

The role of exosomes contents on genetic and epigenetic alterations of recipient cancer cells

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ABSTRACT

Exosomes, as a mediator of cell-to-cell transfer of genetic information, act an important role in intercommunication between tumor cells and their niche including fibroblasts, endothelial cells, adipocytes and monocytes. Several studies have shown that tumor cells can influence their neighboring cells by releasing exosomes. These exosomes provide signaling cues for stimulation, activation, proliferation and differentiation of cells. Exosomes contain mRNAs, microRNAs (miRNA), and proteins that could be transferred to target cells inducing genetic and epigenetic changes. By facilitating the horizontal transfer of bioactive molecules such as proteins, RNAs and microRNAs, they are now thought to have vital roles in tumor invasion and metastases, inflammation, coagulation, and stem cell renewal and expansion. The aim of this review article is to discuss the significance of exosome-mediated intercellular communication within the tumor biology.

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Introduction

Cancer is one of the leading causes of human deaths resulting in mortality about 13 percent. Every year in developing countries more than half the cases of cancer occurred. Cancer incidence rate is increasing due to an aging population and changes in lifestyle (1). Many changes must occur in genes, especially in oncogenes and tumor suppressor genes, until a cancer cell arise out of a healthy cell. Oncogenes promote cellular proliferation and cell growth, while tumor suppressor genes are responsible for the cell division inhibition and survival (2). It has been well documented that over-expression of normal oncogenes, under-expression of tumor suppressor genes and loss of function of such genes, for example genes involved in apoptosis pathways, are three important mechanisms associated with cancer onset (2). Exosomes, 30-100 nm endosomal vesicles, participate in different biological processes, including mediation of cell-cell signaling by carrying genetic materials between cells. Release of the exosome has been reported from a

variety of tumor cells (3). The secreted exosomes can attach to the discrepant receptors on the surfaces of target cells and vacate their components into the second cells after fusing with their membranes (4). This process triggers the functional changes within target cells. Due to horizontal transfer of bioactive materials such as proteins, RNAs, DNA and microRNAs, today, it is believed that they play important roles in various processes during tumor biology such as invasion, metastasis, progression, inflammation, angiogenesis and stem-cell renewal and expansion (Figure 1). In this article, we review the recent approaches documenting the potential roles of exosomes contents in genetic and epigenetic alterations of recipient cells.

Exosomes

The membrane-derived vesicles are generally discriminated by size with two major classes; the larger class is called microvesicles (200–1000 nm) and the smaller class of nanometer size vesicles is called exosomes (30–200 nm) (3).

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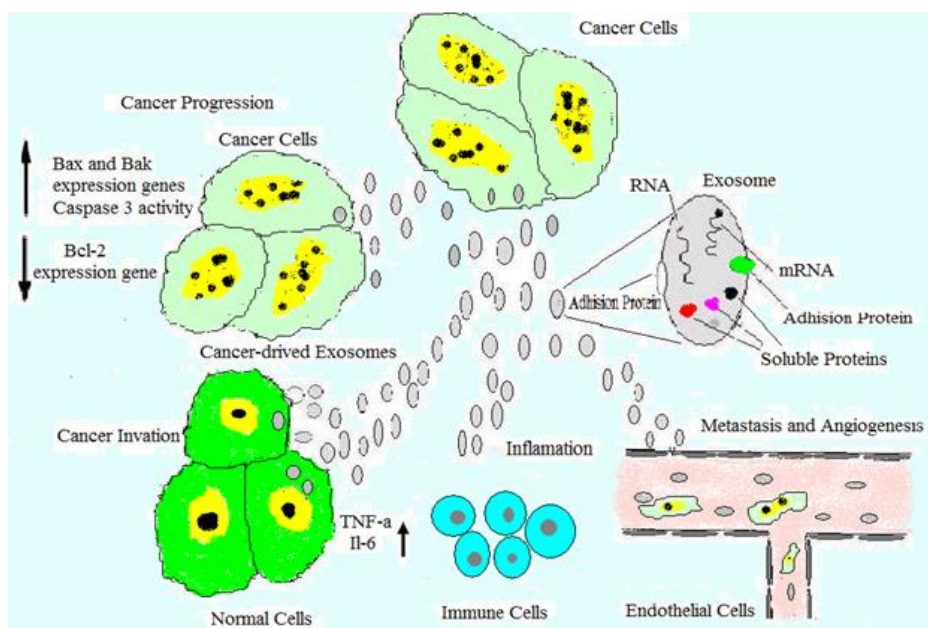


Figure 1. Horizontal transfer of bioactive materials (proteins, RNAs, protein, microRNAs ect), to normal cells, cancer cells (Increase of Bax and Bak expression genes, increase of Caspase 3 activity, Decrease of Bcl-2 expression gene), immune cells (Increase of TNF- α and Il-6) and endothelial cells play important roles in various processes during tumor biology such as invasion, progression, inflammation, angiogenesis and metastasis

Exosomes are released from a variety of cell types such as B-cells, T-cells, mast cells, stem cells, dendritic cells, neurons, adipocytes, platelets, endothelial cells, epithelial cells and cancer cells (4). The composition pattern of each exosome is closely associated with the origin of the cell that is released from. Moreover, this pattern indicates the functionality of the original cell (5). Exosomes have also been isolated from most body fluids, including urine and amniotic fluid, blood, serum, saliva, ascites, breast milk, cerebro-spinal fluid and nasal secretion (4). These vehicles are thought to be produced via the formation of multivesicular bodies (MVB) and secreted through the plasma membrane via exocytosis. Exosomes are released upon fusion of MVB with plasma membrane through endocytotic pathway, and then control a number of signaling pathway that are essential for cell normal functions (6, 7).

This nano vesicle contains a variety of proteins (including heat shock proteins, cytoskeletal proteins, adhesion molecules, membrane transporter and fusion proteins), lipids, mRNAs, different non-coding RNAs and miRNAs. These components are specific to the origin of the exosomes and contribute to cell-cell communications that could be transferred to target cells inducing genetic and epigenetic changes (8, 9). Beside the type of cells, several factors such as pathological and physiological conditions of the origin cells impact on the molecular pattern delivered to exosomes. Additionally, these factors also control the abundance of the production and

secretion of exosomes. It is also reported that sorting the protein cargos to exosomes is modulated by the subcellular compartment. The most abundant proteins among exosomes population are alix, Tgs101, ceramide, flotillin, Rab and tetraspanin family members that contributing to biogenesis of exosomes(10). Two different pathways are utilized to deliver the proteins to exosomes, including ESCRT-dependent and independent pathways, suggesting that selecting cargo within exosomes is a multilevel process (5).

There are few reports about concerning the mechanisms involved in the selection of nucleic acids within exosomes. Carrying nucleic acids through exosomes protects them from enzymes such as RNases, which are present in the extracellular matrix space and biological fluids. The functional mRNAs can translate to protein when are delivered into the target cells. Delivery of mRNAs can profoundly affect the epigenetic profile of the recipient cells and thereby alter their functionality (11, 12).

Surprisingly, three independent studies isolated single stranded DNA, double stranded DNA and retrotransposons from exosomes. Recently, the presence of specific DNA derived from brain tumor cell within exosomes encoding oncogene H-ras that was confirmed by sequencing, could transiently change biological properties of the recipient cells (13). Further studies need to clarify the mechanisms involved in classifying exosomal DNA on the basis of transformation of cells into tumor cells.

By facilitating the horizontal transfer of bioactive molecules, including proteins, RNAs and microRNAs, they are now thought to have critical roles in tumor invasion and metastasis, inflammation, coagulation, and stem-cell renewal and expansion. In addition, due to the redeployment of genetic information, epigenetic alterations are another impact of exosomes which is stimulated in recipient cells (14).

Tumoral Exosome in tumor progression and metastasis

In addition to pro-tumorigenic mutations within cancer cells, the surrounding stroma environment including fibroblasts, blood vessel forming cells, mesenchymal cells, endothelium, immune and inflammatory cells, also exert supportive roles (3). As described before, gene expression in recipient cells is modulated by exosomal mRNA and microRNA (15). Furthermore, most initial studies on exosomal miRNA focused on their function involved in genetic changes, while changes in the epigenetic programming or the cell fate have been less characterized (3).

Besides inappropriate miRNA expression in the course of carcinogenesis, they involve in epigenetic regulation of some genes due to controlling key regulators of epigenetic events including DNA methyl transferases (DNMT) and histone deacetylases. Furthermore, in addition to modulatory roles in the expression of several oncogenes or tumor suppressor genes, miRNAs can respectively act itself as an oncogene and tumor suppressor gene which are named as oncomiRs and oncosuppressor (16). Ohshima and colleagues showed the oncogenesis properties of exosomal let-7 miRNA released from gastric cancer cell line through the activity of potential oncogenes including RAS and HMGA2 (17).

In another study, Rana *et al* observed the regulatory effect of TD-exosomal miR-494 and miR-542-3p on the non-transformed cells via altering the expression of Catherine-17 and matrix metalloproteinases in pre-metastatic tissues (18). Some studies have shown that tumor cells communicate with endothelial cells via transfer of miRNAs in exosome-mediated crosstalk between tumor and endothelial cells that leads to cell migration, angiogenesis, tumor growth and malignancy (19).

Grange *et al* reported that CD105+ renal cancer stem cells secreted the exosomes containing 57 miRNAs that impact on angiogenesis and metastatic process (8). In endothelial cells, miR-27b and let-7f were known as pro-angiogenic miRNAs, whereas miR-221 and miR-222 were described as anti-angiogenic miRNAs (20). Specific microRNAs (miRNAs), such as those of the miR-17-92 cluster, may be responsible for regulating endothelial gene expression during tumor angiogenesis (21). In another study, Tumezu *et al* showed the decrease of integrin $\alpha 5$ expression after transfecting of K562 leukemia cell line with a Cy3-

labeled pre-miR-92a and co-cultured with HUVECs. Their finding suggested that an exosomal miRNA can do like an endogenous miRNA in a recipient cell and support the idea that exosomal miRNAs have an important role in cancer-to-endothelial cell communication (21).

Some miRNAs in exosomes can activate Toll-like receptors, leading the tumor growth to change phenotypes in receiving cells. Fabbri *et al* represented that tumor-secreted miR-21 and miR-29a also can exert their impacts through another mechanism, binding as ligands to the Toll-like receptor (TLR) members. Proverbially, these two described miRNAs can attach to murine TLR7 and human TLR8 in immune cells, triggering a TLR-mediated pro-metastatic inflammatory response that may ultimately leads to tumor growth and metastasis (22). Zhuang *et al* demonstrated the effectiveness of attenuating SOCS5 levels by exogenous miR-9 and promoting a signaling cascade for endothelial cell migration and tumor angiogenesis (23). Table 1 summarizes reports from the role of microRNAs carrying with various tumoral exosomes on the tumor fate.

Epithelial-mesenchymal transition (EMT) plays a critical role in the initiation of tumor metastasis. An increasing number of reports indicate that EMT can be controlled by microRNAs (miRNAs). MiR-26b targets the expression of USP9X in order to inhibit the EMT of hepatocytes. Therefore, the EMT of hepatocellular carcinoma (HCC) affected by miR-26b (24).

Fan *et al* identified that miRNAs (miR-143) involved in promoting prostate cancer metastasis and cancer stem cells are being defined by repressing the expression of FNDC3B (Fibronectin type III domain containing 3B) (25).

Ohshima *et al* have established that cancer cells selectively secrete let-7 miRNAs into the extracellular environment via exosomes, reducing the anti-tumorigenic effect within the cells and facilitating oncogenesis and metastasis (17).

Lim *et al* indicates that dormancy of bone marrow metastasis can be explained by transferring exosomal miRNAs from bone marrow stroma to breast cancer cells (26).

Tumoral exosome involved in angiogenesis

Several miRNAs that are carried by exosomes such as miR-1, miR-17, miR-18, miR-181, let-7, miR-15, miR-16, miR-151 and miR-375 have an angiogenesis, hematopoiesis, exocytosis and tumorigenesis ability which could point the potent role of exosome as a miRNA shuttle to undertake innumerable processes within the cells (27, 28). Recent data viewed the specific impact of miRNAs on modulating the expression of endothelial genes involved in angiogenesis. The miR-17-92 cluster, miR-126 and miR-296 are examples of them (21).

Two independent studies performed by the same group demonstrated that exosome derived from melanoma has pro-angiogenic capacity and are able to generate endothelial spheroids and vascular proliferation (29). Another group of molecule involved in stimulation of angiogenesis is tetraspanins (30). Taken together, the biogenesis of exosomes and tetraspanins can induce the tumor growth due to augmentation of angiogenesis both in tumor tissue and tumor-free tissue (31). Moreover, it has been reported that tetraspanin, named also Tspan8, is involved in exosomal sorting of proteins such as CD106 and CD49d contributing to uptake of exosome by endothelial cells. The presence of several angiogenesis genes, including von Willebrand factor, Tspan8, VEGF, chemokines CXCL5 and MIF, chemokine receptors CCR1, and VEGF receptor 2 were confirmed in exosomes. They are uptaken by Tspan8-CD49d complex which result in augmentation of proliferation, maturation, and migration of endothelial cells (32). Furthermore, exosome contains Notch ligand Delta-like 4(DLL4) which can regulate and develop angiogenesis. In another study, proteins with the angiogenic potential such as angiogenin, FGFa, IL-6, IL-8, VEGF, TIMP-1, and TIMP-2 were isolated from exosomes (33).

Lee *et al* have shown that exosomes secreted from mesenchymal stem cells (MSCs) induced the inhibitory impacts on angiogenesis both *in vitro* and *in vivo* through down regulation of VEGF expression in tumor cell (34). Colorectal cancer cell-derived exosomes are shown to be enriched in cell cycle-related mRNAs and promoted the proliferation of endothelial cells (35).

The angiogenic phenomenon is regulated via several cascades such as the TGF- β , VEGF, FGF and Notch signaling within the endothelial cells (36). What's more, exosomes derived from lung cancer cells can provoke the level of various pro-angiopoietic factors, including IL-11, IL-8, LIF, VEGF, oncostatin M, and matrix metallo proteinase 9 in stromal cells, which involve in promoting invasive manner of tumor such as progression, metastasis, and angiogenesis (37).

Exosomal oncogenic proteins

According to the data available in ExoCarta, numerous oncogene proteins derived from tumor cells such as full-length EGFR, MUC1, Latent membrane protein 1 (LMP1) have been identified within exosomes which are involved in cancer initiation and progression (37). It should be noted that the presence of these oncoproteins are not sufficient, but they are necessary to be functional, ultimately resulting in epigenetic alterations by impact on the recipient cells. It has been reported that challenging with Epstein-Barr virus (EBV) causes an infected cell to release exosomes carrying

the EBV-encoded LMP1 (38). This event triggers several cellular pathways, including apoptosis, cell cycle progression, proliferation, migration. B-cell consequently undergoes transformation to a malignant state (39). EGFR has been purified from pancreatic cell lines (40), brain tumors (41), amphiregulin, TGF α (transforming growth factor α , and EGFR ligands have been found in exosomes secreted by breast and colorectal cell lines (42). Additionally, MUC1 is the cell surface protein presenting in breast cancer exosomes (43).

In addition to oncoproteins, various tumor-suppressor proteins were identified in exosomes. Cellular protein p53 has been known as a significant regulatory factor participating in numerous surveillance signals (44). Mutation of p53 was found in more than 50 percent of tumors (45, 46). p53 carried by exosome leads to modulating cell-cell communication properties (47, 48). On the other hand, p53 triggers the transcription of several genes such as those involved in the exosome production and TSAP6 CHMP4C), stress signals (CAV1 and CHMP4C) etc. (49, 50). Knowing more about other members of p53 family provides an insight into the probable signaling mechanisms that they could be useful to design a potent therapeutic strategy.

Phosphatase and tensin homolog (PTEN) is another tumor suppressor protein regulating PI3K-AKT pathway, and cell cycle, which is mutated in most cancers (51, 52). Reported by Putz and colleagues, tumoral exosomes contain PTEN, with the functional phosphatase inhibit cell proliferation (53). In a study performed on pancreatic cancer cell, it has been shown that tumoral exosomes induce arrest of cell growth in G0/G1 phase and apoptosis. Furthermore, the studies indicated that the down-regulation of Hes-1 as a target in Notch-1 signaling pathway, induced to express PTEN (54). These events emphasize a close regulatory relation between both Notch signaling and PTEN/GSK-3 β , which result in suppressing the Notch-1 survival pathway. This conclusion strongly confirms that functional PTEN is able to exert an inhibitory impact on survival pathways both in origin cells and target cells.

Additionally, carrying adenomatous polyposis coli (APC) and β -catenin, as tumor suppressor proteins, by exosomes leads to inhibition of wnt signaling survival pathways in colorectal cancers that could epigenetically attenuate the progression and development of cancer cells (55, 56).

Post translational modification (PTM)

PTM is the common occurrence changing the structure of amino acids and resulting in protein diversity. This event is considered as an epigenetic change that occurs within cells. Phosphorylation, ubiquitination, methylation, simulation, and

oxidation are the most convenient modifications that cells use to regulate down-stream target signaling, including homeostasis in relation to the physiological and pathogenic function of proteins. Previous reports identified the presence of enzyme with the kinase and phosphatase activity within Exosomes (57-59).

For example, dephosphorylation of the serine-threonine kinase Akt by exosomal PTEN leads to promotion of an anti-proliferative pathway in recipient cells (53). On the other hand, exosomal tyrosine kinase can phosphorylate and activate several proteins involved in proliferative signals and finally leads to cancer. These exosomal enzymes could be a target for therapy. For example, dasatinib repress the phosphorylation of Src carrying by exosome-derived from myeloid leukemia and prevent from angiogenesis (60). Furthermore, protease enzyme within tumoral exosomes plays a significant role in developing metastasis because of disturbing extracellular matrix (61).

Glycosylation is another example of PTM. In the study done on exosome from ovarian carcinoma cell line, high level of mannose and sialic acid were observed. These carbohydrate residues help exosomes to interact with lectin and others extracellular receptors, found to wipe out circulatory exosomes and represent new behaviors in the recipient cell (62). Therefore, PTM on exosomes could be useful for cancer therapy. In addition to epigenetic alteration of proteins, carried by exosomes, PMT has been known as a determinant factor for sorting molecules to exosomes. Ubiquitination, sumoylation, palmitoylation, isoprenylation, phosphorylation, and glycosylation are of PTM involved in selecting proteins, RNA and miRNA for carrying by exosomes (63).

Extracellular matrix and stroma remodeling

The extracellular matrix and the stroma of tumor tissues are two momentous parts, which is known to play role in the tumor biology. Increasing documents further confirmed that tumoral exosomes from discrepant cancers such as mesothelioma, bladder, breast and colorectal can adjust the differentiation status of cells located in stroma. Interestingly, it has been reported that TGF- β proteins, transmembrane receptors, on the surface of tumoral exosome which provoke a Smad-dependent signaling pathway that promote differentiation of myofibroblast, resulting in stroma modification, tumor expansion, vascularization, and metastasis (64). It has also been found that adipose tissue-derived mesenchymal stem cells differentiate into myofibroblast in a TGF β -dependent manner (65). *In vivo* pancreatic cancer model, soluble substances accompanied by tumoral exosomes could involve in initiating and disseminating metastasis. CD44v6 is a key factor in this process and handle the assembly of matrix to activate other factors, such as leukocytes,

stroma and endothelial cell for nucleation of metastasis (66).

Effects on cell death

In contrast to general opinion, the exosome induce cell proliferation. A few studies reported the apoptotic impact of exosome on cancer cells. Ristorcelli *et al* observed that exosome secreted from pancreatic cancer cell lines is able to stimulate apoptosis via mitochondrial pathways (67). In addition, anti-proliferative effect of pancreatic exosomes occurs owing to attenuating hes-1 level and stimulating PTEN and GSK-3 β function (54). It has been suggested that lipid profile of exosomes particularly cholesterol, ceramide, and sphingomyelin results in activation of caspase-3 and caspase-9. Recently, Mahmoodzadeh Hosseini *et al* designed a cyto apoptotic stimuli based on exosome and a potent superantigen, staphylococcal enterotoxin B, via exosome display technique. This compound showed cytotoxic activity against three different cell line, estrogen receptor negative breast cancer cell line (MDA-MB 231) (68), poor-differentiated pancreatic cancer cell line (MIApaca-2) (69) and ovarian cancer cell line (SKOV-3) through activation of intrinsic pathway of apoptosis (70). In addition, Exosome/enterotoxin B could cause to increase necrosis within tumor tissue in murine breast cancer model (71). Furthermore, the studies also revealed the apoptotic impacts of exosomes on immune effector cells including cytotoxic T lymphocytes (CTLs). Exosomes from prostate cancer cell line represent FasL leading to apoptosis in CTLs which evade the immune system (72).

Conclusion

Exosome as a potent cell-cell messenger plays a momentous roles during life time of cells. Each cell receives various types of exosomes released by different type of cells located in discrepant distances. As reviewed above, cancer cells secreted exosomes with the different properties which provoke various genetic and epigenetic impacts in the target cells. Based on previous findings, the functional contents of exosomes such as mRNAs, microRNAs and epigenetic factors, exert as an endogenous agents. Understanding and focusing on the biology of exosomes within the tumor microenvironment is necessary to design a new anti-cancer drugs or selection of therapeutic regimens. The usage of intact exosome for cancer therapy has some limitation. Besides to several unknown materials and compounds in exosomes, they potentially contribute to tumorigenesis and progress of cancer that is harmful for selecting exosomes in order to specific anti-tumor drug delivery. Therefore, today several strategies develop to engineer of modified exosomes, categorized in the second and third generation of

exosomes. These novel exosomes could be efficient for targeted and specific cancer therapy. Therefore, having data concerning exact genetic and

epigenetic properties of natural exosome is useful to mimic a synthetic exosomes with attenuate harmful side effects.

Table1. Summary of clinical and in vitro studies of microRNAs derived from tumor cell exosomes

Exosome source	Potential diagnostic microRNAs	Main findings	Reference
Ovarian cancer	miR-21, miR-141, miR-200a, miR-200c, miR-203, miR-205, miR-214	Exosomal microRNA profiles were similar in ovarian cancer patient samples and distinctly different from benign disease samples. microRNAs were increased in exosomes <i>versus</i> tumor cells	(73)
Breast cancer cells	miR-29a, miR-451, miR-1246	Expression level : up-regulation Selective release of certain microRNA populations was demonstrated in malignant breast cancer cells that are retained in non-malignant mammary epithelial cells.	(74) (75)
Lung cancer cells	miR-17-3p, miR-21, miR-106a, miR-146, miR-155, miR-191, miR-192, miR-203, miR-205, miR-210, miR-212, miR-214, miR-21 and miR-29a	No significant differences in exosome microRNA levels between microRNAs derived from circulating exosomes or from microRNAs from the primary tumor were observed between patient group and control group	(76)
Renal cancer stem cells	miR-92, miR-141, miR-29a, miR-650, miR-151, miR-19b, miR-29c	miR-21 and miR-29a were secreted in exosomes and could bind to murine TLR7 and human TLR8 and trigger a Toll-like receptor (TLR)-mediated prometastatic inflammatory response that could lead to tumor growth and metastasis. Microvesicles were secreted from human renal cell carcinoma that could trigger angiogenesis and premetastatic niche formation in a severe combined immunodeficient (SCID) mouse model.	(22) (8)
Melanoma and normal melanocyte cells	let-7 family, miR-138, miR-125b, miR-130a, miR-34a, miR-196a, miR-199b-3p, miR-25, miR-27a, miR-200b, miR-23b, miR-146a, miR-613, miR-205, miR-149	The first in-depth screening to examine the entire exosome transcriptome, miRNome and proteome. Thousands of mRNAs and 15 microRNAs that are associated with melanoma progression and metastasis were identified.	(77)
Prostate cancer	miR-221, miR-107, miR-130b, miR-141, miR-181a-2, miR-2110, miR-301a, miR-326, miR-331-3p, miR-432, miR-438, miR-574-3p, miR-625	Expression level : up-regulation The levels of 12 microRNAs were different between plasma exosomes of prostate cancer patients compared to control. Eleven microRNAs were present in significantly greater amounts in patients with metastases compared with those without metastasis. The association of exosomal miR-141 and miR-375 with metastases was confirmed in a second patient population.	(78) (79)
Gastric cancer	miR-1, 20a, 27a, 34, 423-5p, let-7 family	Expression level : up-regulation Profiled microRNA expression by microarray in exosomes isolated from gastric cancer cells. Let-7 microRNA family was enriched in exosomes.	(80) (17)
Hepatocellular carcinoma cells	miR-584, miR-517c, miR-378, miR-520f, miR-142-5p, miR-451, miR-518d, miR-215, miR-376a, miR-133b, miR-367, miR-21, miR-122, miR-223	Transmission of exosome microRNAs from hepatocellular carcinoma cells could contribute to the initiation and progression of hepatocellular carcinoma by targeting a tumor suppressor frequently lost in hepatocarcinogenesis.	(81)
Leukemia cells and endothelial cells	miR-92a	Up-regulation of Expression level Leukemia cells released microRNAs from the miR-17-92 cluster and were taken up by human umbilical vein endothelial cells (HUVECs) and repressed a target mRNA. Did not affect the growth of HUVEC cells, but did enhance cell migration and tube formation.	(82) (21)
Mouse dendritic cells	miR-148a, miR-451	Exosomal microRNA from dendritic cells can be transferred to a recipient dendritic cell and repress microRNA target mRNAs in the acceptor cell.	(83)
Human and mouse mast cells	let-7, miR-1, miR-15, miR-16, miR-181, miR-375.	Identified small RNAs, including 121 microRNAs and 1,300 specific mRNAs. Detected mouse exosomal RNA and new mouse proteins in human mast cells after treatment with mouse mast cell exosomes. Coined the term "exosomal shuttle RNA (esRNA)".	(84)

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Conflict of interest

The authors declare that they have no conflict of interest.

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