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The emerging role of exosome-derived non-coding RNAs in cancer biology

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Abstract

Exosomes are a new means of intercellular information exchange that have aroused great research interest. Long neglected in research, exosomes were deemed nonfunctional cellular components to be discarded. However, it has been gradually revealed that exosomes are an important tool for the exchange of intercellular information and material. Exosomes contain specific repertoires of non-coding RNAs

(ncRNAs, including microRNA and lncRNA), indicating that a specific RNA sorting mechanism may exist. Correspondingly, intracellular multivesicular bodies (MVBs) are produced after fusion with the cell membrane to release exosomes rather than inducing autophagy, which reveals that there may be a specific regulatory mechanism for MVB secretion. Cells can trigger cancer-related disorders after the recognition and uptake of circulating exosomal ncRNAs, providing indications for early tumor biopsy and treatment. The use of exosomes as a biological carrier in targeted therapy has been demonstrated. However, there may be a specific, unknown switch for loading drugs. This review focuses on the mechanisms of exosome biogenesis, release, and uptake. We also review the promotion of tumor development by exosomal ncRNAs including chemotherapy resistance，metastasis and the prospective use of exosomes in cancer diagnosis and treatment.

Keywords: Exosomes; MVBs; Tumor; miRNA; lncRNA

Abbreviations:

ncRNAs, non-coding RNAs

TD-exosomes, tumor-derived exosomes

TME, tumor and microenvironment

DCs, dendritic cells

MHC, major histocompatibility complex

hnRNPA2B1, heterogeneous ribonucleoprotein A2B1

SYNCRIP, synaptotagmin-binding cytoplasmic RNA-interacting protein

MVBs, multivesicular bodies

SNARE, soluble N-ethylmaleimide–sensitive factor attachment protein receptor

SM, Sec1/Munc18

Rab11FIPs, Rab11 family of interacting proteins

ctDNA, circulating cell-free tumor DNA

CTCs, circulating tumor cells

OSCC, oral squamous cell carcinoma

1. Introduction

In recent years, the tumor and microenvironment (TME) exchange of information in particular significantly affects tumor occurrence and development, as well as invasion, metastasis, and other malignant biological behavior [1]. Mainly, malignant phenotypic changes in solid tumors occur not only through direct contact and the secretion of soluble factors, but also through the secretion of exosomes, effecting the phenotype of malignant changes via microenvironment nourishment [2, 3]. Remarkably, exosomes, a new discovery in the intercellular communication medium, reveal important cell–cell communication, and tumor cells secrete many exosomes to exchange information between local and distant cells.

Exosomes are lipid vesicles that are 100 times smaller than cells and contain nucleic acids (genes, non-coding RNAs [ncRNAs], DNA), proteins, and lipids. Almost all cells secrete exosomes, which range 30–100 nm in diameter [4-6]. In 1981,

Trams et al. found that shedding vesicles with an average diameter of 40 nm and ranging 500–1000 nm in diameter could be isolated from various normal and tumor cells; this was the first description of exosomes [7]. In 1983, Pan and Johnstone and Harding et al. discovered and defined exosomes [6, 8]. Exosomes were long considered an important metabolic pathway for cellular efflux, until it was shown that dendritic cells (DCS) play an important regulatory role by secreting exosomes containing major histocompatibility complex (MHC) and T cell costimulatory molecules [9]. In 2007, Valadi found that exosomes can carry mRNAs and microRNAs (miRNAs) to transfer genetic material among cells to exchange information between close and distant cells [10]. This discovery sparked research interest in the field of exosomes.

The intensified study of exosomes has revealed that they act as bridges for important information exchange between cells, carrying nucleic acids, proteins, and lipids to the target recipient cells [11, 12]. Tumor cells can treat exosomes as “garbage”, releasing them to promote tumor progression [13, 14]. Overall, tumor cells can collectively release exosomes to achieve this aim. Here, we briefly outline some of the current knowledge on the mechanisms of exosome biogenesis, release, and uptake. We also highlight the critical effects exerted by exosomal ncRNAs on tumor progression and drug resistance, and the prospect of using exosomes in tumor diagnosis and treatment.

2. ncRNA loading into exosomes

The transfer of ncRNA-loaded exosomes plays a key role in cell–cell communication in many cancers [15, 16]. Given the crucial function of Argonaute (AGO) proteins in ncRNAs, they are considered fundamental ncRNA carriers and may be involved in ncRNA loading into exosomes. However, Gibbings et al. found that purified exosomes only contained single-stranded, mature miRNAs, high levels of GW182 (trinucleotide repeat–containing 6A), and low levels of AGO2 protein, and detected neither P-body components nor miRNA-repressible mRNA in the exosomes [17]. This suggests that miRNA loading into exosomes takes place in a miRISC (miRNA-induced silencing complex)-independent manner[18]. Consistent with this, Ostenfeld et al. did not observe any miRNA-processing proteins or miRISC-associated proteins in exosomes [14]. In their study, AGO2 was only detected in one of three replicates. Moreover, immunoblotting revealed no detectable AGO2. In addition, most AGO2 miRNAs were independent of exosomes [19, 20]. These findings indicate that there may be other regulatory mechanisms for ncRNA

loading into exosomes.

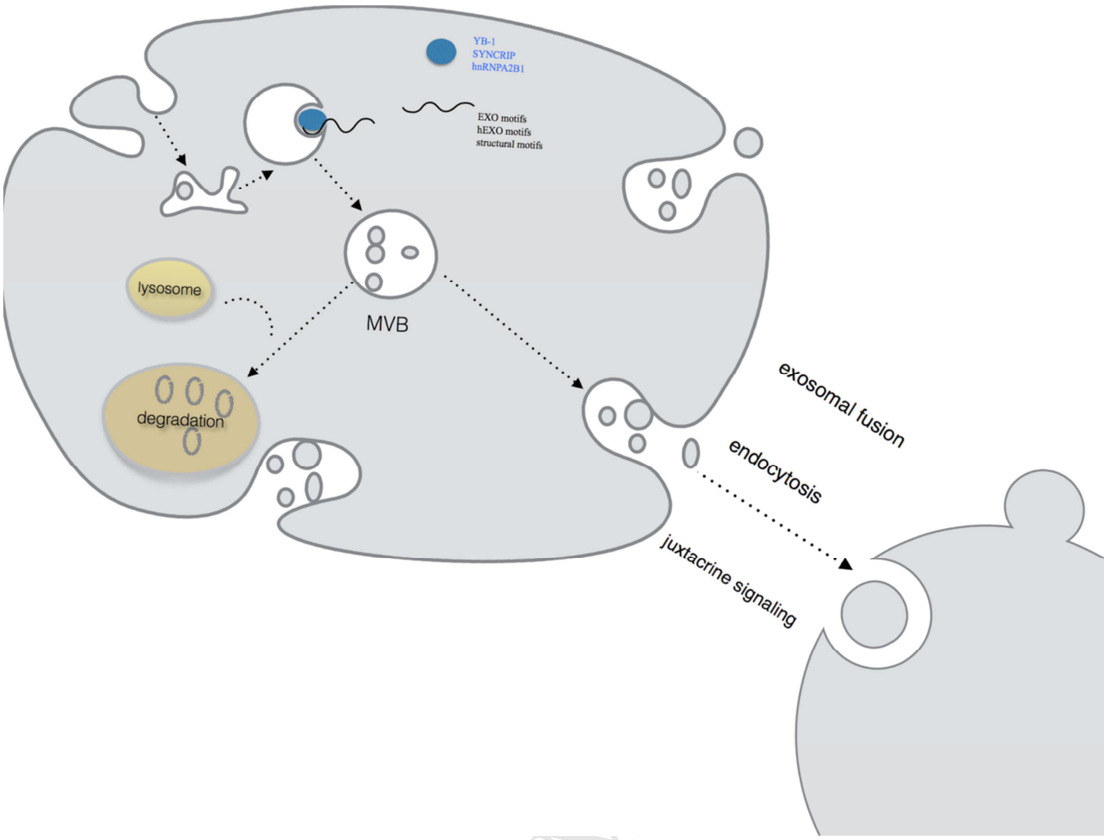

Fig.1. The mechanism of communication between cells by exosomes. ncRNAs contained different RNA motifs can be loaded into multivesicular bodies (MVBs) by different RNA-binding proteins. MVBs can either follow a degradation pathway fusing with lysosomes or release the ILVs as exosomes to the extracellular space. Recipient cells can uptake exosomes by three pathways: exosomal fusion,endocytosis and juxtacrine signaling.

Recent studies have shown that some ncRNAs are enriched in exosomes while others are barely present, suggesting a potential regulatory mechanism for the sorting of specific sets of miRNAs into exosomes（Fig.1）. In T cells, miRNAs enriched in exosomes share the same specific sequence (GGAG), which has been identified as an EXOmotif. EXOmotifs are specifically recognized by hnRNPA2B1 (heterogeneous ribonucleoprotein A2B1) and hnRNPA1, thereby controlling the selective loading of such miRNAs into exosomes. Interestingly, hnRNPA2B1 is mostly sumoylated in exosomes, and sumoylated hnRNPA2B1 is essential for the sorting of miRNAs into exosomes [21]. In hepatocytes, another common extra-seed sequence (GGCU, a

hEXO motif) of miRNAs enriched in exosomes mediates direct binding to the RNA-binding protein SYNCRIP (synaptotagmin-binding cytoplasmic RNA-interacting protein; also known as hnRNP Q or NSAP1) and controls the sorting of such miRNAs into exosomes [22]. SYNCRIP knockdown impairs the exosomal loading of specific exosome-enriched miRNAs. Different from EXOmotifs, hEXO motifs play a positive role in regulating miRNA localization. Embedding a hEXO motif into a miRNA, which is poorly present in exosomes, can enhance its loading into exosomes. Furthermore, although both hnRNPA2B1 and SYNCRIP can interact with a common exosome-sorting motif, they display sequence-specific exosomal sorting capacity in the loading of selected miRNAs [22]. YBX1 (Y-box–binding protein 1) is another protein that might bind specific RNA structural motifs, i.e., ACCAGCCU, CAGUGAGC, and UAAUCCCA of mRNAs, and long ncRNAs (lncRNAs) enriched in exosomes, and control specific ncRNA sorting into exosomes [16] , shown in Fig.1.

Therefore, ncRNAs are selectively loaded into exosomes. Specific proteins act in coordination with specific ncRNA sequences to control ncRNA sorting into exosomes. However, the regulatory mechanisms of ncRNA sorting into exosomes are unknown, so further research is warranted to determine the involvement of other RNA-binding proteins and RNA motifs.

3. Exosome release

The intracellular generation of multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs) is both ESCRT (endosomal sorting complex required for transport)-dependent and ESCRT-independent [15, 23, 24]. After intracellular generation, how MVBs bind to a specific cell membrane region and through plasma membrane fusion ultimately achieve exosome release may involve a series of specific mechanisms of secretion. The mechanisms involved in secretory MVBs have been extensively studied.

Exosome release involves several crucial factors and the synergistic effect of these factors is that MVBs bind to the cell membrane and is a key factor in achieving exosome release after plasma membrane fusion as shown in Fig.2. The most important factor is the small GTPases, including the Rab and RAL GTPases [25-27]. The relatively in-depth study of the Rab GTPase family has found more than 70 subtypes located on different membranes of the surface. Rab GTPases respectively play related roles in vesicle budding, uncoating, motility, tethering, and fusion to coordinate the regulation of vesicle traffic [27].

In general, the role of Rab is indispensable to the regulator and effector proteins [28, 29]. Effector proteins, such as Sl272 and Slb2-b, are also required in Rab27-mediated vesicle transport and fusion [29, 30]. The mechanism of Rab localization in various vesicles after intracellular synthesis is uncertain [28, 31], but the localization of Rab in the vesicles and its recruitment of differently interacting effector proteins confers different biological functions on the vesicles [27]. For example, the transition between early and late endosomes can be achieved by

switching SAND-1, or Mon1, in the Rab conversion process, and the loss of Rab5 is accompanied by the formation of inclusion bodies, marking the obtainment of late endosomal Rab7 [32].

So far, nine small GTPases are associated with secretion (Rab2B, Rab5, Rab7, Rab9A, Rab11, Rab27A, Rab27B, Rab35, RAL). The Rab effect is inseparable from regulators and effectors, as shown by Rab35 and Rab27 [29, 33]. TBC1D10A-C (TBC1 domain family member A–C) regulate the secretion of PLP-EGFP (proteolipid protein 1–enhanced green fluorescent protein)-related exosomes by screening the Rab GAP library in exosome-secreting oligodendrocytes [33], and Rab27 binds to the corresponding effector proteins (Slp4-a, Slac2-b, Munc13-4) to regulate secretory vesicle transport and fusion [29].

Rab mediates MVB transport and plasma membrane fusion by two categories of Rab effector proteins, respectively [29, 34]. Obviously, in mediating exosome secretion first, Rab is in the subcellular position of MVB directional transport, followed by the MVB and plasma membrane docking fusion [35]. Within the cell, the actin and microtubule cytoskeleton is not randomly distributed, and exhibits significant polarity distribution [36, 37]. Actin and Rab in granule secretion have been studied extensively [36, 38]. Correspondingly, the targeted transport of MVBs is associated with the actin and microtubule cytoskeleton, being directly or indirectly bound to the actin and microtubule cytoskeleton, achieving polarized delivery of MVBs via mediation by Rab and the corresponding effector on the MVB membrane and with the aid of a kinetic protein [29, 35] (Fig.2). Interestingly, Rab11

and the Rab11 family of interacting proteins (Rab11FIPs) assist in vesicle transport with actin and kinetic proteins [39], which is defined as juxtanuclear recycling endosomes. Moreover, it has long been confirmed that Rab27A interacts with the corresponding effector (Slac2-a) and the actin-based motor myosin to transport melanosomes along actin filaments [29]. In addition, invadopodia, actin-rich subcellular structures formed by invasive cancer cells that protrude and degrade extracellular matrix (ECM), are specific for Rab27a, CD63-positive MVBs, and key docking and secretion sites; cortactin has a stabilizing effect on invadopodia, and can further enhance the MVB docking site to promote exosome secretion. On the contrary, inhibiting cell-formation invadopodia significantly reduced exosome secretion, all of which indicates the pivotal role of actin in exosome secretion [40, 41].

MVBs not only require the kinetic action of motor proteins in plasma membrane directional transport, but also cannot be separated from the microtubule cytoskeleton “railroad”, MVBs move along the microtubule network to the microtubule plus ends to ultimately achieve MVB and plasma membrane docking fusion [36, 42]. Nevertheless, the specific mechanism spanning MVB and plasma membrane docking fusion to the final release of exosomes is not clear. However, it is currently believed to involve the following steps shown in Fig.2.

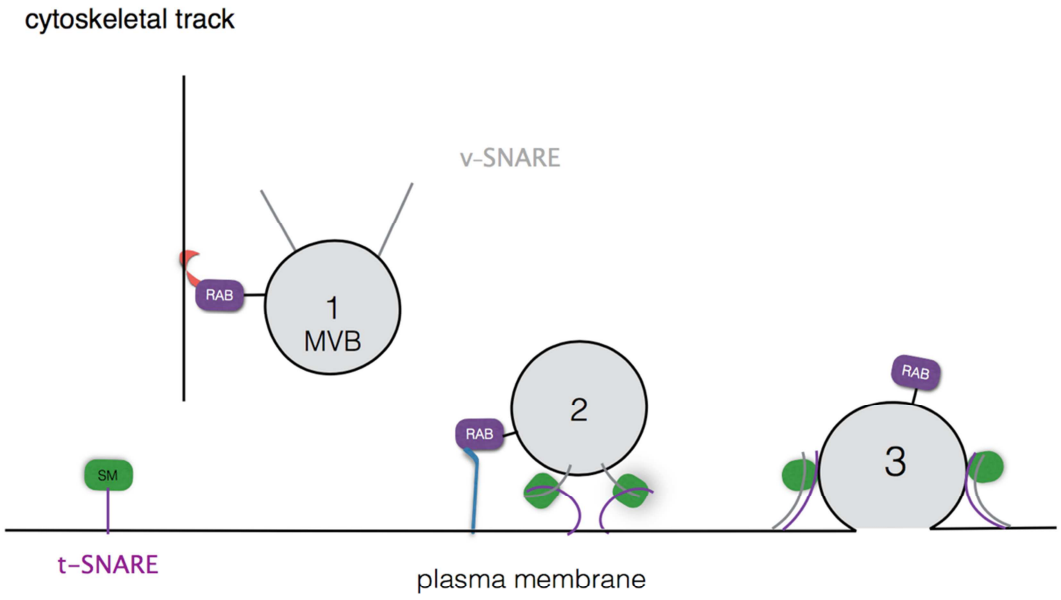

Fig.2. Transport and membrane fusion of the secretory MVBs. Rab GTPases can mediate MVBs transport along actin cytoskeleton (cytoskeletal tracts). When the secretory MVBs close to the plasma membrane, Rab GTPases can promote MVBs adhering by recruiting tethering factors in the target membrane. Meanwhile, the SM protein-bound t-SNAREs is assembled with v-SNAREs to activate membrane fusion.

Small GTPases and the exocyst complex mediate MVB membrane docking to directly or indirectly initiate SNARE complex assembly [43] as shown in Fig.2. Rab27 and the corresponding effector complex play a key role in MVB plasma membrane docking [29]. In addition, Rab27A knockout cells have excessive cortactin expression and unaltered exosome secretion; therefore, Rab27A may be associated with cortactin in mediating MVB docking [29, 41]. At the same time, the exocyst complex is critical for the assembly and control of SNARE complexes [43]. Sec3 (exocyst complex component 1, belonging to the exocyst complex) interacts directly with the target membrane SNARE (t-SNARE, SYX-5) protein Sso2 to initiate t-SNARE assembly plasma membrane fusion [44]. However, RAL1-mediated MVB membrane mating and fusion are often independent of the exocyst complex, and the

active form of RAL1 can activate or recruit SYX-5 at the top plasma membrane to promote MVB fusion, thereby promoting exosome release. Furthermore, when SYX-5 is absent, MVBs accumulate under the plasma membrane [25].

Vesicular SNARE (v-SNARE) and t-SNARE proteins catalyze the fusion of the two membranes (Fig.2), and the ATPase NSF (N-ethylmaleimide sensitive factor) and its adapter proteins disassemble the SNARE complex to recycle SNARE for another round of fusion [45-47]. In K562 cells, VAMP7 (vesicle-associated membrane protein 7, a v-SNARE protein) is involved in MVB plasma membrane fusion and exosome release [47]. In mammals and nematodes, SYX-5 is also involved in MVB fusion to promote exosome release [25]. Tumor cells are usually aerobic glycolytic, and the key enzyme PKM2 (muscle pyruvate kinase) can further stabilize the SNARE complex through SNAP-23 (synaptosome-associated protein 23, t-SNARE) Ser95 phosphorylation to promote exosome release [48]. More interestingly, invadopodia are a key exosome docking site [40]. Additionally, invadopodia formation and maintenance requires the pairing of VAMP7 and SNAP23, and syntaxin 4 (t-SNARE) mediates MMP (matrix metalloproteinase) trafficking to the invadopodia [49]. In summary, these findings show that SNARE is at least partially involved in invadopodia formation, which indirectly affects exosome secretion and suggests that SNARE is involved in plasma membrane docking fusion [40, 49]. The ATPase NSF and its adapter protein, which dismantle the SNARE complex to recycle SNARE, are also necessary for plasma membrane fusion [45, 47, 50].

As shown in Fig.2, SM protein is a soluble factor that may interact with SNARE before and after vesicle attachment. The hook-shaped SM binds to the cell membrane during SNARE-mediated membrane fusion, where it binds to the trans-SNARE complexes to guide fusion [45]. Vps33 (an SM family protein) is also involved in coordinating SNARE complex assembly [51]. In arthrogryposis–renal dysfunction–cholestasis (ARC) syndrome, there is a correlation between vesicular secretion abnormalities associated with *VPS33B* (VPS33B, late endosome- and lysosome-associated) mutations, affecting the SNARE-related pathways [52]. In hepatic stellate cells (HSCs), VPS33B interacts with Rab27A to mediate MVB transport, thereby promoting exosome secretion, which is important for the development of hematopoietic cells and leukemias [53]. This suggests that the SM protein family may be involved in MVB transport, coordinating SNARE to promote multiple roles in MVB and plasma membrane fusion. The vacuolar H+-ATPase (v-ATPase) V0 complex does not depend on ATPase activity (which may act downstream of SNARE) [54, 55]. In flies, VHA100-1 (v-ATPase V0 subunit a1) may act as a modulator of synaptic vesicle fusion efficiency downstream of the SNARE-dependent vesicles [54]. In *Caenorhabditis elegans*, the V0 fraction of V-ATPase mediates the secretion of exosomes containing Hedgehog-related proteins, and the mutation of VHA-5 (the largest subunit of the V0 complex) causes abnormally expanded MVB accumulation, indicating that the complex controls the final step of MVB docking and plasma membrane fusion [55].

4. Exosome ncRNA uptake

Exosome uptake by the recipient cells is an important means of communication in cell–cell interaction. To deliver RNAs or other proteins into recipient cells, exosomes may bind directly to the cell surface and fuse with the cell membrane or be internalized by endocytic pathways (Fig.1). Given the specific recipient cell types, exosomes can be internalized by clathrin- or caveolae-mediated endocytosis, phagocytosis, or macropinocytosis [56]. A recent study has shown that exosome fusion with the recipient cell membrane is more likely to occur in a low pH environment [57]. Interestingly, an acidic extracellular environment can also induce exosome rupture before binding to the recipient cells, and the released RNAs or other material affects the recipient cells in a paracrine manner [58].

In addition, recipient cells can also uptake exosomes via direct exosome binding to cognate receptors on the recipient cell membrane, such as heparan sulfate proteoglycans (HSPGs) and integrin, subsequently activating specific signaling pathways. Christianson et al. found that HSPGs act as true internalizing exosome receptors rather than as cell surface attachment sites; exosome uptake depends on intact HSPG synthesis and HS 2-*O*-sulfation and *N*-sulfation in recipient cells [59]. Furthermore, syntenin can affect HSPGs to enhance complete exosome uptake in the recipient cells [60]. Chen et al. identified integrin αvβ3, integrin α5β1, and HSPG as important receptors and portals for HSC-derived exosome uptake. Exosomes bound to integrin αvβ3 or α5β1 to deliver regulatory miRNAs into HSCs, and then inhibited the expression of activation- and fibrosis-associated genes in the recipient cells [61].

Furthermore, Hoshino et al. have shown that integrins on tumor exosome membranes can be used to predict organ-specific metastasis. For example, the exosomal integrins α6β4 and α6β1 are associated with lung metastasis, while exosomal integrin αvβ5 has been linked to liver metastasis. Targeting integrins α6β4 and αvβ5 decreased exosome uptake [62]. Based on the molecular binding partners of exosomes and the recipient cell membrane, exosomes may be exploited for delivering targeted therapeutic drugs.

5. Exosomes in tumor progression

In the past several years, numerous studies have indicated that ncRNAs play a significant role in tumor progression[63-65]. The influence of ncRNAs on tumor cells involves many aspects. Recently, an increasing number of studies have confirmed that ncRNAs, including miRNAs and lncRNAs[66], can be derived by exosomes or affect tumor cell development, i.e., proliferation, apoptosis, metastasis, chemoresistance, and energy metabolism, through multiple modes[12, 67-70]. A study has found that these exosomal lncRNAs sometimes are enriched for miRNA seed regions for one miRNA family[71]. The non-coding genes transmitted by exosomes can promote tumor cell adaptation to the tumor environment and the obtainment of survival skills[70]. In the following sections, we summarize the influence of autophagy, chemotherapy resistance, and metastasis by ncRNAs, the cargo of exosomes excreted by cancer cells or other stromal cells, on tumor progression.

5.1. Exosomes and autophagy in cancer

Current research shows that autophagy is significantly enhanced in tumor cells, especially in hypoxic conditions, where tumor cells adapt to hypoxia by enhancing autophagy [72, 73]. Interestingly, tumor extracellular release is also significantly increased, especially in hypoxia, exposure to chemotherapy drugs, and other stress situations, while tumor invasion, metastasis, and chemical resistance are promoted [69, 74]. Specifically, hypoxia can promote oral squamous cell carcinoma (OSCC) cell invasion and metastasis by releasing miR-21–rich exosomes [69]. Under the same conditions, lung cancer cells tend to produce exosomes richer in miR-23a, promoting angiogenesis and increasing vascular permeability, which is conducive to tumor cell growth and metastasis [75].

However, in physiological conditions, there is often a dynamic balance between autophagy and exosome secretion in normal cells, that is, intracellular MVB generation, lysosome and autophagosome fusion degradation of digestion, or cell membrane fusion and exosome release are balanced [76] , which process are shown in Fig.1. A more recent study also shows that in the coordination relationship between autophagy and exosome secretion in mouse astrocytes, prion protein (PRNP) regulation of caveolin-1 (CAV1) inhibition of autophagy prevents autophagylase fusion of MVBs, thereby promoting exosome release [77]. Correspondingly, ISGylation of the MVB protein TSG101 (tumor susceptibility gene 101, an ESCRT subunit) in HEK293T cells facilitates lysosomal fusion and degradation of MVBs,

thereby inhibiting exosome secretion [78]. Subsequently, intercellular communication maintains intracellular homeostasis, achieving intracellular balance of material [76]. From the above, what is the relationship between exosome secretion and autophagy in tumor cells? How can exosome secretion and autophagy be mediated? Clearly, much related further experimental research is needed.

5.2. Exosomes and chemotherapy resistance in cancer

Sunitinib resistance is a therapeutic problem for patients with advanced renal cell carcinoma (RCC). Qu et al. found that lncARSR (lncRNA activated in RCC with sunitinib resistance), which is modulated by the AKT/FOXO (forkhead box O) axis, was abundantly expressed in sunitinib-resistant RCC cells compared to parental cells, and demonstrated that lnARSR is required for sunitinib resistance of RCC [12]. Interestingly, lncARSR could be packed into exosomes and transferred sunitinib resistance to recipient cells. This indicates that cancer cells can disseminate survival skills to other recipient cells via exosomes containing lncRNAs. The same phenomenon has been demonstrated in research on pancreatic ductal adenocarcinoma (PDAC). Cancer-associated fibroblasts (CAFs), which form the majority of PDAC tumor bulk, are intrinsically resistant to gemcitabine, the chemotherapeutic standard of care for PDAC [70]. However , gemcitabine-treated pancreatic cancer (PC) cells transfer exosome- delivered miR-155 to recipient cells, which leads to pc cells resistance by downregulating DCK gemcitabine-metabolising gene(DCK)[79].

Exosome-mediated transfer of the lncRNA UCA1 increased tamoxifen resistance in breast cancer cells [80]. CAFs exposed to gemcitabine had significantly increased exosome release. These exosomes increased the chemoresistance-inducing factor, Snail, in recipient epithelial cells and promoted proliferation and drug resistance. Therefore, CAFs can also disseminate survival skills to neighboring cancer cells. In recent years, it has been found that several exosome-transferred miRNAs are related with chemoresistance. Lung cancer studies have reported that exosomal miR-100-5p and miR-96 are involved in cisplatin (DDP) resistance [81, 82]. And studies have shown in non-small cell lung cancer (NSCLC) treatment, A549-gemcitabine-resistant (GR)–derived exosomes were internalized by parental sensitive cells, which can allow the transfer of miR-222-3p enhanced the proliferation, gemcitabine resistance, migration and invasion of receipt cells [83]. Interestingly, in the process of cisplatin-induced drug resistance, the miR-146a-5p significantly decreased in A549/DDP cells and exosomes than A549,And the new biomarker by serum exosomal miR-146a-5p can predict the efficacy of cisplatin for NSCLC patients and real-time monitor drug resistance[84]. What's more, the upregulation of miR-146a-5p could reverse the resistance of A549/DDP. [84]. Zheng et al. found that exosomal transfer of miR-21 derived from tumor-associated macrophages confers DDP resistance in gastric cancer [85]. Mikamori et al. revealed that, in PDAC cells, miR-155 was increased with long-term exposure to gemcitabine, and miR-155 could increase exosome secretion. Subsequently, the exosome-delivered miR-155 could induce chemoresistance in other PDAC cells [86]. In breast cancer cells, exosomes

from adriamycin-resistant breast cancer cells could transmit drug resistance partly by delivering miR-222 [87]. As stated above, these studies suggest an important role for exosomes in drug resistance in several tumor types via ncRNA transfer , which were shown in Table 1.

5.3. Exosomes and metastasis in cancer

Cancer-derived exosomal miRNAs play a key role in the cancer metastatic process. Exosomal miR-105, which is secreted by metastatic breast cancer cells, promotes endothelial cells migration by downregulating tight junctions and destroying the barriers integrity of endothelial monolayers through targeting ZO-1 protein [88]. Li et al. have shown that miR-21 is one of the most upregulated miRNAs in exosomes derived from hypoxic OSCC cells [69], where exosomal miR-21 markedly increased OSCC cell migration and invasion in a hypoxia-inducible factor (HIF)-1α and HIF-2α–dependent manner. Breast cancer cells can reprogram glucose consumption in niche tissues and promote metastasis by secreting vesicles that carry high levels of miR-122 [68]. Zhang et al. found that astrocyte-derived exosomal miRNA primes brain metastasis outgrowth via functional cross-talk between disseminated tumor cells and the brain metastatic microenvironment by losing *PTEN* expression [89]. In breast cancer, exosomes bearing miR-126a released from myeloid-derived suppressor cells (MDSCs) induced by doxorubicin treatment promote lung metastasis [90]. In addition, cancer-derived exosomal miRNAs can also function as ligands,

then bind and activate Toll-like receptors in recipient immune cells to trigger a prometastatic inflammatory response mediated by TLRs and finally lead to tumor growth and metastasis.[91]. The expression of lncRNA MALAT-1 has been found highly expressed in NSCLC patients, and in vitro studies demonstrated that serum exosome-derived long noncoding RNA MALAT-1 promoted the tumor migration. [67]. The above studies are showed in Table1.

Table 1. Exosome ncRNAs promoted drugs resistance and metastasis in different cancers.

ncRNAs	Donor cells	Recipient cells	Function in cancer cells	References
lncARSR	Renal carcinoma cells	Renal carcinoma cells	sunitinib resistance	12
lncRNA UCA	Breast cancer cells	ER+ breast cancer cells	tamoxifen resistance	80
miR-21	TAMs	gastric cancer cells	cisplatin resistance	85
miR-155	PDAC cells	PDAC cells	gemcitabine resistance	86
miR-100-5p	A549/DDP cells	A549 cells	cisplatin resistance	81
miR-96	Lung cancer cells	Lung cancer cells	cisplatin resistance	82
miR-222	MCF-7/Adr	MCF-7/Sss	adriamycin resistance	87
miR-155	Gemcitabine-treated pancreatic cancer	pancreatic cancer	gemcitabine resistance	79
miR-222-3p	A549-gemcitabine-resistant	A549- parental cells	gemcitabine resistance, migration and invasion	83
miR-146a-5p	A549/DDP cells	A549 cells	increasing chemosensitivity to cisplatin	84
miR-21	hypoxic OSCC	normoxic OSCC	migration and invasion	69
miR-122	Breast cancer cells	premetastatic niche cells	reprogrammed glucose metabolism and metastasis	78
miR-17-92 cluster	Astrocyte cells	Disseminated tumor cells	Recruits Iba1+ myeloid cells metastasis outgrowth	89
miR-126a	MDSCs	Breast tumor cells	lung metastasis	90
miR-105	Breast cancer cells	Endothelial cells	migration and destroy the barriers integrity of endothelial monolayers	88

MALAT1	Non-small cell lung cancer	Non-small cell lung cancer	migration	67

6. The prospects of exosomes

Noninvasive early detection of cancer is a perennial hot topic in tumor research. With the clinical progress of global liquid biopsy, clinical trials related to circulating tumor cells (CTCs), circulating cell-free tumor DNA (ctDNA), and exosomes are progressing quickly; the information is available at https://clinicaltrials.gov/. The in-depth study of liquid biopsy detection technology, including that for ctDNA, CTCs, and exosomes [92, 93], has gradually opened the door to early individual treatment. Exosomes have clear advantages: First, they are more easily enriched than CTCs. Second, secretory vesicles can effectively prevent nucleic acid substances from being degraded easily in body fluids [94, 95]. Accordingly, exosomes have indefinite prospects in clinical applications. The cancer treatment prospects of exosomes mainly include the following aspects.

6.1. Blocking biogenesis and circulating elimination of exosomes

The biogenesis of malicious exosomes is blocked by intervening factors or by increasing their metabolic excretion [96]; in this context, it mainly involves inhibiting the exosome biogenesis mechanism. Naturally, the reduction of exosomes will reduce the interference of ESCRT complexes when MVBs are produced with ILVs [23]. VPS4A is involved in the circulatory regulation of the ESCRT complex [24]; silencing

the helper protein VPS4A/B on MCF-7 cells reduced exosome release [97]. In human hepatocellular carcinoma cells, VPS4A is involved in regulating the secretion and uptake of exosome-derived miRNAs [98]. Many of the abovementioned key factors mediating MVB release, i.e., the knockdown of small GTPases, the actin and microtubule cytoskeleton, cortactin, SNARE, SM protein, and V0-ATPase, cause the accumulation or distribution of MVB abnormalities, eventually decreasing exosome release; RNA interference (RNAi) silencing of Rab27A and Rab27B is respectively caused by the accumulation or distribution of MVB abnormalities [34, 40]. Furthermore, n-WASp (neural Wiskott-Aldrich syndrome protein) and Tks5 intervention of invadopodia significantly reduces tumor cell exosome secretion [40]. Several studies have evaluated the selective targeted elimination of circulating exosomes for treating cancer. Malicious exosome biomarkers, including lipids, proteins, and glycoproteins located at the exosome surface, can be used to achieve this. Gold nanoparticle targeting of the construction of specific exosomes provides the option of selective targeted elimination for curing cancer [96].

6.2. Exosome-based carriers

Compared with conventional targeting vectors, exosomes have higher safety and bioavailability, and exhibit lower systemic toxicity and immunogenicity. Furthermore, exosomes improve the therapeutic effect significantly compared to direct drug chemotherapy [99, 100]. Exosomes can not only be loaded with small

interfering RNA (siRNA) and chemotherapy drugs for targeted therapy [101, 102], but can also carry immunoadjuvant–mediated immunotherapy [103]. However, how efficient are exosomes as carriers in actual targeted therapy? Caponnetto et al. have shown that exosome absorption efficiency is significantly size-dependent, where exosomes of different sizes showed different uptake rates [104]. Agrawal et al. demonstrated significant drug efficacy using milk-derived exosomes loaded with paclitaxel [102]. Notably, researchers have sought to identify the best delivery conditions by systematically assessing the impact of key parameters, including incubation time, volume, temperature, and extracellular vesicle:cholesterol-conjugated (EV:cc)-siRNA ratios [101]. Moreover, the use of the evolutionarily conserved late-domain (L-domain) pathway as a mechanism for loading exogenous proteins into exosomes has been reported, where Ndfip1 (Nedd4 family interacting protein 1) expression acts as a molecular switch for exosomal packaging of WW-Cre [105]. Furthermore, exosome-mimetic nanovesicles can be used as an efficient platform for RNAi delivery to the cytoplasm and have been validated in exosomes loaded with siRNA against c-Myc [106, 107].

7. Conclusions

Exosomes are a new means of exchanging information between cells, and also play a significant role in tumor cell development. Tumor-derived exosomes (TD-exosomes) contain specific protein and RNA repertoires. As nanoscale biological

vesicles, exosome content is well-protected and is not degraded. Interestingly, targeted ncRNA delivery can contribute to the communication and exchange of genetic material and exert a significant impact on cell biological behavior. We have focused on discussing the molecular mechanisms of exosomal ncRNA loading, and exosome secretion and uptake, which aids understanding and manipulation of exosomes in intercellular information exchange. Furthermore, the exploration of how cellular uptake of exosomal ncRNAs can lead to proliferation, invasion, metastasis, resistance, and other behavioral changes, and even whether exosomes can serve as potential early fluid biopsy specimens in tumors, continues. The prospect of treatment using exosomes primarily involves controlling the exchange of cell–cell extracellular information and the targeted delivery of pharmaceuticals. Efforts to reveal the specific mechanism of exosome secretion should continue; on the other hand, exosome delivery efficiency requires improvement. Many attempts to this effect have been made, but the existence of doubts and challenges should be acknowledged. However, as studies become more in-depth, we believe they will yield gratifying progress in tumor diagnosis and targeted treatment.

Conflict of interest: None

References

[1] T. Eggert, T.F. Greten, Tumor regulation of the tissue environment in the liver, Pharmacol Ther, 173 (2017) 47-57. doi: 10.1016/j.pharmthera.2017.02.005

[2] M. Tomasetti, W. Lee, L. Santarelli, J. Neuzil, Exosome-derived microRNAs in cancer metabolism: possible implications in cancer diagnostics and therapy, Exp Mol Med, 49 (2017) e285. doi: 10.1038/emm.2016.153

[3] J.N. Frediani, M. Fabbri, Essential role of miRNAs in orchestrating the biology of the tumor microenvironment, Mol Cancer, 15 (2016) 42. doi: 10.1186/s12943-016-0525-3

[4] H. Kalra, G.P. Drummen, S. Mathivanan, Focus on Extracellular Vesicles: Introducing the Next Small Big Thing, Int J Mol Sci, 17 (2016) 170. doi: 10.3390/ijms17020170

[5] R.M. Johnstone, M. Adam, J.R. Hammond, L. Orr, C. Turbide, Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes), J Biol Chem, 262 (1987) 9412-9420.

[6] B.T. Pan, R.M. Johnstone, Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor, Cell, 33 (1983) 967-978.

[7] E.G. Trams, C.J. Lauter, N. Salem, Jr., U. Heine, Exfoliation of membrane ecto-enzymes in the form of micro-vesicles, Biochim Biophys Acta, 645 (1981) 63-70.

[8] C. Harding, J. Heuser, P. Stahl, Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes, J Cell Biol, 97 (1983)

329-339.

[9] C. Thery, L. Duban, E. Segura, P. Veron, O. Lantz, S. Amigorena, Indirect activation of naive CD4+ T cells by dendritic cell-derived exosomes, Nat Immunol, 3 (2002) 1156-1162. doi:10.1038/ni854

[10] H. Valadi, K. Ekstrom, A. Bossios, M. Sjostrand, J.J. Lee, J.O. Lotvall, Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells, Nature cell biology, 9 (2007) 654-659. doi: 10.1038/ncb1596

[11] S.L. Maas, X.O. Breakefield, A.M. Weaver, Extracellular Vesicles: Unique Intercellular Delivery Vehicles, Trends Cell Biol, 27 (2017) 172-188. doi: 10.1016/j.tcb.2016.11.003

[12] L. Qu, J. Ding, C. Chen, Z.J. Wu, B. Liu, Y. Gao, et al, Exosome-Transmitted lncARSR Promotes Sunitinib Resistance in Renal Cancer by Acting as a Competing Endogenous RNA, Cancer cell, 29 (2016) 653-668. doi: 10.1016/j.ccell.2016.03.004

[13] Y. Teng, Y. Ren, X. Hu, J. Mu, A. Samykutty, X. Zhuang, et al, MVP-mediated exosomal sorting of miR-193a promotes colon cancer progression, Nat Commun, 8 (2017) 14448. doi: 10.1038/ncomms14448

[14] M.S. Ostenfeld, D.K. Jeppesen, J.R. Laurberg, A.T. Boysen, J.B. Bramsen, B. Primdal-Bengtson, et al, Cellular disposal of miR23b by RAB27-dependent exosome release is linked to acquisition of metastatic properties, Cancer Res, 74 (2014) 5758-5771. doi: 10.1158/0008-5472.CAN-13-3512

[15] P. Provost, The clinical significance of platelet microparticle-associated

microRNAs, Clin Chem Lab Med, 55 (2017) 657-666. doi: 10.1515/cclm-2016-0895

[16] O.A. Kossinova, A.V. Gopanenko, S.N. Tamkovich, O.A. Krasheninina, A.E. Tupikin, E. Kiseleva, et al, Cytosolic YB-1 and NSUN2 are the only proteins recognizing specific motifs present in mRNAs enriched in exosomes, Biochim Biophys Acta, 1865 (2017) 664-673. doi: 10.1016/j.bbapap.2017.03.010

[17] D.J. Gibbings, C. Ciaudo, M. Erhardt, O. Voinnet, Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity, Nature cell biology, 11 (2009) 1143-1149. doi: 10.1038/ncb1929

[18] Y. Peng, C.M. Croce, The role of MicroRNAs in human cancer, Signal Transduction and Targeted Therapy, 1 (2016) 15004. doi:10.1038/sigtrans.2015.4

[19] J.D. Arroyo, J.R. Chevillet, E.M. Kroh, I.K. Ruf, C.C. Pritchard, D.F. Gibson, et al, Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma, Proceedings of the National Academy of Sciences of the United States of America, 108 (2011) 5003-5008. doi: 10.1073/pnas.1019055108

[20] M.P. Hunter, N. Ismail, X. Zhang, B.D. Aguda, E.J. Lee, L. Yu, T. Xiao, et al, Detection of microRNA expression in human peripheral blood microvesicles, PloS one, 3 (2008) e3694. doi: 10.1371/journal.pone.0003694

[21] C. Villarroya-Beltri, C. Gutierrez-Vazquez, F. Sanchez-Cabo, D. Perez-Hernandez, J. Vazquez, N. Martin-Cofreces, et al, Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs, Nat Commun, 4 (2013) 2980. doi: 10.1038/ncomms3980

[22] L. Santangelo, G. Giurato, C. Cicchini, C. Montaldo, C. Mancone, R. Tarallo, et

al, The RNA-Binding Protein SYNCRIP Is a Component of the Hepatocyte Exosomal Machinery Controlling MicroRNA Sorting, Cell reports, 17 (2016) 799-808. doi: 10.1016/j.celrep.2016.09.031

[23] S. Stuffers, C. Sem Wegner, H. Stenmark, A. Brech, Multivesicular endosome biogenesis in the absence of ESCRTs, Traffic, 10 (2009) 925-937. doi: 10.1111/j.1600-0854.2009.00920.x

[24] J.H. Hurley, P.I. Hanson, Membrane budding and scission by the ESCRT machinery: it's all in the neck, Nat Rev Mol Cell Biol, 11 (2010) 556-566. doi: 10.1038/nrm2937

[25] V. Hyenne, A. Apaydin, D. Rodriguez, C. Spiegelhalter, S. Hoff-Yoessle, M. Diem, et al, RAL-1 controls multivesicular body biogenesis and exosome secretion, J Cell Biol, 211 (2015) 27-37. doi: 10.1083/jcb.201504136

[26] E.E. Kelly, C.P. Horgan, B. Goud, M.W. McCaffrey, The Rab family of proteins: 25 years on, Biochem Soc Trans, 40 (2012) 1337-1347. doi: 10.1042/BST20120203

[27] H. Stenmark, Rab GTPases as coordinators of vesicle traffic, Nat Rev Mol Cell Biol, 10 (2009) 513-525. doi: 10.1038/nrm2728

[28] M.P. Muller, R.S. Goody, Molecular control of Rab activity by GEFs, GAPs and GDI, Small GTPases, (2017) 1-17. doi: 10.1080/21541248.2016.1276999

[29] M. Fukuda, Rab27 effectors, pleiotropic regulators in secretory pathways, Traffic, 14 (2013) 949-963. doi: 10.1111/tra.12083

[30] G. Li, M.C. Marlin, Rab family of GTPases, Methods Mol Biol, 1298 (2015) 1-15. doi: 10.1007/978-1-4939-2569-8_1

[31] B.R. Ali, C. Wasmeier, L. Lamoreux, M. Strom, M.C. Seabra, Multiple regions contribute to membrane targeting of Rab GTPases, J Cell Sci, 117 (2004) 6401-6412. doi: 10.1242/jcs.01542

[32] D. Poteryaev, S. Datta, K. Ackema, M. Zerial, A. Spang, Identification of the switch in early-to-late endosome transition, Cell, 141 (2010) 497-508. doi: 10.1016/j.cell.2010.03.011

[33] C. Hsu, Y. Morohashi, S. Yoshimura, N. Manrique-Hoyos, S. Jung, M.A. Lauterbach, et al, Regulation of exosome secretion by Rab35 and its GTPase-activating proteins TBC1D10A-C, J Cell Biol, 189 (2010) 223-232. doi: 10.1083/jcb.200911018

[34] M. Ostrowski, N.B. Carmo, S. Krumeich, I. Fanget, G. Raposo, A. Savina, et al, Rab27a and Rab27b control different steps of the exosome secretion pathway, Nature cell biology, 12 (2010) 19-30; sup pp 11-13. doi: 10.1038/ncb2000

[35] D. Zhang, L.M. Iyer, F. He, L. Aravind, Discovery of Novel DENN Proteins: Implications for the Evolution of Eukaryotic Intracellular Membrane Structures and Human Disease, Front Genet, 3 (2012) 283. doi: 10.3389/fgene.2012.00283

[36] K.L. Angus, G.M. Griffiths, Cell polarisation and the immunological synapse, Curr Opin Cell Biol, 25 (2013) 85-91. doi: 10.1016/j.ceb.2012.08.013

[37] D. Obino, F. Farina, O. Malbec, P.J. Saez, M. Maurin, J. Gaillard, et al, Actin nucleation at the centrosome controls lymphocyte polarity, Nat Commun, 7 (2016) 10969. doi: 10.1038/ncomms10969

[38] T. Daniele, Y. Hackmann, A.T. Ritter, M. Wenham, S. Booth, G. Bossi, et al, A

role for Rab7 in the movement of secretory granules in cytotoxic T lymphocytes, Traffic, 12 (2011) 902-911. doi: 10.1111/j.1600-0854.2011.01194.x

[39] N.A. Ducharme, A.J. Ham, L.A. Lapierre, J.R. Goldenring, Rab11-FIP2 influences multiple components of the endosomal system in polarized MDCK cells, Cell Logist, 1 (2011) 57-68. doi: 10.4161/cl.1.2.15289

[40] D. Hoshino, K.C. Kirkbride, K. Costello, E.S. Clark, S. Sinha, N. Grega-Larson, et al, Exosome secretion is enhanced by invadopodia and drives invasive behavior, Cell reports, 5 (2013) 1159-1168. doi: 10.1016/j.celrep.2013.10.050

[41] S. Sinha, D. Hoshino, N.H. Hong, K.C. Kirkbride, N.E. Grega-Larson, M. Seiki, et al, Cortactin promotes exosome secretion by controlling branched actin dynamics, J Cell Biol, 214 (2016) 197-213. doi: 10.1083/jcb.201601025

[42] N.B. Martin-Cofreces, F. Baixauli, F. Sanchez-Madrid, Immune synapse: conductor of orchestrated organelle movement, Trends Cell Biol, 24 (2014) 61-72. doi: 10.1016/j.tcb.2013.09.005

[43] M.R. Heider, M. Munson, Exorcising the exocyst complex, Traffic, 13 (2012) 898-907. doi: 10.1111/j.1600-0854.2012.01353.x

[44] P. Yue, Y. Zhang, K. Mei, S. Wang, J. Lesigang, Y. Zhu, et al, Sec3 promotes the initial binary t-SNARE complex assembly and membrane fusion, Nat Commun, 8 (2017) 14236. doi: 10.1038/ncomms14236

[45] T.C. Sudhof, J.E. Rothman, Membrane fusion: grappling with SNARE and SM proteins, Science, 323 (2009) 474-477. doi: 10.1126/science.1161748

[46] Z. Wu, O.D. Bello, S. Thiyagarajan, S.M. Auclair, W. Vennekate, S.S.

Krishnakumar, et al, Dilation of fusion pores by crowding of SNARE proteins, Elife, 6 (2017). doi: 10.7554/eLife.22964

[47] C.M. Fader, D.G. Sanchez, M.B. Mestre, M.I. Colombo, TI-VAMP/VAMP7 and VAMP3/cellubrevin: two v-SNARE proteins involved in specific steps of the autophagy/multivesicular body pathways, Biochim Biophys Acta, 1793 (2009) 1901-1916. doi: 10.1016/j.bbamcr.2009.09.011

[48] Y. Wei, D. Wang, F. Jin, Z. Bian, L. Li, H. Liang, et al, Pyruvate kinase type M2 promotes tumour cell exosome release via phosphorylating synaptosome-associated protein 23, Nat Commun, 8 (2017) 14041. doi: 10.1038/ncomms14041

[49] K.C. Williams, R.E. McNeilly, M.G. Coppolino, SNAP23, Syntaxin4, and vesicle-associated membrane protein 7 (VAMP7) mediate trafficking of membrane type 1-matrix metalloproteinase (MT1-MMP) during invadopodium formation and tumor cell invasion, Mol Biol Cell, 25 (2014) 2061-2070. doi: 10.1091/mbc.E13-10-0582

[50] T. Weber, B.V. Zemelman, J.A. McNew, B. Westermann, M. Gmachl, F. Parlati, et al, SNAREpins: minimal machinery for membrane fusion, Cell, 92 (1998) 759-772.

[51] R.W. Baker, P.D. Jeffrey, M. Zick, B.P. Phillips, W.T. Wickner, F.M. Hughson, A direct role for the Sec1/Munc18-family protein Vps33 as a template for SNARE assembly, Science, 349 (2015) 1111-1114. doi: 10.1126/science.aac7906

[52] P. Gissen, C.A. Johnson, N.V. Morgan, J.M. Stapelbroek, T. Forshew, W.N. Cooper, et al, Mutations in VPS33B, encoding a regulator of SNARE-dependent membrane fusion, cause arthrogryposis-renal dysfunction-cholestasis (ARC)

syndrome, Nat Genet, 36 (2004) 400-404. doi: 10.1038/ng1325

[53] H. Gu, C. Chen, X. Hao, C. Wang, X. Zhang, Z. Li, et al, Sorting protein VPS33B regulates exosomal autocrine signaling to mediate hematopoiesis and leukemogenesis, J Clin Invest, 126 (2016) 4537-4553. doi: 10.1172/JCI87105

[54] P.R. Hiesinger, A. Fayyazuddin, S.Q. Mehta, T. Rosenmund, K.L. Schulze, R.G. Zhai, et al, The v-ATPase V0 subunit a1 is required for a late step in synaptic vesicle exocytosis in Drosophila, Cell, 121 (2005) 607-620. doi: 10.1016/j.cell.2005.03.012

[55] S. Liegeois, A. Benedetto, J.M. Garnier, Y. Schwab, M. Labouesse, The V0-ATPase mediates apical secretion of exosomes containing Hedgehog-related proteins in Caenorhabditis elegans, J Cell Biol, 173 (2006) 949-961. doi: 10.1083/jcb.200511072

[56] T. Tian, Y.L. Zhu, Y.Y. Zhou, G.F. Liang, Y.Y. Wang, F.H. Hu, Z.D. Xiao, Exosome uptake through clathrin-mediated endocytosis and macropinocytosis and mediating miR-21 delivery, The Journal of biological chemistry, 289 (2014) 22258-22267. doi: 10.1074/jbc.M114.588046

[57] M. Record, K. Carayon, M. Poirot, S. Silvente-Poirot, Exosomes as new vesicular lipid transporters involved in cell-cell communication and various pathophysiologies, Biochim Biophys Acta, 1841 (2014) 108-120. doi: 10.1016/j.bbalip.2013.10.004

[58] G. Taraboletti, S. D'Ascenzo, I. Giusti, D. Marchetti, P. Borsotti, D. Millimaggi, et al, Bioavailability of VEGF in tumor-shed vesicles depends on vesicle burst induced by acidic pH, Neoplasia, 8 (2006) 96-103. doi: 10.1593/neo.05583

[59] H.C. Christianson, K.J. Svensson, T.H. van Kuppevelt, J.P. Li, M. Belting, Cancer cell exosomes depend on cell-surface heparan sulfate proteoglycans for their internalization and functional activity, Proceedings of the National Academy of Sciences of the United States of America, 110 (2013) 17380-17385. doi: 10.1073/pnas.1304266110

[60] J. Fares, R. Kashyap, P. Zimmermann, Syntenin: Key player in cancer exosome biogenesis and uptake?, Cell Adh Migr, 11 (2017) 124-126. doi: 10.1080/19336918.2016.1225632

[61] L. Chen, D.R. Brigstock, Integrins and heparan sulfate proteoglycans on hepatic stellate cells (HSC) are novel receptors for HSC-derived exosomes, FEBS letters, 590 (2016) 4263-4274. doi: 10.1002/1873-3468.12448

[62]A. Hoshino, B. Costa-Silva, T.L. Shen, G. Rodrigues, A. Hashimoto, M. Tesic Mark, H. Molina, S. Kohsaka, A. Di Giannatale, S. Ceder, S. Singh, C. Williams, N. Soplop, K. Uryu, L. Pharmer, T. King, L. Bojmar, A.E. Davies, Y. Ararso, T. Zhang, H. Zhang, J. Hernandez, J.M. Weiss, V.D. Dumont-Cole, K. Kramer, L.H. Wexler, A. Narendran, G.K. Schwartz, J.H. Healey, P. Sandstrom, K.J. Labori, E.H. Kure, P.M. Grandgenett, M.A. Hollingsworth, M. de Sousa, S. Kaur, M. Jain, K. Mallya, S.K. Batra, W.R. Jarnagin, M.S. Brady, O. Fodstad, V. Muller, K. Pantel, A.J. Minn, M.J. Bissell, B.A. Garcia, Y. Kang, V.K. Rajasekhar, C.M. Ghajar, I. Matei, H. Peinado, J. Bromberg, D. Lyden, Tumour exosome integrins determine organotropic metastasis, Nature, 527 (2015) 329-335. doi: 10.1038/nature15756

[63]H. Ling, M. Fabbri, G.A. Calin, MicroRNAs and other non-coding RNAs as

targets for anticancer drug development, Nature reviews. Drug discovery, 12 (2013) 847-865. doi: 10.1038/nrd4140

[64]Y. Zheng, L. Liu, G.C. Shukla, A comprehensive review of web-based non-coding RNA resources for cancer research, Cancer Lett, 407 (2017) 1-8. doi: 10.1016/j.canlet.2017.08.015

[65]F.O. Beltran-Anaya, A. Cedro-Tanda, A. Hidalgo-Miranda, S.L. Romero-Cordoba, Insights into the Regulatory Role of Non-coding RNAs in Cancer Metabolism, Front Physiol, 7 (2016) 342. doi: 10.3389/fphys.2016.00342

[66]A. Gallo, S. Vella, M. Miele, F. Timoneri, M. Di Bella, S. Bosi, M. Sciveres, P.G. Conaldi, Global profiling of viral and cellular non-coding RNAs in Epstein-Barr virus-induced lymphoblastoid cell lines and released exosome cargos, Cancer Lett, 388 (2017) 334-343. doi: 10.1016/j.canlet.2016.12.003

[67]R. Zhang, Y. Xia, Z. Wang, J. Zheng, Y. Chen, X. Li, Y. Wang, H. Ming, Serum long non coding RNA MALAT-1 protected by exosomes is up-regulated and promotes cell proliferation and migration in non-small cell lung cancer, Biochem Biophys Res Commun, 490 (2017) 406-414. doi: 10.1016/j.bbrc.2017.06.055

[68]M.Y. Fong, W. Zhou, L. Liu, A.Y. Alontaga, M. Chandra, J. Ashby, A. Chow, S.T. O'Connor, S. Li, A.R. Chin, G. Somlo, M. Palomares, Z. Li, J.R. Tremblay, A. Tsuyada, G. Sun, M.A. Reid, X. Wu, P. Swiderski, X. Ren, Y. Shi, M. Kong, W. Zhong, Y. Chen, S.E. Wang, Breast-cancer-secreted miR-122 reprograms glucose metabolism in premetastatic niche to promote metastasis, Nat Cell Biol, 17 (2015) 183-194. doi: 10.1038/ncb3094

[69]L. Li, C. Li, S. Wang, Z. Wang, J. Jiang, W. Wang, X. Li, J. Chen, K. Liu, C. Li, G. Zhu, Exosomes Derived from Hypoxic Oral Squamous Cell Carcinoma Cells Deliver miR-21 to Normoxic Cells to Elicit a Prometastatic Phenotype, Cancer Res, 76 (2016) 1770-1780. doi: 10.1158/0008-5472

[70]K.E. Richards, A.E. Zeleniak, M.L. Fishel, J. Wu, L.E. Littlepage, R. Hill, Cancer-associated fibroblast exosomes regulate survival and proliferation of pancreatic cancer cells, Oncogene, 36 (2017) 1770-1778. doi: 10.1038/onc.2016.353

[71]A. Ahadi, S. Brennan, P.J. Kennedy, G. Hutvagner, N. Tran, Long non-coding RNAs harboring miRNA seed regions are enriched in prostate cancer exosomes, Sci Rep, 6 (2016) 24922. doi: 10.1038/srep24922

[72] Q. Tan, M. Wang, M. Yu, J. Zhang, R.G. Bristow, R.P. Hill, I.F. Tannock, Role of Autophagy as a Survival Mechanism for Hypoxic Cells in Tumors, Neoplasia, 18 (2016) 347-355. doi: 10.1016/j.neo.2016.04.003

[73] J.P. Pursiheimo, K. Rantanen, P.T. Heikkinen, T. Johansen, P.M. Jaakkola, Hypoxia-activated autophagy accelerates degradation of SQSTM1/p62, Oncogene, 28 (2009) 334-344. doi: 10.1038/onc.2008.392

[74] H.W. King, M.Z. Michael, J.M. Gleadle, Hypoxic enhancement of exosome release by breast cancer cells, BMC Cancer, 12 (2012) 421. doi: 10.1186/1471-2407-12-421

[75] Y.L. Hsu, J.Y. Hung, W.A. Chang, Y.S. Lin, Y.C. Pan, P.H. Tsai, et al, Hypoxic lung cancer-secreted exosomal miR-23a increased angiogenesis and vascular permeability by targeting prolyl hydroxylase and tight junction protein ZO-1,

Oncogene, (2017). doi: 10.1038/onc.2017.105

[76] F. Baixauli, C. Lopez-Otin, M. Mittelbrunn, Exosomes and autophagy: coordinated mechanisms for the maintenance of cellular fitness, Front Immunol, 5 (2014) 403. doi: 10.3389/fimmu.2014.00403

[77] M.V. Dias, B.L. Teixeira, B.R. Rodrigues, R. Sinigaglia-Coimbra, I. Porto-Carreiro, M. Roffe, G.N. Hajj, V.R. Martins, PRNP/prion protein regulates the secretion of exosomes modulating CAV1/caveolin-1-suppressed autophagy, Autophagy, 12 (2016) 2113-2128. doi: 10.1080/15548627.2016.1226735

[78] C. Villarroya-Beltri, F. Baixauli, M. Mittelbrunn, I. Fernandez-Delgado, D. Torralba, O. Moreno-Gonzalo, S. Baldanta, C. Enrich, S. Guerra, F. Sanchez-Madrid, ISGylation controls exosome secretion by promoting lysosomal degradation of MVB proteins, Nat Commun, 7 (2016) 13588. doi: 10.1038/ncomms13588

[79] G.K. Patel, M.A. Khan, A. Bhardwaj, S.K. Srivastava, H. Zubair, M.C. Patton, S. Singh, M. Khushman, A.P. Singh, Exosomes confer chemoresistance to pancreatic cancer cells by promoting ROS detoxification and miR-155-mediated suppression of key gemcitabine-metabolising enzyme, DCK, Br J Cancer, 116 (2017) 609-619. doi: 10.1038/bjc.2017.18

[80] C.G. Xu, M.F. Yang, Y.Q. Ren, C.H. Wu, L.Q. Wang, Exosomes mediated transfer of lncRNA UCA1 results in increased tamoxifen resistance in breast cancer cells, Eur Rev Med Pharmacol Sci, 20 (2016) 4362-4368

[81] X. Qin, S. Yu, L. Zhou, M. Shi, Y. Hu, X. Xu, et al, Cisplatin-resistant lung cancer cell-derived exosomes increase cisplatin resistance of recipient cells in

exosomal miR-100-5p-dependent manner, Int J Nanomedicine, 12 (2017) 3721-3733. doi: 10.2147/IJN.S131516

[82] H. Wu, J. Zhou, S. Mei, D. Wu, Z. Mu, B. Chen, et al, Circulating exosomal microRNA-96 promotes cell proliferation, migration and drug resistance by targeting LMO7, J Cell Mol Med, 21 (2017) 1228-1236. doi: 10.1111/jcmm.13056

[83] F. Wei, C. Ma, T. Zhou, X. Dong, Q. Luo, L. Geng, L. Ding, Y. Zhang, L. Zhang, N. Li, Y. Li, Y. Liu, Exosomes derived from gemcitabine-resistant cells transfer malignant phenotypic traits via delivery of miRNA-222-3p, Mol Cancer, 16 (2017) 132. doi: 10.1186/s12943-017-0694-8

[84] D.L. Yuwen, B.B. Sheng, J. Liu, W. Wenyu, Y.Q. Shu, MiR-146a-5p level in serum exosomes predicts therapeutic effect of cisplatin in non-small cell lung cancer, European review for medical and pharmacological sciences, 21 (2017) 2650-2658.

[85] P. Zheng, L. Chen, X. Yuan, Q. Luo, Y. Liu, G. Xie, et al, Exosomal transfer of tumor-associated macrophage-derived miR-21 confers cisplatin resistance in gastric cancer cells, J Exp Clin Cancer Res, 36 (2017) 53. doi: 10.1186/s13046-017-0528-y

[86] M. Mikamori, D. Yamada, H. Eguchi, S. Hasegawa, T. Kishimoto, Y. Tomimaru, et al, MicroRNA-155 Controls Exosome Synthesis and Promotes Gemcitabine Resistance in Pancreatic Ductal Adenocarcinoma, Sci Rep, 7 (2017) 42339. doi: 10.1038/srep42339

[87] D.D. Yu, Y. Wu, X.H. Zhang, M.M. Lv, W.X. Chen, X. Chen, et al, Exosomes from adriamycin-resistant breast cancer cells transmit drug resistance partly by delivering miR-222, Tumour biology, 37 (2016) 3227-3235. doi:

10.1007/s13277-015-4161-0

[88] M.Y. Fong, W. Zhou, L. Liu, A.Y. Alontaga, M. Chandra, J. Ashby, et al, Breast-cancer-secreted miR-122 reprograms glucose metabolism in premetastatic niche to promote metastasis, Nature cell biology, 17 (2015) 183-194. doi: 10.1038/ncb3094

[89] L. Zhang, S. Zhang, J. Yao, F.J. Lowery, Q. Zhang, W.C. Huang, et al, Microenvironment-induced PTEN loss by exosomal microRNA primes brain metastasis outgrowth, Nature, 527 (2015) 100-104. doi: 10.1038/nature15376

[90] Z. Deng, Y. Rong, Y. Teng, X. Zhuang, A. Samykutty, J. Mu, et al, Exosomes miR-126a released from MDSC induced by DOX treatment promotes lung metastasis, Oncogene, 36 (2017) 639-651. doi: 10.1038/onc.2016.229

[91] M. Fabbri, A. Paone, F. Calore, R. Galli, E. Gaudio, R. Santhanam, F. Lovat, P. Fadda, C. Mao, G.J. Nuovo, N. Zanesi, M. Crawford, G.H. Ozer, D. Wernicke, H. Alder, M.A. Caligiuri, P. Nana-Sinkam, D. Perrotti, C.M. Croce, MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response, Proc Natl Acad Sci U S A, 109 (2012) E2110-2116. doi: 10.1073/pnas.1209414109

[92] G. Siravegna, S. Marsoni, S. Siena, A. Bardelli, Integrating liquid biopsies into the management of cancer, Nat Rev Clin Oncol, (2017). doi: 10.1038/nrclinonc.2017.14

[93] W. Zhang, W. Xia, Z. Lv, C. Ni, Y. Xin, L. Yang, Liquid Biopsy for Cancer: Circulating Tumor Cells, Circulating Free DNA or Exosomes?, Cell Physiol Biochem, 41 (2017) 755-768. doi: 10.1159/000458736

[94] K.L. Morley, M.G. Toohey, D.O. Peterson, Transcriptional repression of a hormone-responsive promoter, Nucleic Acids Res, 15 (1987) 6973-6989.

[95] Z. Szallasi, Another surprising role for exosomes? Improving next-generation sequencing-based cancer diagnostics in liquid biopsies, Ann Oncol, 27 (2016) 557-558. doi: 10.1093/annonc/mdw059

[96] C. Roma-Rodrigues, L.R. Raposo, R. Cabral, F. Paradinha, P.V. Baptista, A.R. Fernandes, Tumor Microenvironment Modulation via Gold Nanoparticles Targeting Malicious Exosomes: Implications for Cancer Diagnostics and Therapy, Int J Mol Sci, 18 (2017). doi: 10.3390/ijms18010162

[97] M.F. Baietti, Z. Zhang, E. Mortier, A. Melchior, G. Degeest, A. Geeraerts, Y. Ivarsson, F. Depoortere, C. Coomans, E. Vermeiren, P. Zimmermann, G. David, Syndecan-syntenin-ALIX regulates the biogenesis of exosomes, Nature cell biology, 14 (2012) 677-685. doi: 10.1038/ncb2502

[98] J.X. Wei, L.H. Lv, Y.L. Wan, Y. Cao, G.L. Li, H.M. Lin, R. Zhou, C.Z. Shang, J. Cao, H. He, Q.F. Han, P.Q. Liu, G. Zhou, J. Min, Vps4A functions as a tumor suppressor by regulating the secretion and uptake of exosomal microRNAs in human hepatoma cells, Hepatology, 61 (2015) 1284-1294. doi: 10.1002/hep.27660

[99] X.C. Jiang, J.Q. Gao, Exosomes as novel bio-carriers for gene and drug delivery, Int J Pharm, 521 (2017) 167-175. doi: 10.1016/j.ijpharm.2017.02.038

[100] D. Ingato, J.U. Lee, S.J. Sim, Y.J. Kwon, Good things come in small packages: Overcoming challenges to harness extracellular vesicles for therapeutic delivery, J Control Release, 241 (2016) 174-185. doi: 10.1016/j.jconrel.2016.09.016

[101] A.J. O'Loughlin, I. Mager, O.G. de Jong, M.A. Varela, R.M. Schiffelers, S. El Andaloussi, M.J. Wood, P. Vader, Functional Delivery of Lipid-Conjugated siRNA by Extracellular Vesicles, Mol Ther, (2017). doi: 10.1016/j.ymthe.2017.03.021

[102] A.K. Agrawal, F. Aqil, J. Jeyabalan, W.A. Spencer, J. Beck, B.W. Gachuki, S.S. Alhakeem, K. Oben, R. Munagala, S. Bondada, R.C. Gupta, Milk-derived exosomes for oral delivery of paclitaxel, Nanomedicine, 13 (2017) 1627-1636. doi: 10.1016/j.nano.2017.03.001

[103] M. Morishita, Y. Takahashi, A. Matsumoto, M. Nishikawa, Y. Takakura, Exosome-based tumor antigens-adjuvant co-delivery utilizing genetically engineered tumor cell-derived exosomes with immunostimulatory CpG DNA, Biomaterials, 111 (2016) 55-65. doi: 10.1016/j.biomaterials.2016.09.031

[104] F. Caponnetto, I. Manini, M. Skrap, T. Palmai-Pallag, C. Di Loreto, A.P. Beltrami, D. Cesselli, E. Ferrari, Size-dependent cellular uptake of exosomes, Nanomedicine, 13 (2017) 1011-1020. doi: 10.1016/j.nano.2016.12.009

[105] U. Sterzenbach, U. Putz, L.H. Low, J. Silke, S.S. Tan, J. Howitt, Engineered Exosomes as Vehicles for Biologically Active Proteins, Mol Ther, (2017). doi: 10.1016/j.ymthe.2017.03.030

[106] S.C. Jang, O.Y. Kim, C.M. Yoon, D.S. Choi, T.Y. Roh, J. Park, J. Nilsson, J. Lotvall, Y.K. Kim, Y.S. Gho, Bioinspired exosome-mimetic nanovesicles for targeted delivery of chemotherapeutics to malignant tumors, ACS Nano, 7 (2013) 7698-7710. doi: 10.1021/nn402232g

[107] T.R. Lunavat, S.C. Jang, L. Nilsson, H.T. Park, G. Repiska, C. Lasser, J.A. Nilsson, Y.S. Gho, J. Lotvall, RNAi delivery by exosome-mimetic nanovesicles - Implications for targeting c-Myc in cancer, Biomaterials, 102 (2016) 231-238. doi: 10.1016/j.biomaterials.2016.06.024

Highlights

1.We summarize mechanisms for the biogenesis,release and uptake of exosomes

2. MVBs fate may contain a specific regulatory mechanism on autophagy and exosomes secretory

3.Exosomal ncRNAs play a vital role in cell to cell communication to promote tumor progression

4.Exosomes may be a promisng application in cancer diagnosis and treatment