Review Article

Multiple Roles of Exosomal Long Noncoding RNAs in Cancers

Wenyuan Zhao, Yuanqi Liu, Chunfang Zhang, and Chaojun Duan

1Department of Oncology, Xiangya Hospital, Central South University, Changsha, China
2Institute of Medical Sciences, Xiangya Hospital, Central South University, Changsha, China
3Department of Thoracic Surgery, Xiangya Hospital, Central South University, Changsha, China
4National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, China

Correspondence should be addressed to Chunfang Zhang; zhcf3801@csu.edu.cn and Chaojun Duan; duancjxy@126.com

Received 1 March 2019; Revised 12 May 2019; Accepted 13 June 2019; Published 7 July 2019

Long noncoding RNAs (lncRNAs) are not transcriptional noise, as previously understood, but are currently considered to be multifunctional. Exosomes are derived from the internal multivesicular compartment and are extracellular vesicles (EVs) with diameters of 30–100 nm. Exosomes play significant roles in the intercellular exchange of information and material. Exosomal lncRNAs may be promising biomarkers for cancer diagnosis and potential targets for cancer therapies, since they are increasingly understood to be involved in tumorigenesis, tumor angiogenesis, and chemoresistance. This review mainly focuses on the roles of emerging exosomal lncRNAs in cancer. In addition, the biogenesis of exosomes, the functions of lncRNAs, and the mechanisms of lncRNAs in exosome-mediated cell-cell communication are also summarized.

1. Introduction

Noncoding RNAs (ncRNAs) account for the majority of transcribed RNA. Long noncoding RNAs (lncRNAs) are ncRNAs that are larger than 200 nucleotides [1, 2]. Rather than being transcriptional noise, lncRNAs regulate biological activities in a variety of ways, including transcriptional regulation, posttranscriptional regulation, translation regulation, and protein cell localization. LncRNAs are also found to play a necessary role in the progression and prognosis of tumors [3, 4]. LncRNAs have important regulatory functions in fundamental pathological and biological processes, which helps to elucidate the use of lncRNAs and their corresponding proteins or peptides for cancer diagnosis and therapy [5]. EVs play an important role in different disease processes, including renal disease [6], osteoarthritis [7], coronary artery disease [8], dermatology [9], and neurodegenerative diseases [10], leukemia [11] and even have immune-modulatory effects on pregnancy and preeclampsia [12]. In addition, EVs are closely related to endothelial damage in sickle-cell disease [13], sinusoidal obstruction syndrome [14], and essential thrombocythemia [15]. The exosome is a kind of vesicle secreted by living cells that has a diameter of 30-100 nm and a bilayer lipid membrane structure. Exosomes are widely present in biological fluids, such as peripheral blood, ascites, urine, saliva, synovial fluid, and cerebrospinal fluid, as well as bronchoalveolar lavage and breast milk [16]. Exosomes can deliver functional molecules, including lipids, proteins, and nucleic acids, to recipient cells. Exosomes participate in intercellular communication and affect various physiological and pathological functions of cells. For example, pancreatic cancer-derived exosomes are involved in the proliferation, progression, and metastasis of pancreatic cancer [17]. However, the mechanisms by which these exosomal elements affect target recipient cells have not been determined to date. Exosomal lncRNAs have been found to participate in the regulation of tumorigenesis, tumor angiogenesis, and drug resistance, which suggests that there are ample opportunities to explore the potential roles of exosomes as biomarkers in cancer therapies. This review summarizes lncRNA functions and exosome biogenesis in exosome-mediated cell-cell communication and specifically focuses on the emerging roles of exosomal lncRNA in cancer. The EVs studied in some articles reviewed have morphological features of exosomes. However, the term EVs was used in these articles since exosomes are a specific subset of vesicles with a distinctive biogenesis.
2. LncRNAs

Currently, increasing evidence suggests that lncRNAs have considerable effects on various molecular mechanisms. Prior studies have indicated that mutations of the noncoding genome are widely involved in common human diseases [18]. Regulatory DNA mutations can widely affect transcription by altering enhancer and promoter activity or chromatin states, which leads to the differential expression of lncRNAs in cancer [19]. Although once considered to be transcriptional noise, lncRNAs exhibit various functions, as illustrated in Figure 1. LncRNAs regulate mRNA selective splicing and stability [20]. Additionally, many LncRNAs regulate gene expression by recruiting chromatin modifiers to special genomic locations, similar to scaffolds [21, 22], or by isolating chromatin modifiers from their regulatory locations, similar to decoys [23]. Moreover, LncRNAs control posttranscriptional regulation by functioning as ceRNAs (competing endogenous RNAs) [24] or miRNA sponges [25]. LncRNAs can also directly interact with important signaling proteins (e.g., phosphorylation) and modulate their functions [26]. Some LncRNAs encode functional micropeptides by small open reading frames (smORFs) [27, 28]. More importantly, Yang and colleagues found several peptides which correspond to nine transcripts annotated as ncRNAs [5]. In addition, two smORFs, which were mainly found in ncRNAs and 5’ untranslated regions (UTRs), could bind several ribosomes and participate in translation. Dysregulated IncRNAs have been reported to be involved in regulating the proliferation, metastases, and recurrence of multiple cancers, including lung cancer [29], prostate cancer [30], hepatocellular cancer [31], and ovarian cancer [32].

3. Exosomes

3.1. Exosome Formation. Exosome biogenesis is observed in various cells, including immune cells, mesenchymal stem cells, neurons, epithelial cells, and endothelial cells (ECs). This process is unlike the formation of microvesicles, which are generated via outward budding at the plasma surface [33]. The underlying mechanism of exosome formation includes several steps. First, an endosome forms through the inward budding of the plasma membrane. Then, further inward budding of the limiting membrane inside the endosome leads to the formation of the multivesicular body (MVB) with a diameter of 30-100 nm, peripheral proteins, cytosolic contents, and the transmembrane, which can be merged into the invaginating membrane through the exocytosis pathway and maintained as extracellular vesicles. MVBs rich in cholesterol fuse with the plasma membrane and then release their contents into the extracellular space. Otherwise, MVBs with deficient cholesterol fuse with lysosomes, causing the degradation of vesicular contents [34]. These released vesicles are known as exosomes. MVB packing was thought to be highly conserved. However, MVB packing is now related to the endosomal sorting complexes required for transport (ESCRT) complex proteins [35]. ESCRT-0, -1, and -II are responsible for recognizing and hiding ubiquitinated membrane proteins in endosomal membranes, and ESCRT-III facilitates cutting and inward budding [36]. However, researchers have observed ESCRT-independent MVB packaging pathways [37] (Figure 2).

3.2. Exosomal Molecular Components. Exosomes contain proteins, RNAs, and DNAs [38]. According to the database [ExoCarta (http://www.exocarta.org)], 9769 proteins, 3408 mRNAs, 2838 miRNAs, and 1116 lipids have been identified in exosomes. Extracellular vesicles (EVs) are composed of a lipid bilayer with transmembrane proteins that enclose cytosolic proteins and RNAs [1]. According to the subcellular origin, EVs include microvesicles (100-1000 nm) and exosomes (30-100 nm), which are derived from the internal MVBs [3]. Employing asymmetric flow field-flow fractionation, researchers identified three exosome subgroups: large exosome vesicles (Exo-L, 90-120 nm), small exosome vesicles (Exo-S, 60-80 nm), and “exomeres” (nonmembranous nanoparticles, ~35 nm). Each subpopulation contains a unique component distribution [39]. Metabolic enzymes and hypoxia, microtubule and coagulation proteins, as well as proteins associated with specific pathways, i.e., glycolysis and mTOR signaling, are abundant in exomeres. The proteins contained in Exo-S and Exo-L are involved in endosomal functions, secretion pathways, the mitotic spindle, and IL-2/STAT5 signaling pathways. Additionally, diverse organ distribution patterns have also been observed among those three subpopulations.

3.3. Exosomal Release and Transportation. Intracellular calcium, Rab GTPases, and SNARE proteins are crucial elements in exosome release. However, the precise coordination of events involved in exosome release has not been determined [40–42]. Rab27A, Rab27B, and Rab11 were observed to participate in MVE docking at the plasma membrane and to act as mediators in exosome releases [43, 44]. Another six small GTPases are also associated with secretions (Rab2B, Rab6, Rab7, Rab9A, Rab35, and RAL) [16, 45]. SNARE proteins may participate in the fusion of MVEs with the plasma membrane to release ILVs as exosomes [46]. Ca++ was observed to be involved in the activation of SNARE complexes in many cell types [47]. However, the precise coordination involved in this event has not been determined.

After being released into the extracellular space, extracellular exosomes can be taken up by the recipient cell membrane, thereby delivering exosomal contents into the cytoplasm. In 2007, Valadi et al. first found that exosomes can function as molecular component cargos after they cocultured HMC-1 human mast cells with exosomes isolated from MC/9 murine mast cells [48]. These researchers found that some RNAs exist in vesicles and can be translated by receptor cells. This exosome-mediated intercellular communication requires several steps: first, exosomes binding to the plasma membrane; second, surface receptor and signaling activation; third, vesicle internalization or fusing with the recipient cells [49]. This binding seems target cell-specific and may be determined by proteins enriched between the exosomal surface and the recipient cell plasma membrane [50]. Several mediators of these interactions are known,
including extracellular matrix, tetraspanins [51], heparin sulfate proteoglycans [52], and lectins [53].

Exosomes with different compositions may have different functions. An example of this phenomenon is that the β-amyloid protein present in exosomes derived from neuroblastoma can be specifically internalized by neurons. However, CD-63-enriched exosomes can bind both neurons and glial cells [54]. Additionally, some special structures at the target cell plasma membrane can influence exosome destiny [55]. Once bound to recipient cells, exosomes can be internalized by endocytosis, phagocytosis, or micropinocytosis [56]. After uptake by recipient cells, exosomes fuse with plasma membrane and release their contents or reach MVBs and undergo digestion by lysosomes [57], whereas some exosomes may escape digestion [58].

3.4. Roles of Exosomes in Cancers. Neighboring or distant cells can communicate through the secretion of exosomes. A variety of biological components have been detected in exosomes, such as proteins, mRNAs, and noncoding RNAs [59]. Recent studies have found that tumor-derived EVs participate in promoting antitumor immune responses, helping metastatic dissemination, creating a microenvironment [60], and assisting tumor angiogenesis [61].

4. Exosomal IncRNAs

Exosomes contain various ncRNAs, including IncRNAs. Exosomal IncRNAs can be released from cancer cells and internalized by recipient cells, which induces various effects. RNA sequencing shows that exosomal RNAs reflect the intercellular RNA compositions, which suggests that the RNAs are selectively packed into exosomes [62]. Moreover, it has been found that exosomal secretions of RNAs show discrepancies between cancer cells and normal cells [63]. In addition, researchers have observed that IncRNAs with low expression levels in cells are enriched in secreted exosomes [64]. These findings suggest that tumor cells can secrete specific IncRNA-enriched exosomes and may effectively influence recipient cells, which further affects tumorigenesis. In addition to tumorigenesis, exosomal IncRNAs also influence brain disorders [65] and cardiovascular diseases [66]. Accumulating evidence has shown that IncRNAs can be packed into vesicles and detected, which enables circulating IncRNAs to serve as biomarkers [67, 68].

4.1. IncRNAs Sorted into Exosomes. The exosomal sorting of RNAs has proven to be highly selective and exhibits cell specificity [69]. Additionally, researchers have noticed that
InncRNA molecules contained in exosomes can reflect the cellular response to stimulation, such as DNA damage. These findings suggest a potential regulatory mechanism of sorting ncRNAs into exosomes. However, the mechanism behind packaging specific biological contents into exosomes is not well-understood at present. Researchers found a specific sequence (GGAG) contained in the exosomal miRNAs, which is identified as the EXOmotif and can be specifically recognized by hnRNPA1 (heterogeneous ribonucleoprotein A1) and hnRNPA2B1, thereby regulating the specific loading of such miRNAs into exosomes [70]. Recently, hnRNPA2B1 has also been found to participate in the sorting of IncRNAs into exosomes by recognizing a specific sequence [71]. Another protein, Y-box–binding protein 1 (YBX1), may also help to sort special RNAs into exosomes via binding to specific structural motifs of RNAs, such as UAAUCCCA and CAGUGAGC of IncRNAs and mRNAs [72].

5. Functions of Exosomal IncRNAs in Cancers

Exosomal IncRNAs can be used as cancer biomarkers and are strongly involved in tumorigenesis, cancer drug resistance, hypoxia signaling, and EMT. These functions of exosomal IncRNAs are listed in Table 1 according to cancer type and are described in the following subsections in detail.

5.1. Cancer Biomarker. The specific IncRNAs contained in cancer cell–derived vesicles may be the measurable and non-invasive clinic biomarkers [73]. Moreover, exosomes prevent proteins and RNAs from being degraded, which renders them intact and functional [74]. In articles published to date, exosomal IncRNAs related to cancer diagnoses and prognoses account for most items.

Serum IncRNAs are commonly used in cancer detection. LncARSR (Ensembl: ENST00000424980) is highly expressed in the plasma of renal cell carcinoma (RCC) patients. In addition, the level of plasma IncRNA-ARSR is decreased after tumor resection and elevated again upon tumor relapse. Correlations between plasma IncRNA-ARSR and progression-free survival (PFS) of RCC patients who underwent sunitinib therapy have also been observed [60]. Exosomal ZFAS1 expression levels are elevated in gastric carcinoma patients and associated with lymphatic metastasis and TNM stage [75]. In addition, with high diagnostic sensitivity and specificity (80.0% and 75.7%), exosomal ZFAS1 is a promising biomarker for gastric cancer diagnosis. Exosomal IncRNAs also exhibit the ability to serve as biomarkers for colorectal

Figure 2: Exosome biogenesis and exosome-mediated delivery of ncRNAs to the recipient cell [37]. A. ncRNAs bind to packing proteins and are selectively secreted. B. Early endosomes are generated from inward budding of the plasma membrane and mature after interacting with Golgi complexes. C. Late endosomes form intraluminal vesicles (ILVs) and incorporate nucleic acids. D. MVB containing ILVs then fuse with the plasma membrane and release exosomes. E. ncRNAs are transferred within exosomes to recipient cells and affect functions.
| Cancer type               | LncRNA | Source     | Function       | Related genes          | Mechanism                                                                 | Reference |
|--------------------------|--------|------------|----------------|------------------------|---------------------------------------------------------------------------|-----------|
| Hepatocellular Carcinoma | Lnc TUC339 | Cell   | Tumorigenesis | None                   | Up or down regulation of TUC339 can effectively influence HCC cell proliferation and metastasis | [83]     |
|                          | Lnc H19 © | Cell   | Tumorigenesis | None                   | Exosomes released by CD90+ cancer cells can affect HUVECs by promoting tube formation and cell-cell adhesion | [86]     |
|                          | Lnc-ROR | Cell   | Chemoresistance | None                   | Lnc-ROR can be selectively enriched in extracellular vesicles by TGFβ1 stimulated HCC/HepG2 cells | [93]     |
|                          | Lnc-ROR © | Cell   | Tumor cell ischemia | MiR-145–HIF-1α | Lnc-ROR can modulate intercellular responses to hypoxia via the transfer of extracellular-vesicle. | [99]     |
|                          | Lnc VLDLR | Cell   | Chemoresistance | None                   | Lnc-VLDLR can be transferred by HCC cell derived EV's and promote chemoresistance in recipient cancer cells | [95]     |
| Lung Cancer              | MALAT-1 © | Serum | Biomarker     | None                   | Serum exosomal MALAT-1 was positively associated with tumor stage and lymphatic metastasis | [69]     |
| Gastric Cancer           | Lnc 00152 © | Serum | Biomarker     | None                   | Serum exosomal Lnc 00152 was significantly elevated in gastric cancer patients | [64]     |
|                          | ZFAS1 © | Serum/Cell | Biomarker/ tumorigenesis | None                   | ZFAS1 enriched exosomes can endow recipient cell with proliferation and migration | [75]     |
|                          | HOTTIP © | Serum | Biomarker     | None                   | Potential biomarker for GC in diagnosis and prognosis                     | [101]    |
| Colorectal Cancer        | CRNDE-h © | Serum | Biomarker     | None                   | CRNDE-h specificity discriminates CRC patients from NC and benign disease group with high sensitivity | [77]     |
|                          | Lnc-PVT1 | Cell | Potential biomarker | C-Myc                   | Lnc-PVT1 shows higher expression in more aggressive colorectal cancer cell line | [89]     |
|                          | KRTAP5-4, MAGEA3, BCAR4 | Serum | Potential biomarker | None                   | Serum exosomal KRTAP5-4, MAGEA3 and BCAR4 provided the greatest predictive ability for colorectal cancer. | [76]     |
| Prostate Cancer          | ELAVL1 and RBMX © | Cell | RNA binding protein binding | None                   | N/A                                                                        | [102]    |
| Cervical Cancer          | LncRNA MALAT1, HOTAIR, MEG3 © | Cervicovaginal lavage | Biomarker     | None                   | RT-PCR in identify different expression LncRNA in cervicovaginal lavage | [79]     |
|                          | H19 | Cell/ Serum | Biomarker | Tumorigenesis | H19 promotes cell proliferation and multicellular tumor spheroid formation | [82]     |
| Cancer type                     | LncRNA                          | Source     | Function               | Related genes | Mechanism                                                                 | Reference |
|--------------------------------|---------------------------------|------------|------------------------|---------------|---------------------------------------------------------------------------|-----------|
| Ovarian cancer                 | Lnc-MEG3                        | Cell       | Drug resistance        | MiR-214       | Enriched in curcumin treated cell/ mediated cisplatin resistance          | [97]      |
|                               | ENST00000444164, ENST00000437683 © | Cell       | NF-κB phosphorylation  | MiR146b-5b/TRA F6/ NF-κB/MMP2 | Activating the phosphorylation of NF-κB in HUVECs and further affecting tumorogenesis | [87]      |
| Colon Cancer                   | LncRNA AC007193.8, RUSC1-AS1, TM 4SF1-ASI, DILGAP1-ASI, SETD5-ASI, DNAJC27-ASI TTC28-ASI © | Cell | None                   | None          | Different lncRNAs enricher in extracellular vesicle subtypes             | [103]     |
| Glioma                         | Lnc-POU3F3 ©                    | Cell       | Endothelial cell angiogenesis | BFGF, VEGF, bFGF, and Angio | Lnc-POU3F3 enriched exosomes may induce HBMEC migration, proliferation, and tube formation | [88]      |
| Bladder Cancer                 | HOTAIR, HOX-AS-2, MALAT1, SOX2, OCT4, Lnc HYMA1, LINC00477, LOC100506688 and OTX2-ASI© | Urine      | Biomarker              | None          | Potentially serving as biomarkers for UBC diagnosis                       | [80]      |
|                               | Lnc-UCA1 ©                      | Cell       | Hypoxic resistance/biomarker | HIF-1α, p27, miR-143 | Hypoxic derived lnc-UCA1 enriched exosome can elevate tumorigenesis and induce cell EMT transformation | [90]      |
|                               | Lnc-UCA1 ©                      | Cell       | Drug resistance        | HIF-1α, p27, miR-143 | Lnc-UCA1 increases the tamoxifen resistance                               | [96]      |
| Laryngeal squamous cell cancer | HOTAIR ©                        | Serum      | Biomarker              | None          | Diagnose combing serum exosomal miR-21 and HOTAIR can have achieve good sensitivity and specificity | [78]      |
| Renal Cancer                   | LncARSR ©                       | Serum/Cell | Biomarker/ Drug resistance | HnRNA2B1, AKT/FOXO axis, miR-34a, miR-449 | LncARSR can be specifically packed into exosomes via hnrRNA2B1; LncARSR enriched exosomes can induce sunitinib sensitivity with resistance. | [60]      |
| Cholangiocarcinoma             | ENST00000588480.1, ENST0000057758.1 © | Bile       | Biomarker              | None          | N/A                                                                       | [71]      |

© refers to the articles which confirmed the usage of the term exosome. Other articles used the term EVs instead although the EVs studied in these articles have morphological features of exosomes.
adenoma [76, 77], laryngeal squamous cell carcinoma [78], non-small-cell lung cancer [69], and cholangiocarcinoma [71].

In addition to serum, exosomal lncRNAs extracted from other bodily fluids were also found to be plausible biomarkers. Exosomal lncRNA MALAT1, HOTAIR, and MEG3 are differentially expressed in cervical cancer cervicovaginal lavage samples, which suggests that these lncRNAs can be promising biomarkers in detecting cervical cancer [79]. In addition, several lncRNAs (HOTAIR, HOX-AS-2, MALAT1, SOX2, OCT4, HYMA1, LINCO00477, LOC100506688, and OTX2-ASI) are enriched in urine exosomes (UEs) from urothelial bladder cancer (UBC) patients [80].

Despite various reports of exosomal lncRNAs functioning as tumor biomarkers, several of these studies did not determine the sensitivity and specificity of the lncRNAs when applied to patients. In addition, many of the studies cannot define the direct relationships of the tested exosomal lncRNAs and cancers. Moreover, methodological differences in EV purification make this approach inadequate in achieving testing reproducibility.

5.2. Tumorigenesis. As mentioned earlier, the expression and function of lncRNAs are associated with various types of cancers [81]. Considering that the roles of lncRNAs in cancer are largely unexplored, research on exosomal lncRNAs is still in its infancy. Most studies investigate the roles of different lncRNAs in tumorigenesis, but they fail to demonstrate that the intercellular transfers of lncRNAs via exosomes play roles in tumorigenesis. For example, Lempride et al. [82] found that lncRNA-H19 enhances the proliferation and spheroid forming ability of cervical cancer cells and is enriched in cell-derived EVs. Similar experiments performed by Kogure et al. show that lncRNA-TUC339 is most highly expressed in hepatocellular carcinoma cells secreting EVs. Up- or downregulation of TUC339 can effectively influence HCC cell proliferation and metastasis [83]. However, these studies did not find direct evidence to demonstrate that exosomes/lncRNAs can directly affect tumorigenesis.

Lei et al. [75] found that lncRNA-ZFAS1 enriched in exosomes can endow recipient cells (low lncRNA-ZFAS1 expression) with increased proliferation and migration ability, which suggests that ZFAS1 can be delivered by exosomes to promote gastric cancer progression.

Dysregulation of angiogenesis occurs in various pathologies and is one of the hallmarks of cancer [84]. Some studies have illustrated that cancer cell-derived exosomes can affect HUVECs in tube formation, in which exosomal lncRNAs may play a pivotal role. CD90+ hepatic cell carcinoma (HCC) has been described with cancer stem-like (CSC) properties [85]. Conigliaro et al. [86] found that exosomes released by CD90+ cancer cells can affect HUVECs by promoting cell-cell adhesion and tube formation. These researchers further found that lncRNA-H19 is enriched in those exosomes. Another study performed by Wu et al. [87] first showed that exosomes isolated from tumor-associated macrophages (TAMs) can incorporate into HUVECs and block the miR146b-5b/TRAF6/NF-κB/MMP2 pathway, which results in efficient reduction of HUVEC migration. In addition, these researchers used SKOV3-derived exosomes and TAM-derived exosomes to costimulate HUVECs and found that inhibition of migration caused by TAM-derived exosomes is overcome. Two exosomal lncRNAs (ENST00000444164, ENST00000437683) were identified as NF-κB pathway-associated genes. A study conducted by Lang et al. [88] found that exosomes enriched in lncRNA-POU3F3 promote angiogenesis in gliomas. Moreover, exosomal lncRNA-POU3F3 has better function in inducing human brain microvascular endothelial cell (HBMEC) migration, proliferation, tube formation, and elevated angio-related gene expression. These results suggest that lncRNAs carried by exosomes can partly influence angiogenesis and further affect tumorigenesis.

5.3. Hypoxia Signaling and EMT. Hypoxia in cancer pathology is considered to be a significant element. Tumor cells frequently utilize hypoxia signaling to maintain the proliferative response in normoxia and escape growth arrest in hypoxia [89]. Takahashi et al. first revealed that lncRNA-ROR is a hypoxia-responsive lncRNA and can promote the survival of cancer cells under ischemic conditions. More importantly, these researchers found that lncRNA-ROR can modulate intercellular responses to hypoxia via the transfer of extracellular vesicles. In addition, hypoxia signaling often stimulates a cellular epithelial-mesenchymal transition (EMT) process, which is a critical regulator of metastasis. Several exosomal lncRNAs have been shown to affect EMT signaling in cancer cells. Xue et al. [90] found that UMUC2 has a positive effect on cell proliferation, migration, and invasion when incubated with hypoxic 5637 cell-derived exosomes. Moreover, compared to the normoxic cell-derived exosomes, lncRNA-UCA1 is enriched in hypoxic cell-derived exosomes. These hypoxia-derived lncRNA-UCA1-enriched exosomes can elevate tumorigenesis, both in vivo and in vitro, and induce cell EMT transformation. Transforming growth factor (TGF)-β can promote epithelial-mesenchymal transition (EMT) and further induce invasion and metastasis in pancreatic cancer [91].

5.4. Drug Resistance. LncRNAs can be transported by exosomes and endow the recipient cells with acquired drug resistance. Some studies have demonstrated that lncRNAs have potential functions in delivering drug resistance in recipient cells. TGF-1 has been shown to be involved in obtaining chemoresistance in various human cancers [92]. The groups of Takahashi found that lncRNA-ROR and lncRNA-VLDLR can be selectively enriched in EVs by TGFβ1-stimulated HCC [93]. HCC-derived exosomes can endow HepG2 cells with increased lncRNA-ROR expression and high chemoresistance. Additionally, these researchers found that lncRNA-ROR knockdown can reverse TGFβ-induced chemoresistance in cancer stem-cell-like CD133+ cells [94]. Another study performed by this team also revealed that lncRNA-VLDLR increases in cells and their EVs under chemotherapeutic stress [95]. These researchers found that lncRNA-VLDLR can be transferred by HCC cell-derived EVs and can promote chemoresistance in recipient cancer cells. Xu et al. [96] found that lncRNA-UCA1 shows
high expression in both tamoxifen-resistant LCC2 cells and their derived exosomes. LCC2-derived exosomes facilitate the breast cell line MCF-7 with an increased ability to resist tamoxifen. Moreover, knocking down UCA1 in exo/LCC reverses this phenomenon.

The above studies have proven that exosomal lncRNAs may function in drug resistance; however, they fail to reveal the underlying mechanism of acquired drug resistance related to exosomal lncRNAs. Other articles may better explain the roles of exosomal RNAs in drug resistance. Zhang et al. [97] demonstrated that curcumin-treated cell-derived EVs can reduce the ability of A2780cp cells to induce chemoresistance. LncRNA-MEG3 showed the greatest upregulation in exosomes after curcumin treatment. MEG3 overexpression after curcumin treatment can clearly inhibit miR-214 expression in cells and EVs. These researchers proved that MEG3 can strengthen EV-mediated transfer of miR-214, thereby downregulating drug resistance in recipient cells. These researchers found direct evidence proving that lncRNA-ARSR can be secreted from sunitinib-resistant cells to sensitive cells and induces sunitinib resistance. Intracellular lncRNA-ARSR elevation is directly due to exosome fusion, rather than an increase in intracellular synthesis. LncRNA-ARSR elevation caused by exosomal delivery functions as competing endogenous RNA for miR-16 and transferring miR-16 in mice [101]. Additionally, in the field of lncRNAs, intercellular transfer of lncRNA-ARSR expression being observed in tumors. A phase II trial has recently evaluated IFNγ-DC-derived exosomes loaded with MHC I/II confined cancer antigens as maintenance immunotherapy after chemotherapy in advanced patients without tumor progression, and exosomes may be used as anticancer vaccines in the future. However, the modulation of lncRNAs in vivo is not easy to achieve; therefore, there have been no lncRNA drugs brought into clinical trials to date.

**6. Conclusion**

In general, exosomes are secreted in almost all types of cells. Exosomes can selectively carry various elements and function as cell-to-cell carriers. LncRNAs secreted by exosomes also play an essential role in cancers. Liquid biopsy through exosomal lncRNAs provides a novel method for diagnosing cancer. Additionally, extracellular lncRNAs packed by exosomes help us evaluate the prognoses and therapeutic effects of the cancers. Moreover, exosomal lncRNAs have been determined to participate in inducing drug resistance in recipient cells, which provides a potential method of cancer therapy. Despite significant progress made in recent years, more work is needed to achieve a better understanding of exosomal lncRNAs in the function and regulation of tumorigenesis.

**7. Perspective**

LncRNAs have shown their utility in the diagnosis and prognosis of some cancers. Unlike commonly used cell-free DNAs (cfDNAs), which originate from dying cells, exosomal nucleic acids (exoNAs), which are derived from living cells, can better reflect the underlying cancer biology [98]. Recently, researchers have presented a novel EGFR T790M assay based on exosomal cfDNAs and RNAs/DNAs from plasma and achieved 92% sensitivity and 89% specificity [99]. However, the use of lncRNAs as biomarkers for cancer diagnosis and prognosis remains limited. First, different methods of isolation, mainly ultracentrifugation-based isolation and exosome precipitation techniques, were used in the aforementioned studies. The methodological differences in exosome isolation and lncRNA extraction make the experimental results difficult to compare. Second, only a small number of lncRNAs have already been investigated, and many of them have been functionally characterized. The construction of an extravascular lncRNA database has greater potential for the study of exosomes.

Moreover, as the natural transporter of functional small RNAs and proteins, exosomes have been suggested to have potential applications in the drug delivery field. It has been demonstrated that specific lncRNAs enriched in exosomes can change the phenotypes of neighboring cells [100]. Moreover, lncRNAs delivered by exosomes can induce drug resistance and angiogenesis in recipient cells. In the field of other exosomal RNAs, researchers have found that MSC-derived exosomes inhibit breast cancer growth by downregulating vascular endothelial growth factor (VEGF) and transferring miR-16 in mice [101]. Additionally, in the field of lncRNAs, intercellular transfer of lncRNA-ARSR through exosomes can significantly dampen the response of RCC xenografts to sunitinib, with increased lncRNA-ARSR expression being observed in tumors. A phase II trial has recently evaluated IFNγ-DC-derived exosomes loaded with MHC I/II confined cancer antigens as maintenance immunotherapy after chemotherapy in advanced patients without tumor progression, and exosomes may be used as anticancer vaccines in the future. However, the modulation of lncRNAs in vivo is not easy to achieve; therefore, there have been no lncRNA drugs brought into clinical trials to date.

**Conflicts of Interest**

The authors have no conflicts of interest.

**Authors’ Contributions**

Wenyuan Zhao and Yuanqi Liu contributed equally to this work and should be considered co-first authors.

**Acknowledgments**

This work was supported by the National Natural Science Foundation of China. (No. 81401901, No. 81372281, No. 81702278, No. 81372515). And we also thank Dr. Suiyu Chen for the helpful discussion.

**References**

[1] Y. Fu, C. Li, Y. Luo, L. Li, J. Liu, and R. Gui, “Silencing of long non-coding RNA MIAT sensitizes lung cancer cells to gefitinib by epigenetically regulating miR-34a,” *Frontiers in Pharmacology*, vol. 9, article 82, 2018.

[2] Y. Hu, Q. N. Zhu, J. L. Deng, Z. X. Li, G. Wang, and Y. S. Zhu, “Emerging role of long non-coding RNAs in cisplatin resistance,” *Onco Targets and Therapy*, vol. 11, pp. 3185–3194, 2018.

[3] T. Cai, Y. Liu, and J. Xiao, “Long noncoding RNA MALAT1 knockdown reverses chemoresistance to temozolomide via...
C. Hsu, Y. Morohashi, S.-I. Yoshimura et al., “Regulation of
H. Valadi, K. Ekström, A. Bossios, M. Sjöstrand, J. J. Lee,
G. VanNiel, G. D’Angelo, and G. Raposo, “Shedding light on the
L. A. Mulcahy, R. C. Pink, and D. R. Carter, “Routes and mech-
G. Raposo, D. Tenza, S. Mecheri, R. Peronet, C. Bonnerot,
N. Rocha, C. Kuijl, R. Van Der Kant et al., “ORP1L contacts the ER protein VAP to control Rab7-RILP-
A. Savina, M. Furlán, M. Vidal, and M. I. Colombo, “Exosome
A. Savina, C. M. Fader, M. T. Damiani, and M. I. Colombo, “Rab11 promotes docking and fusion of multivesicular bodies in a calcium-dependent manner,” Traffic, vol. 6, no. 2, pp. 131–143, 2005.
C. Hsu, Y. Morohashi, S.-I. Yoshimura et al., “Regulation of exosome secretion by Rab35 and its GTPase-activating proteins TBC1D10A-C,” The Journal of Cell Biology, vol. 199, no. 2, pp. 233–232, 2010.
N. Rocha, C. Kuijl, R. Van Der Kant et al., “Cholesterol sensor ORP1L contacts the ER protein VAP to control Rab7-RILP-p150 glued and late endosome positioning,” The Journal of Biological Chemistry, vol. 185, no. 7, pp. 1209–1225, 2009.
R. Jahn and R. H. Scheller, “SNAREs — engines for membrane fusion,” Nature Reviews Molecular Cell Biology, vol. 7, no. 9, pp. 631–643, 2006.
G. Raposo, D. Tenza, S. Mecheri, R. Peronet, C. Bonnerot, and C. Desaymard, “Accumulation of major histocompatibility complex class ii molecules in mast cell secretory granules and their release upon degranulation,” Molecular Biology of the Cell (MBoC), vol. 8, no. 12, pp. 2631–2645, 1997.
H. Valadi, K. Ekström, A. Bossios, M. Sjöstrand, J. J. Lee, and J. O. Löttvall, “Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells,” Nature Cell Biology, vol. 9, no. 6, pp. 654–659, 2007.
G. Van Niel, G. D’Angelo, and G. Raposo, “Shedding light on the cell biology of extracellular vesicles,” Nature Reviews Molecular Cell Biology, vol. 19, no. 4, pp. 213–228, 2018.
L. A. Mülchay, R. C. Pink, and D. R. Carter, “Routes and mechanisms of extracellular vesicle uptake,” Journal of Extracellular Vesicles (JEV), vol. 3, Article ID 24641, 2014.
I. Nazarenko, S. Rana, A. Baumann et al., “Cell surface tetraspanin Tspan8 contributes to molecular pathways of exosome-induced endothelial cell activation,” Cancer Research, vol. 70, no. 4, pp. 1668–1678, 2010.
A. Purushothaman, S. K. Bandari, J. Liu, J. A. Mobley, E. A. Brown, and R. D. Sanderson, “Fibronectin on the surface of myeloma cell-derived exosomes mediates exosome-cell interactions,” The Journal of Biological Chemistry, vol. 291, no. 4, pp. 1652–1663, 2016.
A. E. Morelli, A. T. Larregina, W. J. Shufesky et al., “Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells,” Blood, vol. 104, no. 10, pp. 3257–3266, 2004.
K. Laulagnier, C. Javalet, F. J. Hemming et al., “Amyloid precursor protein products concentrate in a subset of exosomes specifically endocytosed by neurons,” Cellular and Molecular Life Sciences, vol. 75, no. 4, pp. 757–773, 2018.
C. Escrevente, S. Keller, P. Altevogt, and J. Costa, “Interaction and uptake of exosomes by ovarian cancer cells,” BMC Cancer, vol. 11, article 108, 2011.
T. Tian, Y.-L. Zhu, Y.-Y. Zhou et al., “Exosome uptake through clathrin-mediated endocytosis and macropinocytosis and mediating miR-21 delivery,” The Journal of Biological Chemistry, vol. 289, no. 32, pp. 22258–22267, 2014.
T. Tian, Y. Wang, H. Wang, Z. Zhu, and Z. Xiao, “Visualizing of the cellular uptake and intracellular trafficking of exosomes by live-cell microscopy,” Journal of Cellular Biochemistry, vol. III, no. 2, pp. 488–496, 2010.
C. Bissig and J. Gruenberg, “ALIX and the multivesicular endosome: ALIX in wonderland,” Trends in Cell Biology, vol. 24, no. 1, pp. 19–25, 2014.
N. Kosaka, Y. Yoshioka, Y. Fujita, and T. Ochiya, “Versatile roles of extracellular vesicles in cancer,” The Journal of Clinical Investigation, vol. 126, no. 4, pp. 1163–1172, 2016.
L. Qu, J. Ding, C. Chen et al., “Exosome-transmitted IncARSR promotes sunitinib resistance in renal cancer by acting as a competing endogenous RNA,” Cancer Cell, vol. 29, no. 5, pp. 653–668, 2016.
T. L. Whiteside, “Exosomes and tumor-mediated immune suppression,” The Journal of Clinical Investigation, vol. 126, no. 4, pp. 1216–1223, 2016.
D. Koppers-Lalic, M. Hackenberg, I. V. Bijnsdorp et al., “Nontemplated nucleotide additions distinguish the small RNA composition in cells from exosomes,” Cell Reports, vol. 8, no. 6, pp. 1649–1658, 2014.
X. Huang, T. Yuan, M. Tschannen et al., “Characterization of human plasma-derived exosomal RNAs by deep sequencing,” BMC Genomics, vol. 14, no. 1 article 319, 2013.
Q. Li, Y. Shao, X. Zhang et al., “Plasma long non-coding RNA protected by exosomes as a potential stable biomarker for gastric cancer,” Tumor Biology, vol. 36, no. 3, pp. 2007–2012, 2015.
V. Paschon, S. H. Takada, J. M. Ikebara et al., “Interplay between exosomes, microRNAs and toll-like receptors in brain disorders,” Molecular Neurobiology, vol. 53, no. 3, pp. 2016–2028, 2016.
D. Xitong and Z. Xiaorong, “Targeted therapeutic delivery using engineered exosomes and its applications in cardiovascular diseases,” Gene, vol. 575, no. 2 Pt 2, pp. 377–384, 2016.
U. Gezer, E. Özgür, M. Celek, M. Isin, and N. Dalay, “Long non-coding RNAs with low expression levels in cells are enriched in secreted exosomes,” Cell Biology International, vol. 38, no. 9, pp. 1076–1079, 2014.
J. Beermann, M.-T. Piccoli, J. Viereck, and T. Thum, “Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches,” Physiological Reviews, vol. 96, no. 4, pp. 1297–1325, 2016.
R. Zhang, Y. Xia, Z. Wang et al., “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,” Biochemical and Biophysical Research Communications, vol. 490, no. 2, pp. 406–414, 2017.
[70] C. Villarroja-Beltri, C. Gutiérrez-Vázquez, E. Sánchez-Cabo et al., “Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs,” *Nature Communications*, vol. 4, article 2980, 2013.

[71] X. Ge, Y. Wang, J. Nie et al., “The diagnostic/prognostic potential and molecular functions of long non-coding RNAs in the exosomes derived from the bile of human cholangiocarcinoma,” *Oncotarget*, vol. 8, no. 41, pp. 69995–70005, 2017.

[72] O. A. Kossinova, A. V. Gopanenko, S. N. Tamkovich et al., “Cytosolic YB-1 and NSUN2 are the only proteins recognizing specific motifs present in mRNAs enriched in exosomes,” *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, vol. 1865, no. 6, pp. 664–673, 2017.

[73] A. L. S. Revenfeld, R. Bæk, M. H. Nielsen, A. Stensballe, K. Varming, and M. Jørgensen, “Diagnostic and prognostic potential of extracellular vesicles in peripheral blood,” *Clinical Therapeutics*, vol. 36, no. 6, pp. 830–846, 2014.

[74] J. Skog, T. Würdinger, S. van Rijn et al., “Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers,” *Nature Cell Biology*, vol. 10, no. 12, pp. 1470–1476, 2008.

[75] L. Pan, W. Liang, M. Fu et al., “Exosomes-mediated transfer of long noncoding RNA ZFAS1 promotes gastric cancer progression,” *Journal of Cancer Research and Clinical Oncology*, vol. 143, no. 6, pp. 991–1004, 2017.

[76] L. Dong, W. Lin, P. Qi et al., “Circulating long RNAs in serum extracellular vesicles: their characterization and potential application as biomarkers for diagnosis of colorectal cancer,” *Cancer Epidemiology Biomarkers & Prevention*, vol. 25, no. 7, pp. 1158–1166, 2016.

[77] T. Liu, X. Zhang, S. Gao et al., “Exosomal long non-coding RNA CRNDE-h as a novel serum-based biomarker for diagnosis and prognosis of colorectal cancer,” *Oncotarget*, vol. 7, no. 51, pp. 85551–85563, 2016.

[78] J. Wang, Y. Zhou, J. Lu et al., “Combined detection of serum exosomal miR-21 and HOTAIR as diagnostic and prognostic biomarkers for laryngeal squamous cell carcinoma,” *Medical Oncology*, vol. 31, no. 9, article 148, 2014.

[79] J. Zhang, S.-C. Liu, X.-H. Luo et al., “Exosomal long noncoding RNAs are differentially expressed in the cervicovaginal lavage samples of cervical cancer patients,” *Journal of Clinical Laboratory Analysis*, vol. 30, no. 6, pp. 1116–1121, 2016.

[80] C. Berrondo, J. Flax, V. Kuchevor et al., “Expression of the long non-coding RNA HOTAIR correlates with disease progression in bladder cancer and is contained in bladder cancer patient urinary exosomes,” *PLoS ONE*, vol. 11, no. 1, Article ID e0147236, 2016.

[81] T. Gutschner and S. Diederichs, “The hallmarks of cancer: a long non-coding RNA point of view,” *RNA Biology*, vol. 9, no. 6, pp. 703–709, 2012.

[82] T. Lempride, “Long non-coding RNA H19 enhances cell proliferation and anchorage-independent growth of cervical cancer cell lines,” *Experimental Biology and Medicine*, vol. 242, no. 2, pp. 184–193, 2017.

[83] T. Kogure, I. K. Yan, W.-L. Lin, and T. Patel, “Extracellular vesicle-mediated transfer of a novel long noncoding RNA TUC339: a mechanism of intercellular signaling in human hepatocellular cancer,” *Genes & Cancer*, vol. 4, no. 7-8, pp. 261–272, 2013.

[84] S. Goel, D. G. Duda, L. Xu et al., “Normalization of the vasculature for treatment of cancer and other diseases,” *Physiological Reviews*, vol. 91, no. 3, pp. 1071–1121, 2011.

[85] M. B. Herrera, S. Bruno, S. Buttiglieri et al., “Isolation and characterization of a stem cell population from adult human liver,” *Stem Cells*, vol. 24, no. 12, pp. 2840–2850, 2006.

[86] A. Coniglioaro, V. Costa, A. Lo Dico et al., “CD90+ liver cancer cells modulate endothelial cell phenotype through the release of exosomes containing H19 IncRNA,” *Molecular Cancer*, vol. 14, no. 1, article no. 155, 2015.

[87] Q. Wu, X. Wu, X. Ying et al., “Suppression of endothelial cell migration by tumor associated macrophage-derived exosomes is reversed by epithelial ovarian cancer exosomal IncRNA,” *Cancer Cell International*, vol. 17, no. 1, article no. 62, 2017.

[88] H. L. Lang, G. W. Hu, Y. Chen et al., “Glioma cells promote angiogenesis through the release of exosomes containing long non-coding RNA POU3F3,” *European Review for Medical and Pharmacological Sciences*, vol. 21, no. 5, pp. 959–972, 2017.

[89] K. Guo, J. Yao, Q. Yu et al., “The expression pattern of long non-coding RNA PVT1 in tumor tissues and in extracellular vesicles of colorectal cancer correlates with cancer progression,” *Tumor Biology*, vol. 39, no. 4, Article ID 1393390542, 2017.

[90] M. Xue, W. Chen, A. Xiang et al., “Hypoxic exosomes facilitate bladder tumor growth and development through transferring long non-coding RNA-UCA1,” *Molecular Cancer*, vol. 16, no. 1, article no. 143, 2017.

[91] C. J. David, Y.-H. Huang, M. Chen et al., “TGF-β tumor suppression through a lethal EMT,” *Cell*, vol. 164, no. 5, pp. 1015–1030, 2016.

[92] D. Padua and J. Massagué, “Roles of TGFbeta in metastasis,” *Cell Research*, vol. 19, no. 1, pp. 89–102, 2009.

[93] K. Takahashi, I. K. Yan, T. Kogure, H. Haga, and T. Patel, “Extracellular vesicle-mediated transfer of long non-coding RNA ROR modulates chemosensitivity in human hepatocellular cancer,” *FEBS Open Bio*, vol. 4, no. 1, pp. 458–467, 2014.

[94] J. W. Jang, Y. Song, S. H. Kim et al., “CDI33 confers cancer stem-like cell properties by stabilizing EGFR-AKT signaling in hepatocellular carcinoma,” *Cancer Letters*, vol. 389, pp. 1–10, 2017.

[95] K. Takahashi, I. K. Yan, J. Wood, H. Haga, and T. Patel, “Involvement of extracellular vesicle long noncoding RNA (linc-VLDR) in tumor cell responses to chemotherapy,” *Molecular Cancer Research*, vol. 12, no. 10, pp. 1377–1387, 2014.

[96] C. G. Xu, M. F. Yang, Y. Q. Ren, C. H. Wu, and L. Q. Wang, “Exosomes mediated transfer of lncRNA UCA1 results in increased tamoxifen resistance in breast cancer cells,” *European Review for Medical and Pharmacological Sciences*, vol. 20, no. 20, pp. 4362–4368, 2016.

[97] J. Zhang, J. Liu, X. Xu, and L. Li, “Curcumin suppresses cisplatin resistance development partly by modulating extracellular vesicle-mediated transfer of MEG3 and miR-214 in ovarian cancer,” *Cancer Chemistry and Pharmacology*, vol. 79, no. 3, pp. 479–487, 2017.

[98] L. Möhrmann, H. J. Huang, D. S. Hong et al., “Liquid biopsies using plasma exosomal nucleic acids and plasma cell-free DNA compared with clinical outcomes of patients with advanced cancers,” *Clinical Cancer Research*, vol. 24, no. 1, pp. 181–188, 2018.

[99] E. Castellanos-Rizaldos, D. G. Grimm, V. Tadigotla et al., “Exosome-based detection of EGFR T790M in plasma from non-small cell lung cancer patients,” *Clinical Cancer Research*, vol. 24, no. 12, pp. 2944–2950, 2018.

[100] A. Conigliaro, S. Fontana, S. Raimondo, and R. Alessandro, “Exosomes: nanocarriers of biological messages,” *Advances in Experimental Medicine and Biology*, vol. 998, pp. 23–43, 2017.
[101] J.-K. Lee, S.-R. Park, B.-K. Jung et al., “Exosomes derived from mesenchymal stem cells suppress angiogenesis by down-regulating VEGF expression in breast cancer cells,” PLoS ONE, vol. 8, no. 12, Article ID e84256, 2013.

[102] A. Ahadi, S. Brennan, P. J. Kennedy, G. Hutzagner, and N. Tran, “Long non-coding RNAs harboring miRNA seed regions are enriched in prostate cancer exosomes,” Scientific Reports, vol. 6, Article ID 24922, 2016.

[103] B. J. Tauro, D. W. Greening, R. A. Mathias, S. Mathivanan, H. Ji, and R. J. Simpson, “Two distinct populations of exosomes are released from LIM1863 colon carcinoma cell-derived organoids,” Molecular & Cellular Proteomics, vol. 12, no. 3, pp. 587–598, 2013.