levels. It was not exclusively shown that the secreted miRNA was inside exosomes, but as another study has shown that inhibition of nSMase led to unchanged miRNA levels intracellular, but a significant reduction of miRNA in secreted exosomes [20], this is also likely an important factor in the loading of RNA into exosomes.

Together, these data emphasize the importance of the multivesicular bodies and their cooperative proteins as well as sphingomyelin for correct loading and function of miRNA, and this machinery may play an important role in the selection and loading of specific miRNA into exosomes, which can fundamentally influence their biological function.

2.5 Biological Role of Exosomal Shuttle RNA (esRNA)

The biological function of exosomes is fundamentally dependent on the cell that produces these exosomes, as well as the current state of that cell, as this will affect the loading of proteins, mRNA, and miRNA into the exosomes. Exosomes have been shown to have many functions, which primarily have been ascribed to the exosomal proteins, for example, Fas ligand on the surface of tumor exosomes can induce apoptosis in T lymphocytes [37]. Fewer studies have so far determined the biological function of the exosomal RNA. However, as it is predicted that one miRNA can interfere with 100–200 mRNA [38, 39] and also that mRNAs can affect the protein production of the recipient cells, the potential biological role of esRNA is vast.

2.5.1 Protective Effect Against Oxidative Stress

In 2010, Eldh et al. [32] showed, by microarray analysis, that the exosomal mRNA content does not only differ from their donor cells but also depends on which condition they are released under. Furthermore, exosomes released under different conditions have different effects on the recipient cells. Thus, exosomes released from cells grown under the condition of oxidative stress have been shown to provide recipient cells with a tolerance to further oxidative stress, showing that the exosomes condition the recipient cells in some ways. This was shown by a greater viability in cells receiving exosomes released by other cells grown under oxidative stress, compared with cells receiving exosomes released from cells grown under normal condition. The protective effect of the exosomes produced under oxidative

stress was removed by exposing the exosomes to UV light, which destroys the RNA, arguing that the esRNA is involved in the protective effect.

2.6 Induce Invasiveness of Tumor Cells

The microenvironment surrounding a tumor plays an important role in the tumor progression. Large amount of macrophages are often found in breast cancer and an increased macrophage infiltration is commonly associated with a poor prognosis. Macrophages induce proliferation and angiogenesis and enhance the invasiveness and metastasis of tumors [40, 41]. Previously, it has been demonstrated that soluble factors such as cytokines and growth factors released by macrophages can induce these effects. In addition, it was recently shown that exosomal miRNA from macrophages could upregulate the invasiveness of breast cancer cell in vitro [42]. The study by Yang et al. [42] showed that exosomes from IL-4-activated macrophages contained miR-223 and that these exosomes promoted invasion of breast cancer cells (SKBR3) in a transwell invasion assay. When antisense oligonucleotides for miR-223 was added, the invasion induced by the exosomes was decreased. Furthermore, miR-223 could target and reduce *Mef2c*, which led to an increase of β-catenin in the nucleus. The authors suggested that miR-223 was transferred from macrophages to breast cancer cells, via exosomes, where it affected the *Mef2c*-β-catenin pathway, which led to an increased invasiveness of the breast cancer cells. Disruption of the exosomal communication between macrophages and breast cancer cells may play an important role in the prevention of metastasis and could be a potential target in breast cancer therapy.

2.6.1 Increased LDL Levels in Mouse Plasma

The fact that RNA can be transferred between cells via exosomes has taken the cell-to-cell communication concept to another level of complexity. In 2011, this communication mechanism was shown to be even more complex when Zhang et al. [43] found plant microRNA in human serum of healthy Chinese subjects. MIR156a and MIR168a were the two exogenous plant microRNAs that were found in the highest concentrations and have shown to be enriched in rice. They showed that these plant microRNA were upregulated in mice feed with a rice diet, both in the serum and in the liver. Furthermore, more than half of the exogenous plant microRNA found in mouse serum was found to be contained within microvesicles. By in vitro studies they showed that colon cells transfected with MIR168a release microvesicles containing MIR168a. Also, that these microvesicles can be taken up by liver cells and upregulate their MIR168a concentration. Bioinformatics analysis identified low-density lipoprotein reporter adaptor protein 1 (LDLRAP1) as the most conserved putative-binding site for MIR186a. Interestingly, they showed that exogenous plant

microRNA can regulate mammalian gene expression. This was shown by an enrichment of MIR168a in mouse serum and liver, in mice feed with rice, and an inhibition of MIR168a target gene LDLRAP1 in the liver of these mice. Most significantly, this resulted in a decrease in the removal of LDL from the mouse plasma. The authors, therefore, hypothesize that when food is processed in the gastrointestinal tract, the intestinal epithelial cells have the capacity to take up the plant miRNA and pack it into vesicles, which are released into the circulation to reach other organs such as the liver, suggesting that regulatory plant miRNA could travel to and affect different cells [43].

2.6.2 Allow Tumor Cells to Maintain Their Oncogenesis

Let-7 is commonly viewed as a tumor suppressor miRNA and loss of the family members of let-7 indicates poor survival [44]. It has been shown that the metastatic gastric cancer cell line, AZ-P7a, expresses high levels of let-7 miRNAs, both intracellular and in their exosomes. In contrast, in other cancer cell lines analyzed, the level of let-7 miRNAs was high in the cells but low in the exosomes. The authors hypothesized that AZ-P7a cells selectively and actively secreted let-7 miRNA family into the extracellular environment via exosomes, which would decrease the antitumor effect on the inside of the cells and lead them to maintain their oncogenesis and invasiveness, as AZ-P7 is highly metastatic [45].

These findings are interesting as it has been shown that presence of let-7 miRNA in plasma vesicles is related to survival in nonsmall-cell lung cancer [46]. When the patients were divided into let-7f low and high groups, the low-let-7f group had an overall survival rate of 30 % at 46 months, while the high-level group had a survival rate of 8 % (p-value 0.038). Together, these two studies suggest that tumors use exosomes to release let-7f, which may allow the cell to become more tumorigenic, thus affecting survival. Furthermore, the second study is the first to show that the miRNA of extracellular vesicles can be used as a prognostic tool and to predict survival in cancer patients. Importantly, it was not clearly established that the vesicles studied were indeed exosomes and not other larger extracellular vesicles [46].

2.7 Diagnostic and Therapeutic Potential

2.7.1 Biomarkers

Exosomes have shown to have many qualities that make them excellent candidates as biomarkers. Qualities such as being easily sampled from patients by relatively noninvasive means as they can be extracted from several different body fluids such as plasma[47], urine [48], breast milk [49], BAL [50], saliva [24], and NAL [26]. Another feature that makes exosomes a prime candidate for biomarkers is that they

have been shown to be upregulated in serum of patients with ovarian and lung cancer [10, 15]. Furthermore, these tumor exosomes were shown to contain miRNA earlier associated with ovarian and lung tumor, respectively. Thus, the miRNA isolated from the tumor exosomes were shown to reflect the miRNA profile in the tumor tissue. In addition, for the ovarian cancer patients, the miRNA correlating with the tumor miRNA was also upregulated in comparison with both patients with benign ovarian tumors and healthy subjects [10].

Plasma exosomes from patients with glioblastoma have been demonstrated to contain the mRNA for the mutated protein EGFRvIII. The mutated mRNA was found in about 50 % of the tumors, in approximately 25 % of the plasma exosomes of the glioblastoma patients and in 0 % of the healthy controls. Furthermore, the mRNA could not be detected in the plasma exosomes 2 weeks after removal of the tumor, indicating that the tumor was the source of the exosomes [12] and again suggesting a potential role of the RNA content to be used as biomarkers.

In addition, miRNA from exosomes isolated from urine and saliva has also been investigated for the potential use as biomarkers for Sjögren syndrome and prostate cancer, respectively [14, 51], and RNA isolated from exosomes found in amniotic fluid can be used for fetal sex determination [23].

Taken together, multiple studies show that exosomes can be used for profiling of many different diseases such as cancers, and that they can even be used in baby gender determination.

2.7.2 Gene Delivery Vehicles

The fact that exosomes function as natural vectors for the transfer of genetic material between cells has made them interesting candidates as gene therapy vectors. Exosomes have the advantage of being endogenous and to be able to escape the immune system. Vesicles such as viruses, nanoparticles, and liposomes all function as vectors to a certain extent, but will sooner or later be discovered by the immune system and trigger an immune response, which will lead to a rejection or inflammation of affected recipient cells. Another challenge in gene therapy is the delivery, which to have the greatest effect and least side effects, has to be able to be specific. A group in Oxford showed for the first time that RNA-loaded exosomes have therapeutic potential by a knock down of the levels of BACE1 in the brain, which is a gene implicated in Alzheimer's disease [52].By harvesting bone marrow from mice and culturing the immature dendritic cells, they transfected these cells with a peptide-targeting neuron cells, called RVG, fused to a membrane protein, Lamp2b, known to be enriched in exosomal membranes. These transfected cells produced exosomes with the RVG peptide on their surface. The modified exosomes were then loaded with therapeutic siRNA, designed to knock down the BACE1 gene, and injected back into the mice. Mice receiving the exosomes displayed a reduced level of BACE1 in the brain. This study showed both that exosomes can be modified to be able to be derived to a specific cell/organ and that they can be loaded with a cargo, which can be successfully

delivered to the target. Further studies are, of course, required to show any equivalent function in humans, which, of course, raise several methodological and safety concerns and require extensive preparative studies.

In addition, it has also been found that $CD34^+$ cells release exosomes containing proangiogenic miRNA, as $CD34^+$ exosomes induced angiogenic activity in endothelial cells in vitro and vessel growth in vivo in mice. Although it was not established that it was the miRNA that transferred the effect. This natural therapeutic potential of using $CD34^+$ exosomes to induce angiogenesis could improve the outcome of ischemic injuries and improve recovery [53] and also further enhance the potential use of exosomes in therapy.

2.8 Summary and Conclusions

Exosomes have been proven to present an important route of cell-to-cell communication by the delivery of functional RNA species, which fundamentally affect the biological function of a recipient cell. Exosomal shuttle RNA is likely to have vast regulatory functions in the human body under healthy circumstances, but may also be used as biomarkers for many diseases, and may explain progression of severe diseases such as cancers. Last, as exosomes are natural vectors for delivering RNA to cells, they are likely to be useful for the delivery of therapeutic RNAi, such as siRNAs or microRNAs in many diseases. The field of understanding the function and possibilities of esRNA is just beginning its journey and will keep researchers in many fields of biology and medicine occupied for years to come.

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