

Review

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The role of extracellular vesicle microRNAs in cancer biology

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Abstract: microRNAs (miRNAs) constitute a large family of small, approximately 20–22 nucleotide non-coding RNAs that regulate the expression of target genes, mainly at the post-transcriptional level. Multiple studies report that miRNAs are involved in homeostatic maintenance and that aberrant expression of miRNAs is often observed in various types of diseases, including cancer. In cancer biology, miRNAs exert functional roles in tumor initiation, drug resistance, and metastasis. miRNAs are also secreted through small vesicles called exosomes, which are endosome-derived vesicles derived from various cell types including immune and tumor cells. In addition to cellular miRNAs (ce-miRNAs), secreted miRNAs (se-miRNAs) play important roles in cancer development and metastasis. Therefore, se-miRNAs in body fluids have been investigated as a promising biomarkers and therapeutic targets for cancer treatment. In this review, we summarize the current knowledge of miRNA functions in cancer development and discuss the potential clinical applications of se-miRNAs, e.g. as diagnostic markers and therapeutic targets.

Keywords: biomarker; cancer; diagnosis; exosome; microRNA; therapy.

Introduction

Several lines of evidence indicate that microRNA (miRNAs) are critical regulators of global messenger RNA (mRNA) expression in normal and abnormal biological

processes [1–3]. Aberrant expression of miRNAs has been observed in multiple types of cancers and is associated with malignant cancer phenotypes, including metastasis and drug resistance. Therefore, manipulation of miRNA expression represents a promising approach to cancer therapy. Several recent studies report that miRNAs are secreted through exosomes, and that the levels of circulating miRNAs packaged in exosomes differ between cancer patients and healthy donors [4–6]. More importantly, the contents and amount of secreted microRNA (se-miRNAs) are associated with disease stage and regulation of the malignant phenotype [7–9]. In this review, we summarize the major findings regarding se-miRNA function in tumor development, and discuss how detection of se-miRNAs and removal of cancer-derived exosomes could be applied to clinical diagnosis and treatment.

Biosynthesis and functions of miRNAs

miRNAs are approximately 20–22-nucleotide non-coding RNAs that regulate gene expression at the post-transcriptional level by binding to the 3'-untranslated regions (3'UTRs) of target genes or to imperfectly complementary sequences called miRNA response elements (MREs). The binding of miRNAs to target genes mainly leads to the degradation or translational inhibition [10] (Figure 1). miRNAs are transcribed, mainly by RNA polymerase II, as long primary transcripts containing hairpin structures (pri-miRNAs). In the nucleus, these transcripts are cleaved into 70–100 nucleotide precursor miRNAs (pre-miRNAs) by a microprocessor complex containing RNase III Drosha and DGCR8. The pre-miRNAs are exported to the cytoplasm by exportin-5, a member of the Ran-dependent nuclear transport receptor family [11], and further processed into a miRNA:miRNA* complex by a complex containing RNase III Dicer and the transactivating response RNA-binding protein (TRBP).

One of the two strands (the miRNA) is selected as a guide strand, and the complementary strand (miRNA*)

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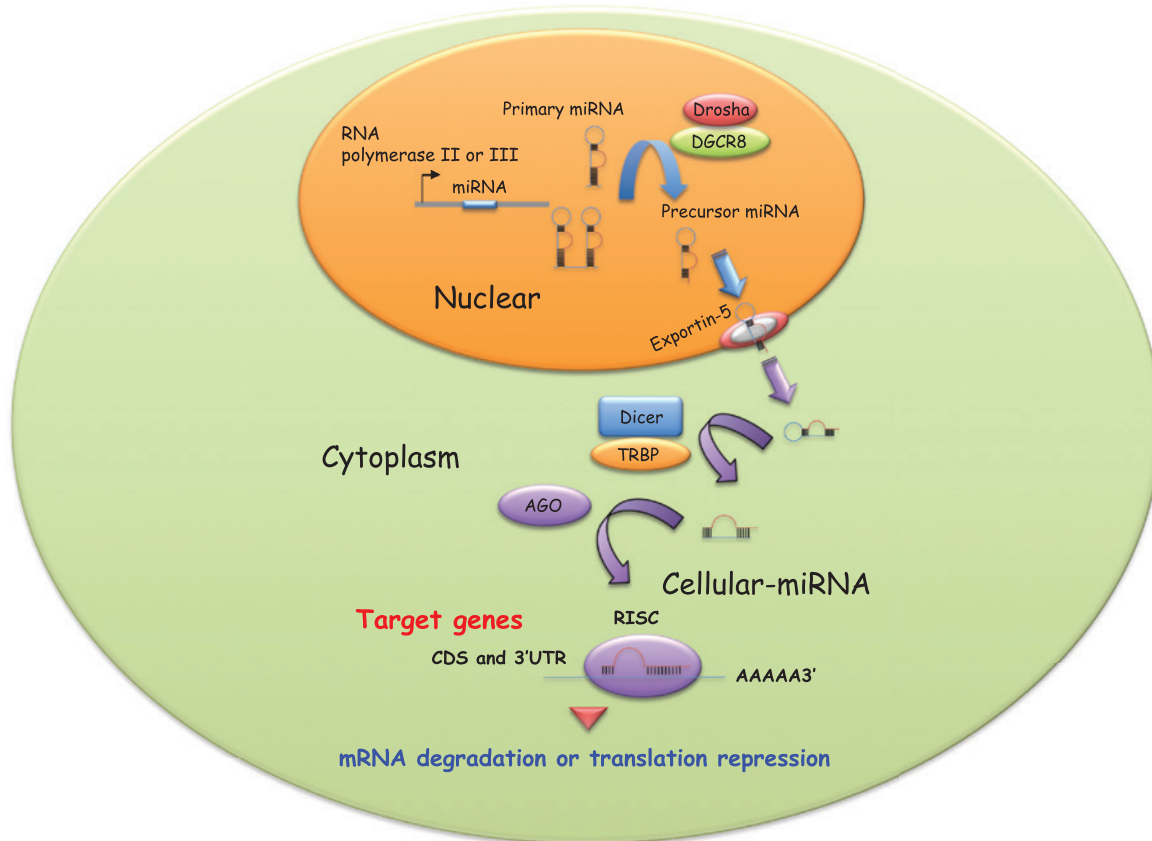


Figure 1: Biogenesis and function of cellular and exosomal microRNA.

miRNAs are transcribed by RNA polymerase II or III as pri-miRNA, and then processed in the nucleus by Drosha-DGCR8 into pre-miRNA. The pre-miRNA is exported to the cytoplasm by exportin-5 and further cleaved by a complex composed of Dicer and TRBP. The functional strand of mature miRNA is incorporated into the RNA-induced silencing complex (RISC), which contains GW182 and Argonaute protein. As a component of this complex, the mature miRNA regulates gene expression by binding to partially complementary sequences in the 3'UTRs or coding regions of target mRNAs, leading to mRNA degradation or translational repression.

is usually degraded [12]. However, recent evidence suggests that miRNA* can also serve as a functional strand, and may play significant biological roles [13]. The mature miRNA is incorporated into the RNA-induced silencing complex (RISC), which contains the GW182 and Argonaute (AGO) proteins. As a component of this complex, the mature miRNA regulates gene expression by binding to partially complementary sequences in the 3'UTRs or MREs of target mRNAs, leading to mRNA degradation or translational repression [12].

Secretion of miRNAs

In 1979, Taylor and Doellgast first reported that several types of tumor cells release or shed intact microvesicles composed of membrane proteins [14]. Subsequently, a number of studies reported that mRNA, miRNAs, and long non-coding RNAs are secreted from different types

of cells through extracellular vesicles (EVs) [15] and play important roles in cell-to-cell communications such as inflammatory response, tissue regeneration, and tumor progression [16–20].

EVs are mainly classified into the following three types: exosomes (30–100 nm), microvesicles (100–1000 nm), and oncosomes (1–10 μ m); each of these three vesicle types has a unique size and function in intracellular communication [21–24]. Although the molecular mechanisms of EV biogenesis and secretion are not fully understood and depend on cell type [25], neutral sphingomyelinase 2 and some Rab GTPases, such as Rab11, Rab27a and Rab27b, have been reported to regulate EV secretion [26–28]. Because EVs are secreted from various types of cells under both physiological and pathological conditions, and their RNA and protein contents differ between patients and healthy donors [29–32], these vesicles are increasingly recognized as promising biomarkers for the diagnosis and prognosis of various diseases.

Se-mRNAs in cancer

Cellular microRNAs (ce-miRNAs) can be classified as tumor-suppressive or oncogenic and play important roles in tumor development such as tumor initiation, acquisition of drug resistance, and metastasis [33–35]. Likewise, se-miRNAs in exosomes (exosomal miRNAs) are also important for tumor progression and metastasis in multiple cancers [7, 9, 20, 36, 37] (Figure 2). In addition to these findings, Melo et al. [8] reported that exosomes derived from breast cancer cells contain pre-miRNAs that interact with the RISC complex and exhibit cell-independent generation of mature miRNAs from pre-miRNAs. In addition, McKenzie et al. [42] reported that the secretion of AGO2 into exosomes depends on the KRAS mutation status of colon cancer cells. Since AGO2 is important for ce-miRNA-mediated gene expression, these findings suggest that se-miRNA function is also regulated by the activity and mutation status of oncogenes in donor cells. Therefore, many cancer researchers are trying to remove or antagonize tumor-derived exosomes as a novel approach to cancer therapy or to develop sensitive systems for detecting se-miRNAs in patients' body fluids.

The roles of se-miRNAs in cancer development

Tumorigenesis

Antonyak et al. [38] demonstrated that EVs from MDA-MB-231 breast cancer and U87 glioblastoma cell lines induce normal fibroblast and epithelial cells to acquire the transformed characteristics of cancer cells, such as anchorage-independent growth and improved survival. Exosomal miRNAs also promote the acquisition of tumor-seeding ability in non-tumorigenic human breast cells [8]. In contrast to exosomes from healthy donors, those from breast cancer patient serum induce MCF10A cells to form tumors in immunodeficient mice. Interestingly, Dicer protein is more abundant in exosomes from cancer patients. Recently, Gutkin et al. [43] reported that hTERT mRNA is also packaged into exosomes and supports the transformation of fibroblasts into telomerase positive cells. Considering these findings, exosomal miRNAs and mRNAs might play an important role in the transformation of normal cells into malignant cells.

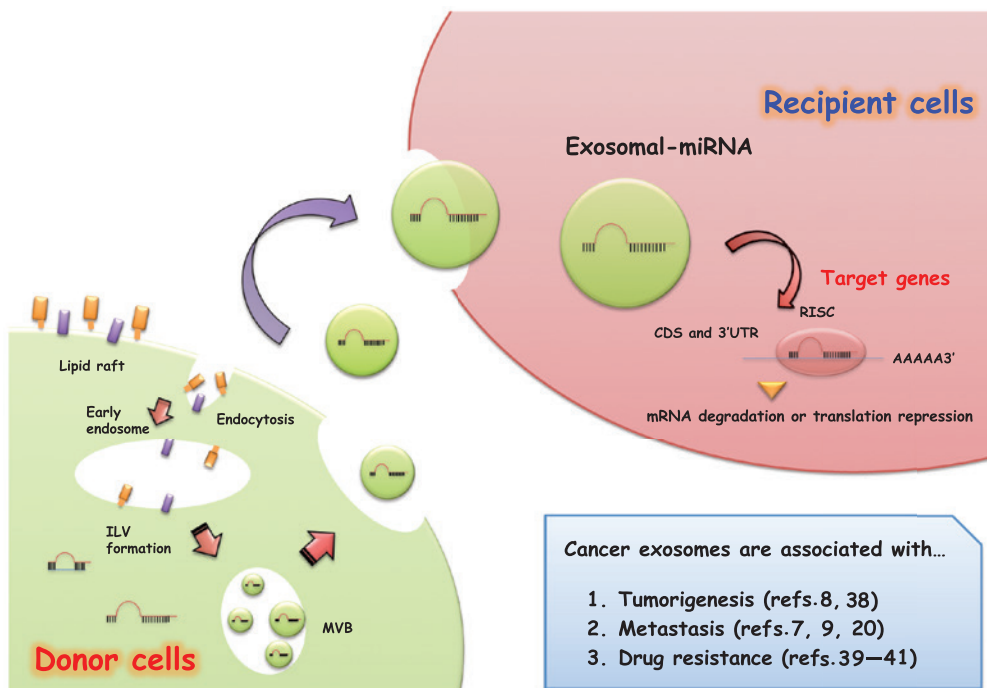


Figure 2: Biogenesis and function of exosomal microRNA.

The endosomal system is composed of primary endocytic vesicles, early endosomes, and multivesicular bodies (MVBs). Primary endocytosed vesicles are recycled to the plasma membrane or transferred to MVBs. The proteins and miRNAs that are entrapped into the limiting membrane of MVBs can be selectively packaged into intraluminal vesicles (ILVs) by invagination of the MVB membrane. MVBs can fuse either with the lysosome for degradation or with the plasma membrane for the release of exosomes into the extracellular space. Exosomal miRNAs are transferred from donor cells to recipient cells and function as cellular miRNAs for gene regulation.

Tumor metastasis

Several studies reported that exosomes are among of the most important factors involved in pre-metastatic niche formation and organotropism in metastasis [36, 44, 45]. In pancreatic cancer, exosomes from pancreatic ductal adenocarcinomas (PDACs) induce liver pre-metastatic niche formation in mice [45]. Exosomes derived from PDACs promote Kupffer cell-mediated TGF- β secretion, which enhances fibronectin production in hepatic stellate cells. Fibronectin accumulation in hepatic stellate cells induces the formation of the pre-metastatic niche by recruiting bone marrow-derived macrophages to the liver.

The organotropisms of metastatic cancer cells are determined by the combination of integrin family proteins displayed on exosomes [44]. While exosomes expressing integrin $\alpha v \beta 5$ specifically bind Kupffer cells and support the liver tropism of colon and pancreatic cancer cells, exosomes expressing integrin $\alpha 6 \beta 4$ and $\alpha 6 \beta 1$ support the lung metastasis of breast cancer cells via binding to lung-resident fibroblasts and epithelial cells.

Exosomal miRNAs are also important for tumor metastasis [7, 9, 20]. Zhou et al. [9] reported that high levels of miR-105 are present in metastatic breast cancer cells and their exosomes, and that exosomal miR-105 promotes tumor metastasis by directly targeting zonula occludens 1 (ZO-1), which regulates tight junction and supports the barrier function of endothelial monolayers. Exosomal miR-181c also promotes the brain metastasis of breast cancer cells by destroying the blood-brain barrier [20]. MiR-181 induces the delocalization of actin fibers via downregulation of 3-phosphoinositide-dependent protein kinase-1, leading to the disruption of intercellular junctions in brain endothelial cells. Fong et al. [7] reported that miR-122 promotes breast cancer metastasis by regulating glucose metabolism in pre-metastatic sites. Interestingly, in contrast to the intracellular level of miR-122, which does not significantly differ between non-metastatic and metastatic cancer cells, the level of exosomal miR-122 is correlated with the metastatic capacity of breast cancer cells. Exosomal miR-122 regulates glucose metabolism through the direct target of pyruvate kinase isozymes M2 (PKM2) in pre-metastatic sites, resulting in the formation of the pre-metastatic niche.

Drug resistance

The main mechanisms underlying the acquisition of drug resistance can be classified into three categories: (1) up-regulation of drug transporters that export anti-cancer

agents [46]; (2) up-regulation of anti-apoptotic genes [47]; and (3) activation of cell survival pathways [48]. In addition to these important pathways related to drug resistance, the epithelial-to-mesenchymal transition (EMT) was originally considered to be important for both tumor metastasis and drug resistance; however, more recent evidence suggests that EMT contributes mainly to drug resistance [49, 50]. Several miRNAs have been identified as critical regulators of drug resistance in various types of cancer through the pathways described above [33, 51–56].

Se-miRNAs are also important for the regulation of drug resistance [39–41]. Chen et al. reported that the level of miR-222 is elevated in exosomes derived from docetaxel-resistant breast cancer cells, and that exosomal miR-222 is transferred into drug-sensitive breast cancer cells [41]. Challagundla et al. [40] reported that exosomal miR-21 and miR-155 promote the acquisition of chemoresistance by human neuroblastoma (NBL) cells. Transfer of exosomal miR-21 from NBL cells to human monocytes and exosomal miR-155 from human monocytes to NBL cells is important for the tumor microenvironment, and such crosstalk promotes resistance to chemotherapy. Exosomal miR-21 from NBL cells induced Toll-like receptor 8 and nuclear factor- κ B (NF- κ B)-dependent up-regulation of miR-155 in monocytes, leading to the production of miR-155-containing exosomes. Exosomal miR-155 promotes CDDP resistance in NBL cells through the direct suppression of TERF1, a component of the shelterin complex and inhibitor of telomerase. Au Yeung et al. [39] reported that exosomal miR-21 from cancer-associated adipocytes (CAAs) and fibroblasts (CAFs) promotes paclitaxel resistance in ovarian cancer cells. Exosomal miR-21 from CAAs and CAFs is transferred into ovarian cancer cells, where it inhibits apoptosis and confers resistance to paclitaxel via direct suppression of APAF1 [39, 57].

Exosome-based strategies for cancer diagnosis and therapy

Cancer diagnosis and therapy targeting exosomes

Given that exosomes and exosomal miRNAs from cancer tissues are important for tumor development processes such as tumor seeding, drug resistance, and metastasis (see Section “The roles of se-miRNAs in cancer development”), many clinical oncologists and cancer researchers are currently trying to develop novel methods for cancer

diagnosis and therapies based on detection and removal (or antagonism) of tumor-derived exosomes.

Diagnosis

Because exosomal miRNAs and circulating RNAs are associated with secreted proteins such as CD63, Ago proteins, and HDL [17, 58, 59], they are stable in body fluids and easily detectable using quantitative PCR methods. Several studies have shown that exosomal miRNAs can be detected in a variety of body fluids, from blood to breast milk, and that their levels are correlated with patient status [4, 5, 60–73] (Table 1). On January 21, 2016, cancer diagnosis using exosomes from blood became commercially available in the US (<http://www.exosomedx.com>) [6].

Like cellular miRNA expression profiles, the expression profiles of cell-free and circulating miRNAs are also correlated with the pathological condition of cancer patients [4, 60]. Therefore, a considerable amount of research has been expended in trying to develop novel methods for the detection and characterization of microvesicles in the blood of patients classified according to clinical stage [74–76]. Im et al. [74] developed a surface plasmon resonance-based system for the detection of exosomal proteins. Using this system, they identified CD24 and EpCAM as specific markers for tumor-derived exosomes derived from ovarian cancers. Yoshioka et al. [75] also established a highly sensitive and rapid analytical

method, ExoScreen, for detecting and characterizing exosomes from blood samples of colon cancer patients. These reports suggest that exosomes are promising biomarkers for the early detection of cancers as well as for monitoring therapy response and patient condition.

Several studies have reported that specific exosomal miRNAs are associated with the condition of cancer patients [5, 67, 73]. In epithelial ovarian cancer (EOC), serum exosome levels are elevated, and high levels of exosomal miR-200b and miR-200c are observed mainly in patients with advanced EOC [5]. Ogata-Kawata et al. [67] found that in comparison to healthy controls, colon cancer patients, even in the early stages, exhibited significantly higher levels of seven exosomal miRNAs (let-7a, miR-1229, miR-1246, miR-150, miR-21, miR-223, and miR-23a). Shi et al. [73] reported that increases in the level of exosomal miR-21 in cerebrospinal fluid correlate with poor prognosis and cancer recurrence in glioma patients.

Exosomal lincRNA and miRNA are also detected in the urine of prostate and bladder cancer patients [77–79]. Bryzgova et al. [77] reported that the level of miR-19b, normalized to that of miR-16, in urine-derived exosomes was significantly lower in prostate cancer patients than in healthy donors. Armstrong et al. also reported that in bladder cancer, high levels of miR-21 and miR-4454 are commonly detected in whole blood cell, tumor, and urine. Recently, lincRNA HOX transcript antisense RNA (HOTAIR), which is associated with tumor progression and poor prognosis in several types of cancers [80–82], was reported in urinary exosomes from

Table 1: Profiles of se-miRNAs in cancer patients.

Cancer	Body fluids	microRNAs	Reference
Glioma	Cerebrospinal fluid (exosome)	↑ miR-21	[73]
Oral cancer	Blood (plasma)	↑ miR-181c	[61–63]
	Blood (plasma)	↑ miR-21	
	Blood (plasma)	↑ miR-196	
Lung cancer	Blood (plasma/exosome)	↑ miR-205, -19a, -19b, -30b, and -20a	[64–66]
	Blood (exosome)	↑ miR-378a, miR-379, miR-139-5p, and miR-200b-5p	
	Blood (plasma)	Down-regulation after RT ↑ miR-29a and miR-150	
Colon cancer	Blood (exosome)	↑ let-7a, miR-1229, miR-1246, miR-150, miR-21, miR-223, and miR-23a	[67–69]
	Blood (Serum)	↑ miR-23a, miR-27a, miR-142-5p and miR-376c	
	Blood (Serum)	↑ miR-203	
Ovarian cancer	Blood (exosome)	↑ miR-21, miR-141, miR-200a, miR-200b, miR-203, miR-205 and miR-214	[4, 5]
	Blood (exosome)	↑ miR-200b and 200c	
Breast Cancer	Blood (Serum)	Combination of miRNA profile	[60]
		↑ miR-1246, miR-1307-3p and miR-6861-5p ↓ miR-4634 and miR-6875-5p	
Gastric cancer	Blood (plasma)	1 miR-26a, miR-142-3p, miR-148a, and miR-195	[71]
Prostate cancer	Urine (exosome)	T miR-574-3p, miR-141-5p and miR-21-5p	[72]

RT, radiation therapy.

bladder cancer patients. Since urinary exosomes are a non-invasive source, combinatorial methods designed to detect simultaneously urinary miRNAs and lncRNA might provide a more precise and simple approach for the diagnosis of prostate and bladder cancers [83].

Exosome targeting therapy

Several studies report that exosomes from cancer cells attenuate the anti-tumor effects of immunotherapeutic drugs such as rituximab and trastuzumab by acting as a decoy for cancer cells [84, 85]. Therefore, cancer researchers are attempting to remove such exosomes from patient blood. Ciravolo et al. [84] also reported that the exosomes from HER2-positive breast cancer cells display HER2 protein on their surface, thereby competitively inhibiting the interaction between breast cancer cells and trastuzumab, and ultimately leading to tumor cell proliferation and poor prognosis. Aung et al. [85] found that lymphoma-derived exosomes that display CD20 protein on their surface inhibit the anti-tumor effects of the anti-CD20 chimeric antibody rituximab by direct binding. These findings suggest that reduction or removal of cancer exosomes would support immunotherapy against cancer-specific antigens.

Another approach is to trap metastatic cells using a 3D scaffold device, called an “M-Trap” [86]. Cancer exosomes from ovarian cancer patient ascites are embedded on the M-Trap, acting as a pre-metastatic niche. Therefore, this device effectively impairs the interaction between metastatic ovarian cancer cells and their niche. In addition to the exciting findings about the roles of exosomes in cancer metastasis described in subsection “Tumor metastasis” [20, 44, 45], these results suggest that exosomes are critical mediators in the crosstalk and homing of metastatic tumor cells to the niche in various types of cancers.

Conclusions

In this review, we summarized the roles of exosomal miRNA in cancer biology, including tumor initiation, drug resistance, and metastasis. Given that several lines of evidence demonstrate that miRNA profiles, not only in tumor tissues but also in body fluids, reflect the condition and tumor stage of cancer patients, development of highly sensitive and rapid systems for detecting exosomes and their RNAs should contribute to improvements in cancer diagnosis. In addition, targeting and removal of cancer exosomes represents a novel and promising approach for treatment of metastatic cancer. Accordingly, increasing

our understanding of the molecular mechanisms of miRNA and exosome secretion, along with profiling of exosomal miRNAs in each tissues and body fluid, is an important goal for both basic and clinical cancer research.

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