Exosomal cargoes in OSCC: Current findings and potential functions

Chengzhi Zhao [Equal first author, 1], **Geru Zhang** [Equal first author, 1], **Jialing Liu** [2], **Chenghao Zhang** [2], **Wen Liao** [Corresp. 2]

[1] State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases, West China School of Stomatology, Sichuan University, Chengdu, China

[2] State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases, Department of Orthodontics, West China Hospital of Stomatology,Sichuan University, Chengdu, Sichuan, China

Corresponding Author: Wen Liao
Email address: liaowenssw@126.com

Oral squamous cell carcinoma (OSCC) is the most prevalent malignancy in head and neck cancer, with high recurrence and mortality. Early diagnosis and efficient therapeutic strategies are vital for the treatment of OSCC patients. Exosomes can be isolated from a broad range of different cell types, implicating them as important factors in the regulation of human physiological and pathological processes. Due to their abundant cargo including proteins, lipids, and nucleic acids, exosomes have played a valuable diagnostic and therapeutic role across multiple diseases, including cancer. In this review, we summarize recent findings concerning the content within, and participation of, exosomes relating to OSCC and their roles in tumorigenesis, proliferation, migration, invasion, metastasis, and chemoresistance. We conclude this review by looking ahead to their potential utility in providing new methods for treating OSCC to inspire further research in this field.

Exosomal cargoes in OSCC: current findings and potential functions.

Chengzhi Zhao[1#], Geru Zhang[1#], Jialing Liu[2], Chenghao Zhang[2], Wen Liao[3*]
# These two authors contribute equally to the manuscript.
[1] State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases, West China School of Stomatology, Sichuan University, Chengdu, Sichuan, China
[2] State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases, Department of Orthodontics, West China School of Stomatology, Sichuan University, Chengdu, Sichuan, China
[3] State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases, Department of Orthodontics, West China Hospital of Stomatology, Sichuan University, Chengdu, Sichuan, China

Corresponding Author:
Wen Liao[3]
No.14, 3Rd Section of Ren Min Nan Rd. Chengdu, Sichuan 610041,China
Email address: liaowenssw@126.com

Abstract

Oral squamous cell carcinoma (OSCC) is the most prevalent malignancy in head and neck cancer, with high recurrence and mortality. Early diagnosis and efficient therapeutic strategies are vital for the treatment of OSCC patients. Exosomes can be isolated from a broad range of different cell types, implicating them as important factors in the regulation of human physiological and pathological processes. Due to their abundant cargo including proteins, lipids, and nucleic acids, exosomes have played a valuable diagnostic and therapeutic role across multiple diseases, including cancer. In this review, we summarize recent findings concerning the content within, and participation of, exosomes relating to OSCC and their roles in tumorigenesis, proliferation, migration, invasion, metastasis, and chemoresistance. We conclude this review by looking ahead to their potential utility in providing new methods for treating OSCC to inspire further research in this field.

Introduction

Extracellular vesicles (EVs) are membrane-bound organelles actively secreted by cells[1, 2]. First recognized as cell dust, EVs were considered to be part of a mechanism for disposal of needless cellular components[3]. With the recent discovery that EVs contain an abundant amount of important cargo (such as proteins, lipids, and nucleic acids), they are no longer considered carriers of cellular waste. Additionally, since the finding that cargo-laden EVs can be released by

nearly all cell types and detected in many body fluids (e.g., breast milk, saliva, urine, and cerebrospinal fluid), they have gradually been recognized as a mechanism for intercellular communication[2, 4]. EVs are classified into different groups, namely microvesicles (MVs), exosomes, and apoptotic bodies, based on their morphological features and contents (Figure 1)[5]. MVs, also known as ectosomes[6], generally range from 50–1,000 nm in diameter and are generated by the outward budding of the plasma membrane[4]. Apoptotic bodies, ranging from 500–1000 nm in diameter, peel off from dying cells[7], while exosomes have a diameter of <150 nm[2]. Exosomes originate from intraluminal vesicles (ILVs) secreted by multivesicular bodies (MVBs) through fusing with the plasma membrane[4]. To the best of our knowledge, EVs have been defined based on their size; however, recent consensus has recognized vesicular biogenesis and cargo as defining characteristics of the vesicles, which may ultimately be more precise criteria for classification[2, 8, 9].

As previously mentioned, exosomes were first considered waste compartments for cells[10]. We now know that exosomes play critical roles in intercellular communication. The various types of exosomal cargo, including proteins, lipids, and nucleic acids within and on the exosome surface[11], vary depending on their parental cells of origin and extracellular environment. Exosomes secreted by the same cell type can differ based on the types of stimuli that the cells are experiencing, which implies that exosomes can be indicative of different cellular states[7, 12]. Exosomes have been shown to play significant roles in regulating physiological and pathological processes, in addition to having great potential in therapeutic development[7, 11-13].

Exosome function has been unveiled in many diseases, including cancer[14-18]. Exosome participation in numerous phases of the cancer process has been observed, including *in situ* tumorigenesis[19], tumor growth[20, 21], angiogenesis[22], evasion of immune system[23, 24], resistance to chemotherapeutic agents[11], and metastasis[11, 24, 25]. In addition, antitumor effects have also been observed[26, 27].

OSCC, usually preceded by white or red mucosal changes known as leukoplakia or erythroplakia, respectively, or sometimes a combination of red and white features, is the most prevalent malignancy of the head and neck[28] characterized by high recurrence and poor prognosis. There are approximately 350,000–400,000 new cases each year with high risk of recurrence (20% to 30%)[29]. Some risk factors contribute to the occurrence of OSCC, such as tobacco consumption, alcohol consumption, HPV infection, and other systemic or environmental factors[28].

Recently, a close association between OSCC and exosome biology has been reported. Exosomes act as transporters between cells and their microenvironment[30, 31], directly promoting the initiation and progression of OSCC. Exosomes can also modulate OSCC by regulating the immune system, causing metabolic dysfunction and chemoresistance[32]. They have also been developed for applications in the clinic, including as biomarkers for early diagnosis and drug delivery. However, there is limited research focused on exosomes in OSCC. Therefore, we wrote this review by looking ahead to their potential utility in providing new methods for treating OSCC and intend to inspire further research on exosomes in accordance with OSCC in the field.

Survey methodology

We systematically searched with PubMed Advanced Search Builder with the following keywords: (1) OSCC and exosomes, (2) HNSCC and exosomes, (3) proteins in exosomes and OSCC, (4) lipid and exosomes and OSCC, (5) nucleic acids and exosomes in OSCC, (6) non-coding RNA and exosomes in OSCC, (7) mitochondrial DNA and exosomes in OSCC, (8) exosomes and clinic use and OSCC. By reading the titles and abstracts, papers nonrelated with either exosomal cargoes or OSCC or HNSCC were excluded. Besides, papers not published in English were excluded. Our search was not refined by publishing date, journal or impact factor of the journal, authors or authors affiliations.

1. Exosome biogenesis

The biogenesis and release of exosomes to the extracellular environment is an ordered process. The first step for exosome biogenesis is the formation of early endosomes (EEs). By inward budding or endocytosis, primary endocytic vesicles and their contents fuse with each other to form EEs[7, 33]. After the process of primary endocytic vesicles delivering their contents and membranes to EEs in the peripheral cytoplasm over 8–15 minutes, EEs will then go to their destination[33]. One possible destination for EEs is recycling to the plasma membrane, directly or with the help of recycling endosomes[33, 34]. The other possibility is their conversion to late endosomes (LEs) for additional processing. LEs accumulate ILVs formed by the inward budding of the endosomal membrane[8], which results in the conversion of LEs to MVBs[7, 8]. ILV formation is exquisitely regulated by mechanisms that remain to be fully elucidated, but it is reported to mainly rely on endosomal-sorting complex required for transport (ESCRT)-dependent machinery. Multiprotein ESCRT complexes are composed of ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III. These localize on the cytoplasmic side of the endosomal membrane and play unique roles in sorting proteins into ILVs with assistance of accessory proteins (ALIX, VPS4, and VTA1)[35]. Although the ESCRT-dependent pathway is considered the main mechanism regulating ILV biogenesis, some evidence has revealed that there are alternative, independent pathways[11, 35, 36]. The type of cargo that is sorted into ILVs may relate to the mechanisms involved. With MVBs, there are two notable fates: to either degrade by fusing with lysosomes, or having their ILVs released as exosomes by fusion with the plasma membrane[37, 38]. A variety of mechanisms involved in the release of exosomes have been proposed. Rab GTPases, including RAB11, RAB35, RAB27A, RAB27B, and RAB7, with assistance from ALIX and syntenin, are recognized to play an important role[39, 40]. In addition, some mechanisms independent of Rab GTPases may also play a significant role in this process[41]. For instance, the soluble N-ethyl maleimide (NEM)-sensitive factor attachment protein receptor (SNARE) complex drives membrane fusion and thus exosome secretion[11].

Exosome biogenesis starting from the formation of EEs to the final release into extracellular environment is a complex process (Figure 2). To our knowledge, it can differ between cell types

and their cellular physiological or pathological status[7, 12], but much remains to be discovered to fully exploit the potential for exosomes as future clinical therapies[11].

2. Exosome capsuled protein in OSCC

It is known that exosomes encapsulate genetic material as well as proteins from their cells of origin. Traditionally, the proteins that are encapsulated or transported by exosomes include structural and functional proteins that maintain the basic structure of exosomes and are responsible for regulating fusion, migration, and adhesion to target cells, such as transmembrane protein families (CD9, CD63, CD81 and CD82), molecular chaperones (Hsp70, Hsp90), multi-capsule synthesis proteins (TSG101 and ALIX), membrane carried fusion proteins, and others[42-45]. Some exosomes can carry specific proteins that allow their identification and differentiation from other exosomes, which could help to determine their unique function and potentially act as biomarkers for detecting disease progression[46-48].

Numerous studies have already revealed that epidermal growth factor receptor (EGFR) plays an important role in tumorigenesis and drug resistance[49-51]. In OSCC, overexpression of EGFR activates many signal pathways, such as RAS/MEK/ERK, PI3K/AKT, and JAK/STAT pathways[52-55]. Fujiwara et al. found that OSCC can secrete exosomes with encapsulated EGFR. Healthy epithelial cells internalized these EGFR-containing exosomes, resulting in the epithelial-mesenchymal transition (EMT) of OSCC[56]. Principe et al. reported that cancer-associated-fibroblasts (CAF) participate in the tumorigenesis of oral tongue squamous cell carcinoma (OTSCC). They also demonstrated that MFAP5 was abundant in exosomes originating from CAF, and would activate OTSCC cell growth and migration via activation of MAPK and AKT pathways[57]. Additionally, proteins loaded into exosomes can regulate stromal cell metabolism to support cancer cell proliferation. Jiang et al. reported that exosomes derived from tumor cells could transport p-ERK1/2 to stromal fibroblasts, resulting in the degradation of CAV1 and glucometabolic reprogramming. The energy produced by this reaction could be absorbed and utilized by surrounding tumor cells[58].

As previously reported, the interaction between tumor cells and the immune system can play a dual role. On one hand, immune cells can recognize and eliminate tumor cells in the early stages of tumor development[59-61]. In contrast, excessive infiltration of immune cells is related with poor prognosis[62, 63]. Exosomes can be viewed as a bridge between tumor and immune cells. The Fas/FasL pathway regulates proinflammatory processes and promotes the apoptosis of immune cells in vivo. Kim et al. revealed that FasL+ exosomes in OSCC patient serum induced apoptosis in Jurkat and T cells[64]. Wang et al. demonstrated that exosomes derived from OSCC were rich with NF-κB-activating kinase-associated protein 1 and could strengthen the proliferation and cytotoxicity of natural killer (NK) cells through the interferon regulatory factor 3 pathway[65]. Chen and colleagues found that thrombospondin 1 (THBS1) was transported by OSCC-derived exosomes and could stimulate macrophage transformation into M1-like tumor associated macrophages (TAMs), which were capable of controlling OSCC cell migration[66]. Moreover, exosomes can also facilitate communication carrier between tumor cells and healthy cells. Recent research indicates that transforming growth factor-beta (TGF-β) is important for

tumorigenesis and immunosuppression in the tumor microenvironment[67]. South and colleagues isolated TGF-βII+ exosomes from the OSCC microenvironment and claimed these exosomes could stimulate TGF-β signaling between tumor cells and their surrounding microenvironment, but the mechanism remains unknown[68]. They also showed that OSCC-derived exosomes were loaded with the C-terminal fragment of desmoglein 2, a highly expressed protein in many kinds of malignant tumors. DSG2 expression in OSCC could promote exosome secretion through the modification of metalloproteases and repartitioning of Cav1[69]. Moreover, these DSG2-CTF-positive exosomes could modulate the microenvironment by converting nearby fibroblasts into TAMs[69].

Currently, patients can receive various kinds of antitumor therapies like radiotherapy, chemotherapy, and surgery. However, metastasis is the major cause of death in most cases. The pathological generation of blood and lymphatic vessels is closely associated with tumor development and progression. These tubular tissues provide nutrients to tumor cells and allow them to spread to distal organs far from the tumor origin. Vascular endothelial growth factor C (VEGF-C) is universally acknowledged as one of the most effective proteins in promoting lymphatic vessels. It is abundant in lymph node positive metastatic OSCC patients. Zhong et al. discovered that increased VEGF-C expression associated with an increased number of salivary exosomes in OSCC tissues[70]. Kozaki and colleagues discovered an increased invasive capacity when HSP90-rich exosomes were abundant; moreover, the knockdown of HSP90α and HSP90β decreased the metastatic capacity and survivability of OSCC cells[71]. Observing that OSCC LN1-1 cells were more aggressive in lymphatic node metastasis than OEC-M1 cells, Wang et al. used stable isotope amino acid labeling to reveal that higher laminin-332 protein levels in tumor cell-derived exosomes was a major cause for the superior lymphangioge
nesis ability of OSCC LN1-1 cells, as laminin-332 promotes lymphatic endothelial cell migration and tube formation[72]. Li et al. reported that PF4V1, CXCL7, F13A1, and ApoA1 could affect OSCC lymph node metastasis[73].

EMT is also an important biological process in which malignant tumor cells derived from epithelial cells acquire migration and invasion capabilities. Dayan et al. showed that caveolin-1 was transported by OSCC-derived exosomes and occurs in tongue squamous cell carcinoma[74]. Additional studies revealed that CAV1 is an important EMT regulator and could drive the transition of fibroblasts into cancer-associated fibroblasts[75-77].

3. Lipid cargo in OSCC exosomes

Exosomes are an alternative means to carrier proteins and lipoproteins for transporting lipids[78]. There are approximately 2000 lipid species identified through comparative lipidomic analyses[79], which can localize to the membrane and lumen of exosomes. They play critical roles in exosomes biogenesis and release[78, 80]. The roles for several kinds of lipids have been reported, including BMP (Bismonoacylglycerophosphate), cholesterols, ceramides, and phosphatidic acid[78]. BMP is recognized as a lipidic molecule required for MVB formation[81] and ILV biogenesis[82], however, it is irrelevant to the formations of MVs, which indicates its potential as a biomarker to distinguish exosomes from MVs[80]. The lipid contents of exosomes

are usually different from their parental cells. The discrimination of lipids between exosomes and parental cells could play significant roles in many pathophysiologies such as cancer. Llorente et al. quantified 280 species of lipids from PC-3 prostate cancer cells and their exosomes and found some differences in their lipid composition[83].

Over the past few years, lipid metabolic abnormalities have been identified as a feature of tumor cells. Inhibiting key metabolic pathways of lipids may therefore be a promising therapeutic strategy. Using bitter melon extract to treat the head and neck squamous carcinoma cell (HNSCC) line Cal27, Sur et al. observed a significant reduction to fatty acid biogenesis-related genes at the level of both mRNA and protein[84]. Hu et al. found that some lipid metabolism-related genes in OSCC patients could be used for prognostication[85]. Lipid uptake can also be enhanced in cancer[86]. Pascual et al. observed a similar phenomenon in OSCC, as they documented a subpopulation of CD44bright OSCC cells that express high levels of lipid metabolic genes and can initiate metastasis.

Arachidonic acid (AA) is a free fatty acid that can be transported by exosomes[87], and its metabolism is a major dysregulated pathway in cancer cells[88]. As the precursor of both leukotrienes and prostaglandins, AA can be transferred between tissues by exosomes contributing to tumor growth and progression[89]. Exosomes secreted from AsPC-1 cells, a highly metastatic pancreatic ductal adenocarcinoma cell line, were reported to deliver AA to macrophages. The fusogenicity of AsPC-1 exosomes decreased when pretreatment with PLA2 caused the removal of AA, indicating that exosomal AA may enhance crosstalk between cancer cells and TAMs, thus contributing to tumor progression[89].

Leukotrienes (LTs), a product of AA, are involved in various pathophysiologies such as inflammatory asthma, atherosclerosis, and cancer[78]. Several types of LTs, such as LTB4, LTC4, cysteinyl leukotriene LTC4, and LTC4 synthase, are enriched in exosomes[90, 91]. Exosomes appear to play a role in the biogenesis of LTs. For example, LTA4, the precursor of leukotrienes, only has a five-second half-life in in vitro buffer, but with the protection of exosomes, their half-life can be elongated to several minutes[90]. Lukic et al. found that exogenous LTC4 generated by monocytic cells can be transformed into pro-tumorigenic LTD4 through gamma-glutamyl transpeptidase 1. GGT-1 is contained within both exosomes and primary cancer cells, which stimulates cancer cell migration and survival[90]. Since the 5-lipoxygenase (5-Lox) and cyclooxygenase (COX)-2 pathways of arachidonic acid metabolism are involved in oral carcinogenesis[92], LTs in exosomes may also play an important role in OSCC.

Prostaglandins (PG) are another product of AA. Exosomes with high concentrations of prostaglandins transport more PGE2 to neighboring cells, enhancing the overall presence of PGE2 in the microenvironment[87]. Cell motility and metastatic status are impacted by extracellular levels of PGE2[93]. Evidence has shown that exosomes rich in PGE2 participate in tumor immune evasion and promote tumor growth[78]. PGE2-mediated inflammation contributing to OSCC at different stages of carcinogenesis, invasion and metastasis contributes to patient morbidity and mortality[94]. Abrahao et al. showed that HNSCC cells secrete PGE2.

The overexpression of COX-2 in tumor and inflammatory cells, and subsequent increased production of PGE2, may promote HNSCC growth in an autocrine and paracrine way in the microenvironment. Exosomes can therefore be considered a potential medium for autocrine and paracrine regulation[95].

4. Nucleic acids in OSCC exosomes

A diverse collection of nucleic acids, including DNA, coding mRNA, non-coding RNA (ncRNA), micro RNA (miRNA), circular RNA (circRNA), and long non-coding RNA (lncRNA) have been identified in exosomes[8]. The nucleic acid content of exosomes is believed to play a significant role in promoting cancer pathogenesis through the oncogenic transformation and transfer of cancer-specific genetic material[12]. Understanding how nucleic acids transported by exosomes mediate the process of OSCC is critical, and could illuminate strategies for exosome-based targeted therapy.

4.1 DNA

DNA in exosomes has been observed in cell culture supernatant as well as human and mouse biological fluids such as blood, seminal fluid, and urine[12]. Based on cell origin, it is likely that different types of exosomes contain distinct types of DNA, such as single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), mitochondrial DNA (mtDNA), and of varying states (e.g., fragment length, chromosome-bound)[12]. Among these various types, dsDNA is the most evaluated[96]. Cancer cell-derived exosomes contain more DNA, which may be indicative toward their potential functions in tumorigenesis. Exosomal DNA has been found to be involved in immunity regulation[97, 98]. When tumors were treated by different strategies (e.g., antitumor drugs or radiotherapy), the induced secretion of cancer cell-derived exosomes containing DNA triggered dendritic cell (DC) activation and cytokine production, both of which can have antitumor effects by regulating immune responses[97, 98]. The unique characteristics of DNA in exosomes may be used as biomarkers for tumor diagnosis. For example, the same mutations in susceptibility genes were found in exosomal DNA and parental cells of pheochromocytomas and paragangliomas[99], and mutant KRAS, TP53, NOTCH1, and BRCA2 DNA in exosomes from pancreatic cancer were also detected[100, 101]. Exosomes in OSCC may play similar roles, as mutant genes were also discovered in OSCC cells[102, 103]. Because of the inherent stability of DNA within exosomes, it has become an attractive candidate biomarker[12].

Viral DNA has also been detected in exosomes from cancer patients[104]. Meckes et al. identified that ERK and PI3K/AKT signaling pathways can be activated if the recipient cells were exposed to exosomes containing major EBV oncogene LMP1[105]. Human papillomavirus (HPV) is considered a risk factor for OSCC, and its DNA has been found in exosomes from HeLa cells[106]. Exosomes containing HPV are also involved in HPV-associated carcinogenesis, indicating a potential role in OSCC although there is no direct evidence noted[107]. HPV DNA in plasma-derived exosomes was detected in rectal squamous cell carcinoma patients[108]. Ambrosio et al. isolated exosomes from the HPV DNA-positive cell line CaSki, which can transfer DNA to normal cell lines. Moreover, circulating exosome-encapsulated HPV DNA in the blood of neoplastic patients was verified to be transferred to normal and tumor cells at least

in vitro. It is noteworthy that this dynamic process led by exosomes might be involved at other anatomical sites[108].

Mitochondria are major energy generators in cells, and increasing studies have revealed their role in tumor development. Previously, scientists discovered that mitochondria could pass between cells through vesicles[109]. Subsequent studies showed that mitochondria and mitochondrial DNA could spread through tumors and surrounding non-tumor tissue to repair mitochondrial damage caused by a highly metabolic environment in cancers such as melanoma and breast cancer[110-113]. Currently, scientists are deepening our understanding of the connections between mitochondria and tumors. For example, cancer cells can obtain mitochondria from healthy cells to achieve chemical resistance[114], and mitochondrial DAMP provides tumor cells with a possible immune escape mechanism[115, 116]. The transfer of mitochondria or mtDNA is not completed independently, but is carried out by the transporting mediators between cells, such as exosomes and secretory vesicles. In oral cancer, whether exosomal mtDNA participates in tumorigenesis, tumor proliferation, and migration is still vague. However, Uzawa et al. proposed a novel method for detecting mtDNA in OSCC patients and reported significant differences in serum mtDNA levels before and after OSCC patient treatment. They also identified mtDNA as a promising molecular marker for OSCC prognostication[117]. Despite these advances, further exploration into exosome-derived mtDNA and its application in the diagnosis and treatment of OSCC is necessary.

4.2 mRNA

Coding mRNA has been found in exosomes. Transferring mRNA within exosomes can enable mRNA translation into proteins in recipient cells[118, 119], giving mRNA a potentially powerful role in cell-to-cell communication. Its function has been studied in both healthy and pathological states[119, 120]. As a means for cell-to-cell transportation, the mRNA profiles of tumor-derived exosomes have been evaluated in various cancers such as melanoma[121], glioblastoma[122], prostate cancer[123], and colorectal cancer[124]. Moreover, these studies revealed that mRNAs in tumor-derived exosomes can play vital roles in promoting malignant tumor growth, proliferation, and metastasis through suppressing immune responses and resisting antitumor treatment [121, 122, 125].

Salivary liquid biopsy has emerged as an excellent method for disease detection, which is also applied to OSCC[126]. Evaluation of the salivary transcriptome from OSCC patients has helped to identify some mRNA biomarkers[127]. Qadir et al. characterized and compared the transcriptome profiles between exosomes isolated from primary human normal oral keratinocytes (HNOK) and HNSCC cell lines. The results showed that in HNSCC-derived exosomes, the expression of matrix remodeling (EFEMP1, DDK3, SPARC), cell cycle (EEF2K), membrane remodeling (LAMP2, SRPX), differentiation (SPRR2E), apoptosis (CTSC), and transcription/translation (KLF6, PUS7) factors showed significant differences from healthy cell-derived exosomes[128], indicating that cancer cells may confer transcriptome reprogramming through exosomes to enhance cancer-associated pathologies.

4.3 ncRNA

Most of the human genome is considered biologically active. However, only a minor fraction of DNA encodes proteins. ncRNA represents the majority of RNA that is not translated into proteins[129]. ncRNA is a category of exosomal cargo under investigation for its complex role in regulating gene expression[130]. ncRNA interactions are often interconnected which, when deregulated, could eventually drive tumorigenesis and progression[131]. Identifying ncRNAs and their interactions will help to provide robust biomarkers and new therapeutic targets for more effective cancer therapies, better outcomes, and greater survival[130, 131].

4.3.1 Regulatory ncRNA

4.3.1.1 miRNA

miRNA are small ncRNAs around 22 nucleotides long. They perform their post-transcriptional regulatory effects by binding to specific sites known as miRNA response elements (MREs) on their target transcripts, leading to either transcript degradation or translational inhibition[131]. miRNA regulatory activity in cancer has been widely studied[132-134], as miRNA can be divided into oncogenic miRNA and tumor suppressor miRNA[135].

miRNA function in OSCC has been thoroughly discussed[136]. Since they are secreted from various types of healthy and tumor cells[137], miRNA can be detected in exosomes based on their cell of origin. Dickman et al. found that miR-142-3p secreted from oral cancer cells promotes cancer cell growth by eliminating the miRNA tumor suppressive effect. Exosomes also promote tumor angiogenesis by releasing miR-142-3p to its microenvironment[138]. Overexpression of miR-6887-5p in SCC/OSCC cells inhibited tumor growth according to Higaki et al[139]. Similar results document targeting miRNA as a treatment strategy to inhibit tumor growth. Lower levels of miR-3188 were detected in CAFs than normal fibroblasts, and loss of miR-3188 promoted malignant phenotypes in head and neck cancer cells, supporting its consideration as a therapeutic target[140].

miRNA in CAF-derived exosomes not only promote or inhibit tumor growth, but have also been shown to participate in OSCC cell migration and invasion. Sun et al. found that miR3825p was overexpressed in CAFs compared with fibroblasts of adjacent normal tissue, and miR3825p overexpression was an important regulatory factor in OSCC cell migration and invasion[141]. By comparing miRNA profiles in non-invasive SQUU-A and highly invasive SQUU-B tongue cancer cell clones, it was observed that hsa-miR-200c-3p acts within a key pro-invasion role in OSCC. The transfer of miR-200c-3p in exosomes derived from a highly invasive OSCC line can also accelerate the invasion potential of non-invasive counterparts[142]. Normoxic and hypoxic OSCC-derived exosomes yielded different miRNA profiles; miR-21 showed its most significant role under hypoxic conditions. The loss of miR-21 in hypoxic OSCC cells downregulated miR-21 levels in exosomes and significantly reduced cell migration and invasion. Restoration of miR-21 expression in HIF-1α- and HIF-2α-depleted exosomes rescued OSCC cell migration and invasion[143].

Metastasis is a great challenge in our effort to fight cancer, and it is one feature of OSCC[141]. Some studies have noticed that cancer stem cell-derived extracellular vesicles are enriched with miR-21-5p, which is associated with increased potential of OSCC metastasis[144]. Some

miRNAs have been revealed as performing anti-cancer roles as well. By examining the miRNA profiles of CAF- and normal fibroblast (NF)-derived exosomes, miR-34a-5p expression was found to be significantly decreased, making it an anti-cancer therapeutic target for OSCC[145]. The tumor microenvironment (TME) plays a vital role in the progression of OSCC. Recent research has revealed that tumor-derived exosomes (TEX) accumulate in the TME and interact between tumor and healthy stromal cells[146]. Cai et al. cocultured exosomes extracted from OSCC cell lines (SCC-9 and CAL-27) with macrophages. Their results showed that the upregulation of miR-29a-3p in OSCC-derived exosomes is related to M2 subtype macrophage polarization. After interfering with miR-29a-3p from OSCC, M2 subtype macrophage polarization was inhibited by OSCC-derived exosomes[147]. Tumor angiogenesis is a hallmark in tumor development[148]. Rosenberger et al. demonstrated a significant antitumor effect of when the intra-tumoral injection of mesenchymal stem cell (MSC)-derived exosomes was associated with a loss of tumor vasculature[146]. However, the outcomes of treated tumors showing diverse levels of VEGF mRNA, as well as smaller tumor volumes, indicates that VEGF-independent mechanisms exist to regulate this antitumor reaction. Considering the multifunctional roles and complex regulatory network, exosomal miRNA may be involved in this process[149].

Chemoresistance is a significant challenge for OSCC treatment with no clear mechanism. Several studies have shown that miRNA in exosomes of both healthy and tumor cells can manipulate this phenomenon[150-152]. Some miRNAs are upregulated during chemotherapy, which can enhance chemoresistance against antitumor drugs such as cisplatin (CIS) and docetaxel (DTX). Kirave et al. found that when transferring exosomes from CIS-resistant to CIS-sensitive cells, miR-155 was significantly upregulated in the recipient CIS-sensitive cells[150]. Exosomes isolated from CIS-resistant cell lines contained a higher concentration of miR-21 in accordance with the parental cells' increased cisplatin resistance, which indicates that miR-21 may be a potential target against chemoresistance[153]. The underlying mechanism is considered related to EMT and decreased DNA damage in cancer cells[150, 151]. Some miRNAs were downregulated during chemoresistance. For example, downregulation of miR-200c increased resistance to DTX, when miR-200c was transported by exosomes, the results showed the increase of the sensitivity to DTX both *in vitro* and *in vivo*, indicating miR-200c could be a therapeutic target of OSCC[151].

4.3.1.2 lncRNA and circRNA

Beyond miRNA, there are other regulatory ncRNAs that perform complex roles in cancer[154]. One kind, lncRNA, ranging from 200 to >1000 nucleotides, is a novel class in the human genome with seldom to no coding potential[155]. They participate in various diseases through interacting with DNA[156], RNA, or proteins[157]. lncRNA may be involved in cancer cell proliferation[158, 159], migration[158], invasion[160], metastasis[160, 161], and antitumor drug resistance[159]. Xu et al. found that the expression of LINC00662 in OSCC positively correlated with tumor size, stage, and lymph node metastasis. It is capable of inducing the proliferation, migration, and invasion of OSCC cells by regulating the Wnt/beta-catenin pathway[158]. Zhang

et al. discovered that knockdown of lncRNA UCA1 significantly suppressed TGFβ1-induced tongue cancer cell invasion and eventually induced EMT[162]. The regulatory effect of lncRNA is reported to act as "sponge" for miRNAs[162, 163]. TGFβ1-induced EMT and invasion in OSCC are consistent with increased JAG1, whereas miR-124 inhibits its expression. UCA1 binds to miR-124 directly and can downregulate miR-124 expression. This is the basis for lncRNA UCA1's protumor effect through sponge-like lncRNA-miRNA-mRNA regulation[162]. lncRNA TIRY was also found to act as a miRNA sponge in OSCC by downregulating miR-14 expression in CAF-derived exosomes[161]. lncRNA FLJ22447 (lnc-CAF) secreted from CAFs regulates NFs to CAFs, and tumor cells increased lnc-CAF levels in stromal fibroblasts via exosomal lnc-CAF as well[164]. CircRNA consists of a closed continuous loop structure without 5'-3' polarity or a poly-A tail, which enables its resistance to RNases and higher stability compared with linear RNA[165]. Similar to lncRNA, circRNA also functions as a miRNA sponge[166]. Although the roles of circRNA in exosomes remains unknown, a hypothesis has been introduced by Bai et al[165]. Some circRNAs may bind to and transport with miRNAs by exosomes. After entering target cells, miRNAs are released to regulate target genes[161]. CircRNAs in exosomes may therefore enter the recipient cells, bind to miRNAs, and regulate target genes. Several researchers have investigated the role of circRNAs in OSCC[167], and differences in circRNAs profiles between OSCC patients and healthy people have also been distinguished[168, 169]. However, there is still a lack of direct evidence for exo-circRNA regulating OSCC.

4.3.2 Other ncRNA

Besides regulatory ncRNA, tRNA and rRNA make up another group of ncRNA referred to as housekeeping ncRNA[129]. Baglio et al. defined the exosome-enclosed RNA species from the full small RNAome of MSC-produced exosomes[170]. Adipose and bone marrow MSC subtypes secrete different tRNA species that may be relevant to clinical applications; however, how tRNAs are transported through exosomes and their influence on the microenvironment in a cell type-dependent manner remains clear[170]. Crescitelli et al. analyzed RNA profiles in different EVs including exosomes. According to their findings, rRNA was primarily detectable in apoptotic bodies, but smaller RNAs without prominent ribosomal RNA peaks in exosomes[171]. This indicates that exosomes are potentially not carriers of rRNA. Collectively, there is little evidence surrounding the exosomal transportation of tRNA and rRNA, let alone their potential function in modulating cancer and their microenvironment.

5. Clinical use of exosomes

5.1 Exosomes as biomarkers for diseases diagnosis

Combining their stability and accessibility in various biological fluids, exosomes can be used as biomarkers for various diseases. For example, exosomes carrying Glypican-1 are considered a sensitive indicator of pancreatic cancer in blood samples[47]. In gastric cancer patients, the presence of CD63+ exosomes in tumor cells but not stromal cells indicates a worse prognosis than if CD63+ exosomes were found in both cell types[172]. In oral squamous cell cancers, exosomes have been discovered in the tumor microenvironment and regarded as very promising

in understanding tumorigenesis, tumor metastasis, tumor invasion, and communication between tumor cells. Studies have shown that the morphology of exosomes isolated from patients is very different from that of healthy people, suggests the possibility of using exosomes for disease liquid biopsy[173]. By using high-resolution AFM, Sharma et al. found the salivary exosomes of oral cancer patients were much larger and amorphous compared with those of healthy people, and also found that CD63 was significantly increased on the surface of cancer exosomes[174]. In a study by Rabinowits, tongue squamous cell carcinoma tissue and normal tissue were collected in pairs. They isolated exosomes and found different miRNA loading patterns similar to the loading patterns of blood exosomes, suggesting that circulating exosomes can be a more reliable method in evaluating tumors[175]. Sanada et al. examined the expression levels of secreted lysyl-oxidase-like 2 (LOXL2) in pharyngeal and tongue cancer patient serum and found that elevated serum exosome LOXL2 levels are associated with low-grade oral cancer[176]. The contents of exosomes can indicate tumor invasion capacity and occurrence of distant metastasis. Li and colleagues found that miR-21-rich exosomes are associated with increased OSCC invasiveness, and that these exosomes are delivered to normoxic cells to promote prometastatic behaviors[143]. Nakashima et al. utilized integrated microarray profiling technology to analyze the different expression patterns of miRNA between non-invasive and highly invasive tongue cancer cells, observing that hsa-miR-200c-3p was the crucial point in spreading invasive ability[142]. Different tumor cells have different secretion patterns, which determines the type of cargo carried by exosomes. Therefore, exosomes can be used in non-invasive examination for tumor staging. Ludwig et al. suggest that tumor staging can be understood by exploring the interaction between OSCC cell-derived exosomes and lymphocytes. Exosomes in the plasma of patients with tumors in an uncontrolled phase have greater induction of T cell apoptosis and inhibition of lymphocyte proliferation, which differs from patients without significant disease[177]. The quantity and content of exosomes could predict the prognosis of treated OSCC patients. Liu and Tian compared serum exosomes between laryngeal squamous cell carcinoma patients and vocal cord nodule patients, finding that the expression levels of miR-21 and HOTAIR were higher in exosomes of malignant lesions. Moreover, the serum exosomes of patients with laryngeal cancer in stage III/IV also showed a high level of miR-21 and HOTAIR in exosomes[178], suggesting an association between the level of exosomal content and prognosis. In a clinical study by Zorrilla et al., the expression of CD63+ plasma exosomes were significantly lower after surgical treatment than before, which indicated a longer life expectancy[179].

5.2 Exosomes as therapeutic mediums

Exosomes can not only be used as disease biomarkers, but also in the drug delivery for therapeutic cargo. In terms of exosomal structure, they consist of biogenic lipid bilayers similar to cell membranes, which protect cargo from degradation. The surface diameter of exosomes is only 40–150 nm, so they are small enough to access most tissue without consumption and degradation by macrophages[12, 180-183]. Moreover, they may exhibit inherent targeting properties, which is determined by lipid composition and protein content[184]. With these

advantages, they are considered as potential drug delivery systems. At present, clinical trials using exosomes as a drug delivery method against cancer have been gradually increasing. In lung cancer, tumor cell-derived exosomes were extracted from the pleural effusion of lung cancer patients. After modification and loading with the chemotherapy drug methotrexate, they were reinjected into the patient's chest cavity. It has been observed that exosomes have a safe inhibitory effect on the growth of tumor cells[185]. In colon cancer, exosomes with carcinoembryonic antigen were isolated from ascites fluid. After combining them with granulocyte macrophage colony stimulating factor, they served as a vaccine to induce a beneficial tumor-specific antitumor cell toxic T lymphocyte response[186]. In OSCC, the current drug-loading process is mainly based on different carrier systems, such as nanoparticles, nanolipids, and hydrogels, which can alleviate the disadvantage of poor water solubility for oral cancer anti-cancer drugs to a certain extent[187-189]. Studies have shown that exosomes have been used as carriers for chemotherapeutic agents such as curcumin, DOX, and PTX, thereby reducing their side effects and improving therapeutic efficiency[190-192]. Despite this progress, research on exosomes as a drug-loading system is still limited, mainly due to their limited ability to deliver high-dose therapeutic drugs, insufficient basic experiments, and a lack of effective, standardized separation and purification methods[182, 193].

Conclusions

Many studies have confirmed the importance of exosomes in cancer tumorigenesis, proliferation, migration, invasion, metastasis, and chemoresistance, with research continuing to improve. Studies concerning the content of exosomes and their role in OSCC are also growing. Because of their unique secretion patterns, exosomes can be used as ideal biomarkers for OSCC diagnosis. Their unique surface markers and lipid coating also allows them to be used as a drug delivery system, which may be applied to OSCC. However, it is worth noting that there is no consistent test standard and separation method to examine the cargo carried by exosomes. The application of exosomes in OSCC has great potential for cancer diagnosis and treatment. The type and abundance of cargo found in OSCC-related exosomes varies among different states of health and disease, and a comprehensive understanding would help us elucidate disease mechanisms and provide opportunities for the diagnosis and treatment of OSCC. Greater research efforts on the different types of cargo in OSCC-related exosomes are needed. From our perspective, discovering different molecules in OSCC exosomes and determining their roles and mechanisms will be needed to develop better diagnostics and therapeutic strategies.

Acknowledgements

Thank Dr. Qiang Peng for his valuable suggestion.

References

1. Shao, H., et al., New Technologies for Analysis of Extracellular Vesicles. *Chem Rev,* **2018**. *118(4)*: p. 1917-1950.

2. Shah, R., T. Patel, and J.E. Freedman, Circulating Extracellular Vesicles in Human Disease. *N Engl J Med,* **2018**. *379(10)*: p. 958-966.
3. Johnstone, R.M., et al., Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *J Biol Chem,* **1987**. *262(19)*: p. 9412-20.
4. van Niel, G., G. D'Angelo, and G. Raposo, Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol,* **2018**. *19(4)*: p. 213-228.
5. Gyorgy, B., et al., Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. *Cell Mol Life Sci,* **2011**. *68(16)*: p. 2667-88.
6. Minciacchi, V.R., M.R. Freeman, and D. Di Vizio, Extracellular vesicles in cancer: exosomes, microvesicles and the emerging role of large oncosomes. *Semin Cell Dev Biol,* **2015**. *40*: p. 41-51.
7. Liao, W., et al., Exosomes: The next generation of endogenous nanomaterials for advanced drug delivery and therapy. *Acta Biomater,* **2019**. *86*: p. 1-14.
8. Abels, E.R. and X.O. Breakefield, Introduction to Extracellular Vesicles: Biogenesis, RNA Cargo Selection, Content, Release, and Uptake. *Cell Mol Neurobiol,* **2016**. *36(3)*: p. 301-12.
9. Mateescu, B., et al., Obstacles and opportunities in the functional analysis of extracellular vesicle RNA - an ISEV position paper. *J Extracell Vesicles,* **2017**. *6(1)*: p. 1286095.
10. Pan, B.T. and R.M. Johnstone, Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. *Cell,* **1983**. *33(3)*: p. 967-78.
11. Mashouri, L., et al., Exosomes: composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. *Mol Cancer,* **2019**. *18(1)*: p. 75.
12. Kalluri, R. and V.S. LeBleu, Discovery of Double-Stranded Genomic DNA in Circulating Exosomes. *Cold Spring Harb Symp Quant Biol,* **2016**. *81*: p. 275-280.
13. Ferguson, S.W. and J. Nguyen, Exosomes as therapeutics: The implications of molecular composition and exosomal heterogeneity. *J Control Release,* **2016**. *228*: p. 179-190.
14. Li, L.M., Z.X. Liu, and Q.Y. Cheng, Exosome plays an important role in the development of hepatocellular carcinoma. *Pathol Res Pract,* **2019**. *215(8)*: p. 152468.
15. Malm, T., S. Loppi, and K.M. Kanninen, Exosomes in Alzheimer's disease. *Neurochem Int,* **2016**. *97*: p. 193-9.
16. Kadota, T., et al., Extracellular Vesicles in Chronic Obstructive Pulmonary Disease. *Int J Mol Sci,* **2016**. *17(11)*.
17. Zhan, C., et al., Exosomes and other extracellular vesicles in oral and salivary gland cancers. *Oral Dis,* **2019**.
18. Hadavand, M. and S. Hasni, Exosomal biomarkers in oral diseases. *Oral Dis,* **2019**. *25(1)*: p. 10-15.
19. Milman, N., L. Ginini, and Z. Gil, Exosomes and their role in tumorigenesis and anticancer drug resistance. *Drug Resist Updat,* **2019**. *45*: p. 1-12.
20. Matei, I., H.S. Kim, and D. Lyden, Unshielding Exosomal RNA Unleashes Tumor Growth And Metastasis. *Cell,* **2017**. *170(2)*: p. 223-225.
21. Xue, M., et al., Hypoxic exosomes facilitate bladder tumor growth and development through transferring long non-coding RNA-UCA1. *Mol Cancer,* **2017**. *16(1)*: p. 143.
22. Zeng, Z., et al., Cancer-derived exosomal miR-25-3p promotes pre-metastatic niche formation by inducing vascular permeability and angiogenesis. *Nat Commun,* **2018**. *9(1)*: p. 5395.
23. Chen, G., et al., Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. *Nature,* **2018**. *560(7718)*: p. 382-386.

24. Kulkarni, B., et al., Exosomal miRNA in chemoresistance, immune evasion, metastasis and progression of cancer. *Drug Discov Today,* **2019**. *24(10)*: p. 2058-2067.
25. Hoshino, A., et al., Tumour exosome integrins determine organotropic metastasis. *Nature,* **2015**. *527(7578)*: p. 329-35.
26. Pakravan, K., et al., MicroRNA-100 shuttled by mesenchymal stem cell-derived exosomes suppresses in vitro angiogenesis through modulating the mTOR/HIF-1alpha/VEGF signaling axis in breast cancer cells. *Cell Oncol (Dordr),* **2017**. *40(5)*: p. 457-470.
27. Xie, C., et al., Exosomes derived from microRNA-101-3p-overexpressing human bone marrow mesenchymal stem cells suppress oral cancer cell proliferation, invasion, and migration. *Mol Cell Biochem,* **2019**. *458(1-2)*: p. 11-26.
28. Chi, A.C., T.A. Day, and B.W. Neville, Oral cavity and oropharyngeal squamous cell carcinoma--an update. *CA Cancer J Clin,* **2015**. *65(5)*: p. 401-21.
29. Gupta, B., N.W. Johnson, and N. Kumar, Global Epidemiology of Head and Neck Cancers: A Continuing Challenge. *Oncology,* **2016**. *91(1)*: p. 13-23.
30. Razzo, B.M., et al., Tumor-derived exosomes promote carcinogenesis of murine oral squamous cell carcinoma. *Carcinogenesis,* **2019**.
31. Momen-Heravi, F. and S. Bala, Extracellular vesicles in oral squamous carcinoma carry oncogenic miRNA profile and reprogram monocytes via NF-kappaB pathway. *Oncotarget,* **2018**. *9(78)*: p. 34838-34854.
32. Cui, J., et al., Exosomal miR-200c suppresses chemoresistance of docetaxel in tongue squamous cell carcinoma by suppressing TUBB3 and PPP2R1B. *Aging,* **2020**. *12(8)*: p. 6756-6773.
33. Huotari, J. and A. Helenius, Endosome maturation. *Embo j,* **2011**. *30(17)*: p. 3481-500.
34. Morelli, A.E., et al., Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. *Blood,* **2004**. *104(10)*: p. 3257-66.
35. Trajkovic, K., et al., Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science,* **2008**. *319(5867)*: p. 1244-7.
36. McNally, E.K. and C.L. Brett, The intralumenal fragment pathway mediates ESCRT-independent surface transporter down-regulation. *Nat Commun,* **2018**. *9(1)*: p. 5358.
37. Hessvik, N.P. and A. Llorente, Current knowledge on exosome biogenesis and release. *Cell Mol Life Sci,* **2018**. *75(2)*: p. 193-208.
38. Grant, B.D. and J.G. Donaldson, Pathways and mechanisms of endocytic recycling. *Nat Rev Mol Cell Biol,* **2009**. *10(9)*: p. 597-608.
39. Hsu, C., et al., Regulation of exosome secretion by Rab35 and its GTPase-activating proteins TBC1D10A-C. *J Cell Biol,* **2010**. *189(2)*: p. 223-32.
40. Baietti, M.F., et al., Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. *Nat Cell Biol,* **2012**. *14(7)*: p. 677-85.
41. Alonso, R., et al., Diacylglycerol kinase alpha regulates the formation and polarisation of mature multivesicular bodies involved in the secretion of Fas ligand-containing exosomes in T lymphocytes. *Cell Death Differ,* **2011**. *18(7)*: p. 1161-73.
42. Li, W., et al., Role of exosomal proteins in cancer diagnosis. *Mol Cancer,* **2017**. *16(1)*: p. 145.
43. Li, P., et al., Progress in Exosome Isolation Techniques. *Theranostics,* **2017**. *7(3)*: p. 789-804.
44. Hemler, M.E., Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. *Annu Rev Cell Dev Biol,* **2003**. *19*: p. 397-422.
45. van Niel, G., et al., Exosomes: a common pathway for a specialized function. *J Biochem,* **2006**. *140(1)*: p. 13-21.

46. Zhou, H., et al., Exosomal Fetuin-A identified by proteomics: a novel urinary biomarker for detecting acute kidney injury. *Kidney Int,* **2006**. *70(10)*: p. 1847-57.
47. Melo, S.A., et al., Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature,* **2015**. *523(7559)*: p. 177-82.
48. DeRita, R.M., et al., c-Src, Insulin-Like Growth Factor I Receptor, G-Protein-Coupled Receptor Kinases and Focal Adhesion Kinase are Enriched Into Prostate Cancer Cell Exosomes. *J Cell Biochem,* **2017**. *118(1)*: p. 66-73.
49. Shostak, K. and A. Chariot, EGFR and NF-κB: partners in cancer. *Trends Mol Med,* **2015**. *21(6)*: p. 385-93.
50. Sigismund, S., D. Avanzato, and L. Lanzetti, Emerging functions of the EGFR in cancer. *Mol Oncol,* **2018**. *12(1)*: p. 3-20.
51. Yang, C.C. and K.W. Chang, Eicosanoids and HB-EGF/EGFR in cancer. *Cancer Metastasis Rev,* **2018**. *37(2-3)*: p. 385-395.
52. Nakamura, H., et al., Relationship Between EGFR Expression in Oral Cancer Cell Lines and Cetuximab Antibody-dependent Cell-mediated Cytotoxicity. *Anticancer Res,* **2019**. *39(3)*: p. 1275-1282.
53. Zhang, M., et al., EGFR confers radioresistance in human oropharyngeal carcinoma by activating endoplasmic reticulum stress signaling PERK-eIF2α-GRP94 and IRE1α-XBP1-GRP78. *Cancer Med,* **2018**. *7(12)*: p. 6234-6246.
54. Byeon, H.K., M. Ku, and J. Yang, Beyond EGFR inhibition: multilateral combat strategies to stop the progression of head and neck cancer. *Exp Mol Med,* **2019**. *51(1)*: p. 1-14.
55. Oliveira-Silva, R.J., et al., Anti-EGFR Therapy: Strategies in Head and Neck Squamous Cell Carcinoma. *Recent Pat Anticancer Drug Discov,* **2016**. *11(2)*: p. 170-83.
56. Fujiwara, T., et al., Carcinogenic epithelial-mesenchymal transition initiated by oral cancer exosomes is inhibited by anti-EGFR antibody cetuximab. *Oral Oncol,* **2018**. *86*: p. 251-257.
57. Principe, S., et al., Proteomic Analysis of Cancer-Associated Fibroblasts Reveals a Paracrine Role for MFAP5 in Human Oral Tongue Squamous Cell Carcinoma. *J Proteome Res,* **2018**. *17(6)*: p. 2045-2059.
58. Jiang, E., et al., Tumoral microvesicle-activated glycometabolic reprogramming in fibroblasts promotes the progression of oral squamous cell carcinoma. *Faseb j,* **2019**. *33(4)*: p. 5690-5703.
59. Teng, M.W., et al., From mice to humans: developments in cancer immunoediting. *J Clin Invest,* **2015**. *125(9)*: p. 3338-46.
60. Borst, J., et al., CD4(+) T cell help in cancer immunology and immunotherapy. *Nat Rev Immunol,* **2018**. *18(10)*: p. 635-647.
61. Gardner, A. and B. Ruffell, Dendritic Cells and Cancer Immunity. *Trends Immunol,* **2016**. *37(12)*: p. 855-865.
62. Gonzalez, H., I. Robles, and Z. Werb, Innate and acquired immune surveillance in the postdissemination phase of metastasis. *Febs j,* **2018**. *285(4)*: p. 654-664.
63. Mantovani, A., et al., Tumour-associated macrophages as treatment targets in oncology. *Nat Rev Clin Oncol,* **2017**. *14(7)*: p. 399-416.
64. Kim, J.W., et al., Fas ligand-positive membranous vesicles isolated from sera of patients with oral cancer induce apoptosis of activated T lymphocytes. *Clin Cancer Res,* **2005**. *11(3)*: p. 1010-20.
65. Wang, Y., et al., Oral cancer-derived exosomal NAP1 enhances cytotoxicity of natural killer cells via the IRF-3 pathway. *Oral Oncol,* **2018**. *76*: p. 34-41.
66. Xiao, M., et al., M1-like tumor-associated macrophages activated by exosome-transferred THBS1 promote malignant migration in oral squamous cell carcinoma. *J Exp Clin Cancer Res,* **2018**. *37(1)*: p. 143.

67. Batlle, E. and J. Massagué, Transforming Growth Factor-β Signaling in Immunity and Cancer. *Immunity,* **2019**. *50(4)*: p. 924-940.
68. Languino, L.R., et al., Exosome-mediated transfer from the tumor microenvironment increases TGFβ signaling in squamous cell carcinoma. *Am J Transl Res,* **2016**. *8(5)*: p. 2432-7.
69. Overmiller, A.M., et al., Desmoglein 2 modulates extracellular vesicle release from squamous cell carcinoma keratinocytes. *Faseb j,* **2017**. *31(8)*: p. 3412-3424.
70. Zhong, W.-Q., et al., Increased salivary microvesicles are associated with the prognosis of patients with oral squamous cell carcinoma. *Journal of cellular and molecular medicine,* **2019**. *23(6)*: p. 4054-4062.
71. Ono, K., et al., HSP-enriched properties of extracellular vesicles involve survival of metastatic oral cancer cells. *Journal of cellular biochemistry,* **2018**. *119(9)*: p. 7350-7362.
72. Wang, S.-H., et al., Laminin γ2-enriched extracellular vesicles of oral squamous cell carcinoma cells enhance in vitro lymphangiogenesis via integrin α3-dependent uptake by lymphatic endothelial cells. *International journal of cancer,* **2019**. *144(11)*: p. 2795-2810.
73. Li, C., et al., Potential Markers from Serum-Purified Exosomes for Detecting Oral Squamous Cell Carcinoma Metastasis. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology,* **2019**. *28(10)*: p. 1668-1681.
74. Vered, M., et al., Caveolin-1 accumulation in the tongue cancer tumor microenvironment is significantly associated with poor prognosis: an in-vivo and in-vitro study. *BMC cancer,* **2015**. *15*: p. 25.
75. Campos, A., et al., Cell Intrinsic and Extrinsic Mechanisms of Caveolin-1-Enhanced Metastasis. *Biomolecules,* **2019**. *9(8)*.
76. Nwosu, Z.C., et al., Caveolin-1 in the regulation of cell metabolism: a cancer perspective. *Mol Cancer,* **2016**. *15(1)*: p. 71.
77. Núñez-Wehinger, S., et al., Caveolin-1 in cell migration and metastasis. *Curr Mol Med,* **2014**. *14(2)*: p. 255-74.
78. Record, M., et al., Exosomes as new vesicular lipid transporters involved in cell-cell communication and various pathophysiologies. *Biochim Biophys Acta,* **2014**. *1841(1)*: p. 108-20.
79. Haraszti, R.A., et al., High-resolution proteomic and lipidomic analysis of exosomes and microvesicles from different cell sources. *J Extracell Vesicles,* **2016**. *5*: p. 32570.
80. Record, M., et al., Extracellular vesicles: lipids as key components of their biogenesis and functions. *J Lipid Res,* **2018**. *59(8)*: p. 1316-1324.
81. Subra, C., et al., Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. *Biochimie,* **2007**. *89(2)*: p. 205-12.
82. Falguières, T., D. Castle, and J. Gruenberg, Regulation of the MVB pathway by SCAMP3. *Traffic,* **2012**. *13(1)*: p. 131-42.
83. Llorente, A., et al., Molecular lipidomics of exosomes released by PC-3 prostate cancer cells. *Biochim Biophys Acta,* **2013**. *1831(7)*: p. 1302-9.
84. Sur, S., et al., Inhibition of the key metabolic pathways, glycolysis and lipogenesis, of oral cancer by bitter melon extract. *Cell Commun Signal,* **2019**. *17(1)*: p. 131.
85. Hu, Q., et al., Obesity and genes related to lipid metabolism predict poor survival in oral squamous cell carcinoma. *Oral Oncol,* **2019**. *89*: p. 14-22.
86. Tousignant, K.D., et al., Lipid Uptake Is an Androgen-Enhanced Lipid Supply Pathway Associated with Prostate Cancer Disease Progression and Bone Metastasis. *Mol Cancer Res,* **2019**. *17(5)*: p. 1166-1179.
87. Subra, C., et al., Exosomes account for vesicle-mediated transcellular transport of activatable phospholipases and prostaglandins. *J Lipid Res,* **2010**. *51(8)*: p. 2105-20.

88. Gatto, F., A. Schulze, and J. Nielsen, Systematic Analysis Reveals that Cancer Mutations Converge on Deregulated Metabolism of Arachidonate and Xenobiotics. *Cell Rep,* **2016**. *16(3)*: p. 878-95.
89. Linton, S.S., et al., Tumor-promoting effects of pancreatic cancer cell exosomes on THP-1-derived macrophages. *PLoS One,* **2018**. *13(11)*: p. e0206759.
90. Esser, J., et al., Exosomes from human macrophages and dendritic cells contain enzymes for leukotriene biosynthesis and promote granulocyte migration. *The Journal of allergy and clinical immunology,* **2010**. *126(5)*.
91. Lukic, A., et al., Exosomes and cells from lung cancer pleural exudates transform LTC4 to LTD4, promoting cell migration and survival via CysLT1. *Cancer Lett,* **2019**. *444*: p. 1-8.
92. Lai, Y.H., et al., MiR-31-5p-ACOX1 Axis Enhances Tumorigenic Fitness in Oral Squamous Cell Carcinoma Via the Promigratory Prostaglandin E2. *Theranostics,* **2018**. *8(2)*: p. 486-504.
93. Nasry, W.H.S., J.C. Rodriguez-Lecompte, and C.K. Martin, Role of COX-2/PGE2 Mediated Inflammation in Oral Squamous Cell Carcinoma. *Cancers (Basel),* **2018**. *10(10)*.
94. Harada, T., et al., Wnt5b-associated exosomes promote cancer cell migration and proliferation. *Cancer Sci,* **2017**. *108(1)*: p. 42-52.
95. Luga, V., et al., Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. *Cell,* **2012**. *151(7)*: p. 1542-56.
96. Kahlert, C., et al., Identification of double-stranded genomic DNA spanning all chromosomes with mutated KRAS and p53 DNA in the serum exosomes of patients with pancreatic cancer. *J Biol Chem,* **2014**. *289(7)*: p. 3869-75.
97. Kitai, Y., et al., DNA-Containing Exosomes Derived from Cancer Cells Treated with Topotecan Activate a STING-Dependent Pathway and Reinforce Antitumor Immunity. *J Immunol,* **2017**. *198(4)*: p. 1649-1659.
98. Diamond, J.M., et al., Exosomes Shuttle TREX1-Sensitive IFN-Stimulatory dsDNA from Irradiated Cancer Cells to DCs. *Cancer Immunol Res,* **2018**. *6(8)*: p. 910-920.
99. Wang, L., et al., Exosomal double-stranded DNA as a biomarker for the diagnosis and preoperative assessment of pheochromocytoma and paraganglioma. *Mol Cancer,* **2018**. *17(1)*: p. 128.
100. Yang, S., et al., Detection of mutant KRAS and TP53 DNA in circulating exosomes from healthy individuals and patients with pancreatic cancer. *Cancer Biol Ther,* **2017**. *18(3)*: p. 158-165.
101. San Lucas, F.A., et al., Minimally invasive genomic and transcriptomic profiling of visceral cancers by next-generation sequencing of circulating exosomes. *Ann Oncol,* **2016**. *27(4)*: p. 635-41.
102. Natsuizaka, M., et al., Interplay between Notch1 and Notch3 promotes EMT and tumor initiation in squamous cell carcinoma. *Nat Commun,* **2017**. *8(1)*: p. 1758.
103. Biswas, N.K., et al., Lymph node metastasis in oral cancer is strongly associated with chromosomal instability and DNA repair defects. *Int J Cancer,* **2019**. *145(9)*: p. 2568-2579.
104. Yang, Y., et al., Exosomes mediate hepatitis B virus (HBV) transmission and NK-cell dysfunction. *Cell Mol Immunol,* **2017**. *14(5)*: p. 465-475.
105. Meckes, D.G., Jr., et al., Human tumor virus utilizes exosomes for intercellular communication. *Proc Natl Acad Sci U S A,* **2010**. *107(47)*: p. 20370-5.
106. Mata-Rocha, M., et al., Presence of HPV DNA in extracellular vesicles from HeLa cells and cervical samples. *Enferm Infecc Microbiol Clin,* **2020**. *38(4)*: p. 159-165.
107. Guenat, D., et al., Exosomes and Other Extracellular Vesicles in HPV Transmission and Carcinogenesis. *Viruses,* **2017**. *9(8)*.

108. Ambrosio, M.R., et al., Putative Role of Circulating Human Papillomavirus DNA in the Development of Primary Squamous Cell Carcinoma of the Middle Rectum: A Case Report. *Front Oncol,* **2019**. *9*: p. 93.
109. Acquistapace, A., et al., Human mesenchymal stem cells reprogram adult cardiomyocytes toward a progenitor-like state through partial cell fusion and mitochondria transfer. *Stem Cells,* **2011**. *29(5)*: p. 812-24.
110. Tan, A.S., et al., Mitochondrial genome acquisition restores respiratory function and tumorigenic potential of cancer cells without mitochondrial DNA. *Cell Metab,* **2015**. *21(1)*: p. 81-94.
111. Wang, X. and H.H. Gerdes, Transfer of mitochondria via tunneling nanotubes rescues apoptotic PC12 cells. *Cell Death Differ,* **2015**. *22(7)*: p. 1181-91.
112. Herst, P.M., C. Grasso, and M.V. Berridge, Metabolic reprogramming of mitochondrial respiration in metastatic cancer. *Cancer Metastasis Rev,* **2018**. *37(4)*: p. 643-653.
113. Berridge, M.V., L. Dong, and J. Neuzil, Mitochondrial DNA in Tumor Initiation, Progression, and Metastasis: Role of Horizontal mtDNA Transfer. *Cancer Res,* **2015**. *75(16)*: p. 3203-8.
114. Pasquier, J., et al., Preferential transfer of mitochondria from endothelial to cancer cells through tunneling nanotubes modulates chemoresistance. *J Transl Med,* **2013**. *11*: p. 94.
115. Zhang, Q., et al., Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature,* **2010**. *464(7285)*: p. 104-7.
116. Zhang, Q., K. Itagaki, and C.J. Hauser, Mitochondrial DNA is released by shock and activates neutrophils via p38 map kinase. *Shock,* **2010**. *34(1)*: p. 55-9.
117. Uzawa, K., et al., Quantitative detection of circulating tumor-derived mitochondrial NADH subunit variants as a potential prognostic biomarker for oral cancer. *Int J Oncol,* **2015**. *47(3)*: p. 1077-83.
118. Huang, X., et al., Characterization of human plasma-derived exosomal RNAs by deep sequencing. *BMC Genomics,* **2013**. *14*: p. 319.
119. Valadi, H., et al., Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol,* **2007**. *9(6)*: p. 654-9.
120. Feng, Y., et al., Urinary Exosomes and Exosomal CCL2 mRNA as Biomarkers of Active Histologic Injury in IgA Nephropathy. *Am J Pathol,* **2018**. *188(11)*: p. 2542-2552.
121. Del Re, M., et al., PD-L1 mRNA expression in plasma-derived exosomes is associated with response to anti-PD-1 antibodies in melanoma and NSCLC. *Br J Cancer,* **2018**. *118(6)*: p. 820-824.
122. Skog, J., et al., Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol,* **2008**. *10(12)*: p. 1470-6.
123. Lázaro-Ibáñez, E., et al., Distinct prostate cancer-related mRNA cargo in extracellular vesicle subsets from prostate cell lines. *BMC Cancer,* **2017**. *17(1)*: p. 92.
124. Hao, Y.X., et al., KRAS and BRAF mutations in serum exosomes from patients with colorectal cancer in a Chinese population. *Oncol Lett,* **2017**. *13(5)*: p. 3608-3616.
125. Geis-Asteggiante, L., et al., Differential Content of Proteins, mRNAs, and miRNAs Suggests that MDSC and Their Exosomes May Mediate Distinct Immune Suppressive Functions. *J Proteome Res,* **2018**. *17(1)*: p. 486-498.
126. Yakob, M., et al., Salivary biomarkers for detection of oral squamous cell carcinoma - current state and recent advances. *Curr Oral Health Rep,* **2014**. *1(2)*: p. 133-141.
127. Brinkmann, O., et al., Oral squamous cell carcinoma detection by salivary biomarkers in a Serbian population. *Oral Oncol,* **2011**. *47(1)*: p. 51-5.
128. Qadir, F., et al., Transcriptome reprogramming by cancer exosomes: identification of novel molecular targets in matrix and immune modulation. *Mol Cancer,* **2018**. *17(1)*: p. 97.

129. Romano, G., et al., Small non-coding RNA and cancer. *Carcinogenesis,* **2017**. *38(5)*: p. 485-491.
130. Zhang, J., et al., Exosome and exosomal microRNA: trafficking, sorting, and function. *Genomics Proteomics Bioinformatics,* **2015**. *13(1)*: p. 17-24.
131. Chan, J.J. and Y. Tay, Noncoding RNA:RNA Regulatory Networks in Cancer. *Int J Mol Sci,* **2018**. *19(5)*.
132. He, L., et al., Salivary exosomal miR-24-3p serves as a potential detective biomarker for oral squamous cell carcinoma screening. *Biomed Pharmacother,* **2020**. *121*: p. 109553.
133. Li, W., et al., Oral mucosal mesenchymal stem cellderived exosomes: A potential therapeutic target in oral premalignant lesions. *Int J Oncol,* **2019**. *54(5)*: p. 1567-1578.
134. Sakha, S., et al., Exosomal microRNA miR-1246 induces cell motility and invasion through the regulation of DENND2D in oral squamous cell carcinoma. *Sci Rep,* **2016**. *6*: p. 38750.
135. Svoronos, A.A., D.M. Engelman, and F.J. Slack, OncomiR or Tumor Suppressor? The Duplicity of MicroRNAs in Cancer. *Cancer Res,* **2016**. *76(13)*: p. 3666-70.
136. Langevin, S., et al., Comprehensive microRNA-sequencing of exosomes derived from head and neck carcinoma cells in vitro reveals common secretion profiles and potential utility as salivary biomarkers. *Oncotarget,* **2017**. *8(47)*: p. 82459-82474.
137. Tkach, M. and C. Théry, Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. *Cell,* **2016**. *164(6)*: p. 1226-1232.
138. Dickman, C.T., et al., Selective extracellular vesicle exclusion of miR-142-3p by oral cancer cells promotes both internal and extracellular malignant phenotypes. *Oncotarget,* **2017**. *8(9)*: p. 15252-15266.
139. Higaki, M., et al., Eldecalcitol (ED-71)-induced exosomal miR-6887-5p suppresses squamous cell carcinoma cell growth by targeting heparin-binding protein 17/fibroblast growth factor-binding protein-1 (HBp17/FGFBP-1). *In Vitro Cell Dev Biol Anim,* **2020**. *56(3)*: p. 222-233.
140. Wang, X., et al., Loss of exosomal miR-3188 in cancer-associated fibroblasts contributes to HNC progression. *J Exp Clin Cancer Res,* **2019**. *38(1)*: p. 151.
141. Sun, L.P., et al., Cancer‐associated fibroblast‐derived exosomal miR‐382‐5p promotes the migration and invasion of oral squamous cell carcinoma. *Oncol Rep,* **2019**. *42(4)*: p. 1319-1328.
142. Kawakubo-Yasukochi, T., et al., miR-200c-3p spreads invasive capacity in human oral squamous cell carcinoma microenvironment. *Mol Carcinog,* **2018**. *57(2)*: p. 295-302.
143. Li, L., et al., Exosomes Derived from Hypoxic Oral Squamous Cell Carcinoma Cells Deliver miR-21 to Normoxic Cells to Elicit a Prometastatic Phenotype. *Cancer Res,* **2016**. *76(7)*: p. 1770-80.
144. Chen, J.H., et al., Ovatodiolide Suppresses Oral Cancer Malignancy by Down-Regulating Exosomal Mir-21/STAT3/β-Catenin Cargo and Preventing Oncogenic Transformation of Normal Gingival Fibroblasts. *Cancers (Basel),* **2019**. *12(1)*.
145. Li, Y.Y., et al., Cancer-associated fibroblasts contribute to oral cancer cells proliferation and metastasis via exosome-mediated paracrine miR-34a-5p. *EBioMedicine,* **2018**. *36*: p. 209-220.
146. Ludwig, N., et al., Exosomes from HNSCC Promote Angiogenesis through Reprogramming of Endothelial Cells. *Mol Cancer Res,* **2018**. *16(11)*: p. 1798-1808.
147. Cai, J., et al., Oral squamous cell carcinoma-derived exosomes promote M2 subtype macrophage polarization mediated by exosome-enclosed miR-29a-3p. *Am J Physiol Cell Physiol,* **2019**. *316(5)*: p. C731-c740.
148. Viallard, C. and B. Larrivée, Tumor angiogenesis and vascular normalization: alternative therapeutic targets. *Angiogenesis,* **2017**. *20(4)*: p. 409-426.

149. Rosenberger, L., et al., Stem cell exosomes inhibit angiogenesis and tumor growth of oral squamous cell carcinoma. *Sci Rep,* **2019**. *9(1)*: p. 663.
150. Kirave, P., et al., Exosome mediated miR-155 delivery confers cisplatin chemoresistance in oral cancer cells via epithelial-mesenchymal transition. *Oncotarget,* **2020**. *11(13)*: p. 1157-1171.
151. Cui, J., et al., Exosomal miR-200c suppresses chemoresistance of docetaxel in tongue squamous cell carcinoma by suppressing TUBB3 and PPP2R1B. *Aging (Albany NY),* **2020**. *12(8)*: p. 6756-6773.
152. Qin, X., et al., Exosomal miR-196a derived from cancer-associated fibroblasts confers cisplatin resistance in head and neck cancer through targeting CDKN1B and ING5. *Genome Biol,* **2019**. *20(1)*: p. 12.
153. Liu, T., et al., Exosomes containing miR-21 transfer the characteristic of cisplatin resistance by targeting PTEN and PDCD4 in oral squamous cell carcinoma. *Acta Biochim Biophys Sin (Shanghai),* **2017**. *49(9)*: p. 808-816.
154. Morris, K.V. and J.S. Mattick, The rise of regulatory RNA. *Nat Rev Genet,* **2014**. *15(6)*: p. 423-37.
155. Yang, Y., L. Wen, and H. Zhu, Unveiling the hidden function of long non-coding RNA by identifying its major partner-protein. *Cell Biosci,* **2015**. *5*: p. 59.
156. Lin, K., et al., Identification of cancer hallmark-associated gene and lncRNA cooperative regulation pairs and dictate lncRNA roles in oral squamous cell carcinoma. *J Cell Mol Med,* **2020**. *24(9)*: p. 5213-5223.
157. Zhang, H., et al., Long noncoding RNA-mediated intrachromosomal interactions promote imprinting at the Kcnq1 locus. *J Cell Biol,* **2014**. *204(1)*: p. 61-75.
158. Xu, D., et al., Long non-coding RNA LINC00662 promotes proliferation and migration in oral squamous cell carcinoma. *Onco Targets Ther,* **2019**. *12*: p. 647-656.
159. Wang, X., H. Li, and J. Shi, LncRNA HOXA11-AS Promotes Proliferation and Cisplatin Resistance of Oral Squamous Cell Carcinoma by Suppression of miR-214-3p Expression. *Biomed Res Int,* **2019**. *2019*: p. 8645153.
160. Xiong, H.G., et al., Long noncoding RNA MYOSLID promotes invasion and metastasis by modulating the partial epithelial-mesenchymal transition program in head and neck squamous cell carcinoma. *J Exp Clin Cancer Res,* **2019**. *38(1)*: p. 278.
161. Jin, N., et al., Long non-coding RNA TIRY promotes tumor metastasis by enhancing epithelial-to-mesenchymal transition in oral cancer. *Exp Biol Med (Maywood),* **2020**. *245(7)*: p. 585-596.
162. Zhang, T.H., et al., LncRNA UCA1/miR-124 axis modulates TGFβ1-induced epithelial-mesenchymal transition and invasion of tongue cancer cells through JAG1/Notch signaling. *J Cell Biochem,* **2019**. *120(6)*: p. 10495-10504.
163. Tan, J., L. Xiang, and G. Xu, LncRNA MEG3 suppresses migration and promotes apoptosis by sponging miR-548d-3p to modulate JAK-STAT pathway in oral squamous cell carcinoma. *IUBMB Life,* **2019**. *71(7)*: p. 882-890.
164. Ding, L., et al., A novel stromal lncRNA signature reprograms fibroblasts to promote the growth of oral squamous cell carcinoma via LncRNA-CAF/interleukin-33. *Carcinogenesis,* **2018**. *39(3)*: p. 397-406.
165. Bai, H., et al., Exo-circRNAs: a new paradigm for anticancer therapy. *Mol Cancer,* **2019**. *18(1)*: p. 56.
166. He, T., et al., Overexpressed circPVT1 in oral squamous cell carcinoma promotes proliferation by serving as a miRNA sponge. *Molecular medicine reports,* **2019**. *20(4)*: p. 3509-3518.
167. Han, L., J. Cheng, and A. Li, hsa_circ_0072387 Suppresses Proliferation, Metastasis, and Glycolysis of Oral Squamous Cell Carcinoma Cells by Downregulating miR-503-5p. *Cancer Biother Radiopharm,* **2020**.

168. Wei, T., et al., Circular RNA expression profiling identifies specific circular RNAs in tongue squamous cell carcinoma. *Mol Med Rep,* **2020**. *21(4)*: p. 1727-1738.
169. Qiu, X., et al., Profiling and bioinformatics analyses reveal differential expression of circular RNA in tongue cancer revealed by high-throughput sequencing. *J Cell Biochem,* **2019**. *120(3)*: p. 4102-4112.
170. Baglio, S.R., et al., Human bone marrow- and adipose-mesenchymal stem cells secrete exosomes enriched in distinctive miRNA and tRNA species. *Stem Cell Res Ther,* **2015**. *6(1)*: p. 127.
171. Crescitelli, R., et al., Distinct RNA profiles in subpopulations of extracellular vesicles: apoptotic bodies, microvesicles and exosomes. *J Extracell Vesicles,* **2013**. *2*.
172. Miki, Y., et al., Clinico-pathological significance of exosome marker CD63 expression on cancer cells and stromal cells in gastric cancer. *PLoS One,* **2018**. *13(9)*: p. e0202956.
173. Zlotogorski-Hurvitz, A., et al., Morphological and molecular features of oral fluid-derived exosomes: oral cancer patients versus healthy individuals. *J Cancer Res Clin Oncol,* **2016**. *142(1)*: p. 101-10.
174. Sharma, S., et al., Quantitative nanostructural and single-molecule force spectroscopy biomolecular analysis of human-saliva-derived exosomes. *Langmuir,* **2011**. *27(23)*: p. 14394-400.
175. Rabinowits, G., et al., Comparative Analysis of MicroRNA Expression among Benign and Malignant Tongue Tissue and Plasma of Patients with Tongue Cancer. *Front Oncol,* **2017**. *7*: p. 191.
176. Sanada, T., et al., Elevated exosomal lysyl oxidase like 2 is a potential biomarker for head and neck squamous cell carcinoma. *Laryngoscope,* **2020**. *130(5)*: p. E327-e334.
177. Ludwig, S., et al., Suppression of Lymphocyte Functions by Plasma Exosomes Correlates with Disease Activity in Patients with Head and Neck Cancer. *Clin Cancer Res,* **2017**. *23(16)*: p. 4843-4854.
178. Wang, J., et al., Combined detection of serum exosomal miR-21 and HOTAIR as diagnostic and prognostic biomarkers for laryngeal squamous cell carcinoma. *Med Oncol,* **2014**. *31(9)*: p. 148.
179. Rodríguez Zorrilla, S., et al., A Pilot Clinical Study on the Prognostic Relevance of Plasmatic Exosomes Levels in Oral Squamous Cell Carcinoma Patients. *Cancers (Basel),* **2019**. *11(3)*.
180. Surman, M., et al., Extracellular Vesicles as Drug Delivery Systems - Methods of Production and Potential Therapeutic Applications. *Curr Pharm Des,* **2019**. *25(2)*: p. 132-154.
181. Vader, P., et al., Extracellular vesicles for drug delivery. *Adv Drug Deliv Rev,* **2016**. *106(Pt A)*: p. 148-156.
182. Yang, M. and S.Y. Wu, The Advances and Challenges in Utilizing Exosomes for Delivering Cancer Therapeutics. *Front Pharmacol,* **2018**. *9*: p. 735.
183. de Jong, O.G., et al., Drug Delivery with Extracellular Vesicles: From Imagination to Innovation. *Acc Chem Res,* **2019**. *52(7)*: p. 1761-1770.
184. Murphy, D.E., et al., Extracellular vesicle-based therapeutics: natural versus engineered targeting and trafficking. *Exp Mol Med,* **2019**. *51(3)*: p. 1-12.
185. Guo, M., et al., Autologous tumor cell-derived microparticle-based targeted chemotherapy in lung cancer patients with malignant pleural effusion. *Sci Transl Med,* **2019**. *11(474)*.
186. Dai, S., et al., Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer. *Mol Ther,* **2008**. *16(4)*: p. 782-90.
187. Ketabat, F., et al., Controlled Drug Delivery Systems for Oral Cancer Treatment-Current Status and Future Perspectives. *Pharmaceutics,* **2019**. *11(7)*.

973 188. Luo, C., et al., Emerging integrated nanohybrid drug delivery systems to facilitate the
974 intravenous-to-oral switch in cancer chemotherapy. *J Control Release,* **2014**. *176*: p. 94-
975 103.
976 189. Poonia, M., et al., Nanotechnology in oral cancer: A comprehensive review. *J Oral*
977 *Maxillofac Pathol,* **2017**. *21(3)*: p. 407-414.
978 190. Batrakova, E.V. and M.S. Kim, Using exosomes, naturally-equipped nanocarriers, for
979 drug delivery. *J Control Release,* **2015**. *219*: p. 396-405.
980 191. Sun, D., et al., A novel nanoparticle drug delivery system: the anti-inflammatory activity
981 of curcumin is enhanced when encapsulated in exosomes. *Mol Ther,* **2010**. *18(9)*: p.
982 1606-14.
983 192. Tian, Y., et al., A doxorubicin delivery platform using engineered natural membrane
984 vesicle exosomes for targeted tumor therapy. *Biomaterials,* **2014**. *35(7)*: p. 2383-90.
985 193. Ludwig, N., T.L. Whiteside, and T.E. Reichert, Challenges in Exosome Isolation and
986 Analysis in Health and Disease. *Int J Mol Sci,* **2019**. *20(19)*.
987

Table 1(on next page)

NcRNAs regulating the process of OSCC in exosomes

1 **Table 1. NcRNAs regulating the process of OSCC in exosomes**

NcRNAs	Types of ncRNAs	Pro /Anti-tumor	Target/Signal pathway	Functions	Origin of exosomes	Ref.
miR8485	miRNA	Pro-tumor	—	Promote the carcinogenesis of premalignant lesions, proliferation, migration and invasion of tumor cells	MSCs	[133]
miR-6887-5p	miRNA	Anti-tumor	HBp17/FGFBP-1	Inhibit tumor cell proliferation, colony formation, then tumor growth	A431 cells	[139]
miR-142-3p	miRNA	Pro-tumor	TGFBR1	Cause tumor-promoting changes	Oral dysplasia and OSCC cell lines	[138]
miR-24-3p	miRNA	Pro-tumor	PER1	Maintain the proliferation of OSCC cells	Saliva in OSCC patients	[132]
miR-3188	miRNA	Anti-tumor	BCL2	The loss of miR-3188 in exosomes contributes to the malignant phenotypes of HNC cells through the depression of BCL2	CAFs	[140]
miR-34a-5p	miRNA	Anti-tumor	AXL AKT/GSK-3beta/beta-catenin signaling pathway	MiR-34a-5p binds to direct downstream target AXL to suppress OSCC cell proliferation and metastasis	CAFs	[145]
miR3825p	miRNA	Pro-tumor	—	Responsible for OSCC cell migration and invasion	CAFs	[141]
miR-21-5p	miRNA	Pro-tumor	—	Increase metastasis, stemness, chemoresistance and poor survival in patients with OSCC	CAL27 and SCC-15 OSCC cells	[144]
miR-1246	miRNA	Pro-tumor	DENND2D ERK/AKT	Increase cell motility and invasive ability	HOC313-LM OSCC cells	[134]

			pathway			
miR-21	miRNA	Pro-tumor	miR-21/HIF-1alpha/HIF-2alpha-dependent pathway	MiR-21 can be delivered to normoxic cells to promote prometastatic behaviors	Hypoxic OSCC cells	[143]
		Pro-tumor	PTEN, PDCD4	Induce cisplatin resistance of OSCC cells	HSC-3-R and SCC-9-R	[153]
miR-200c-3p	miRNA	Pro-tumor	CHD9, WRN	Spread invasive capacity by exosomes in tumor microenvironment	SQUU-B tongue cancer cell clones	[142]
miR-155	miRNA	Pro-tumor	—	Lead to mesenchymal transition and increase migratory potential and acquire cells drug-resistant phenotype	Cisplatin resistant OSCC cells	[150]
miR-200c	miRNA	Anti-tumor	TUBB3, PPP2R1B	Increase the sensitivity of Docetaxel (DTX) resistant HSC-3 cells to DTX	normal tongue epithelial cells (NTECs)	[151]
miR-101-3p	miRNA	Anti-tumor	COL10A1	Overexpression of miR-101-3p inhibit oral cancer progression and provide a therapeutic target	human bone marrow mesenchymal stem cells (hBMSCs)	[27]
miR-29a-3p	miRNA	Pro-tumor	SOCS1	Promote M2 subtype macrophage polarization, tumor cell proliferation and invasion	SCC-9 and CAL-27	[147]
FLJ22447	lncRNA	Pro-tumor	Lnc-CAF/IL-33	Reprogram normal fibroblast to CAFs and promote OSCC development	CAFs	[164]

2

Figure 1

Different classification of EVs

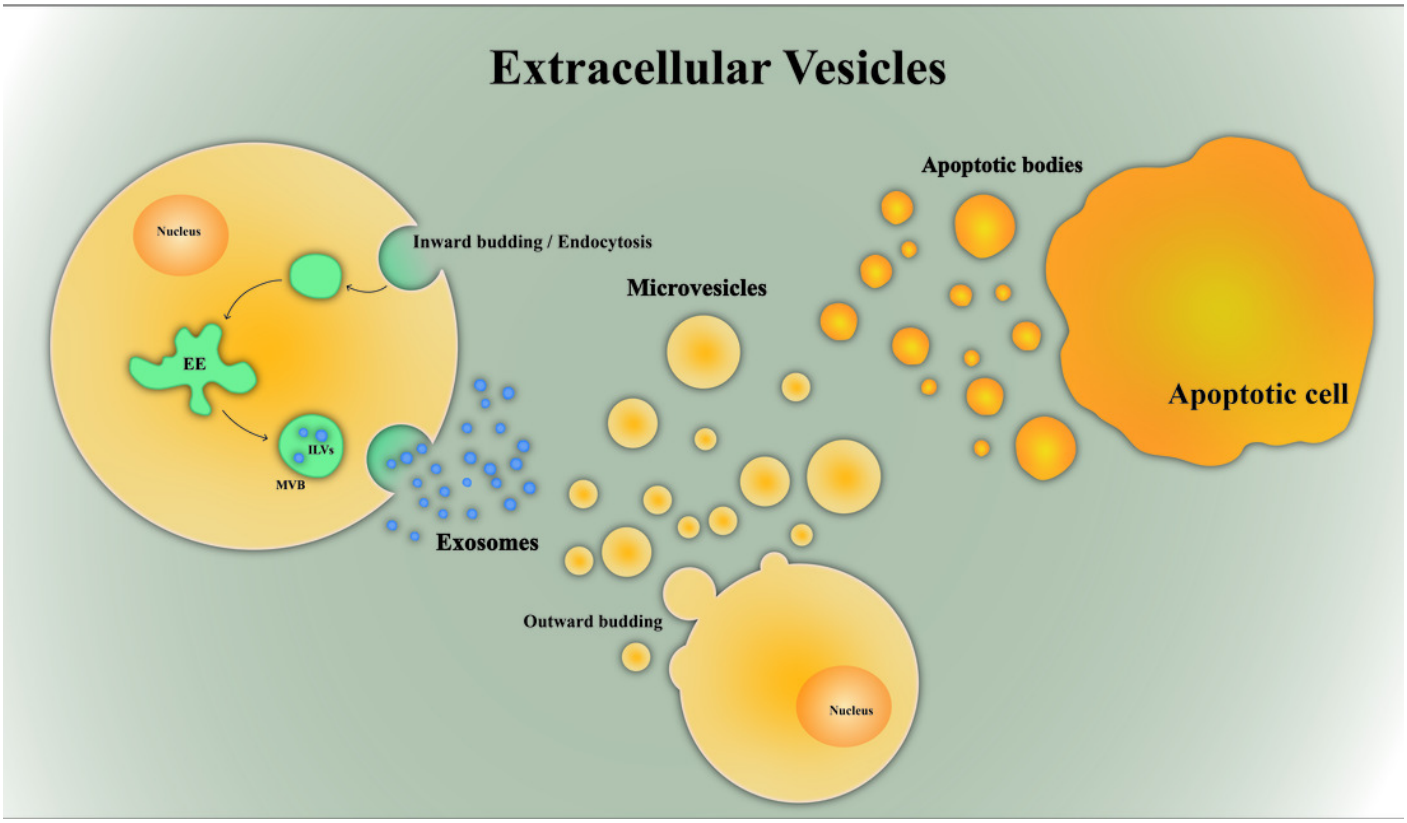

Figure 2

Exosomes biogenesis and secretion within endosomal system

This figure is reprinted from ref. [42] according to open access licence CC BY 4.0

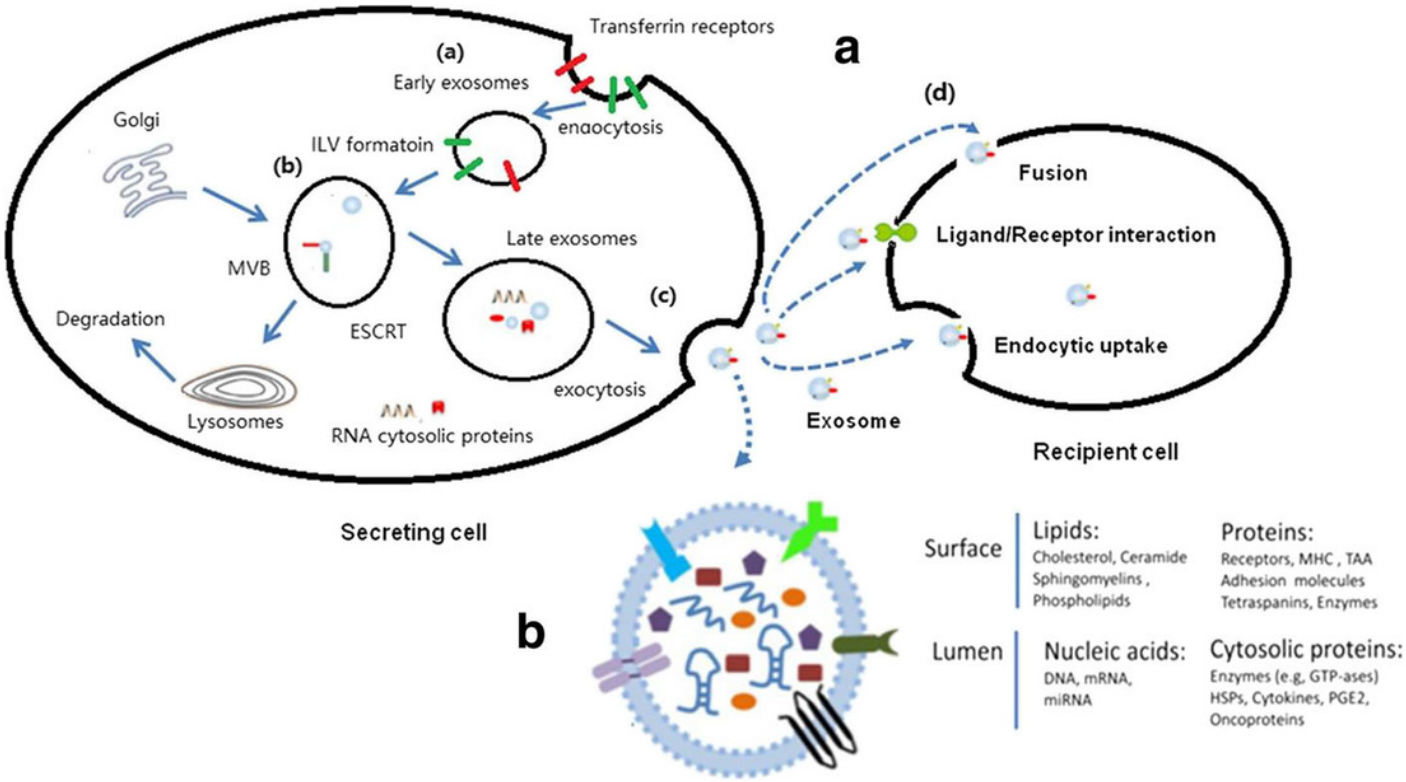

Figure 3

Functions of exosomes capsuled proteins in OSCC

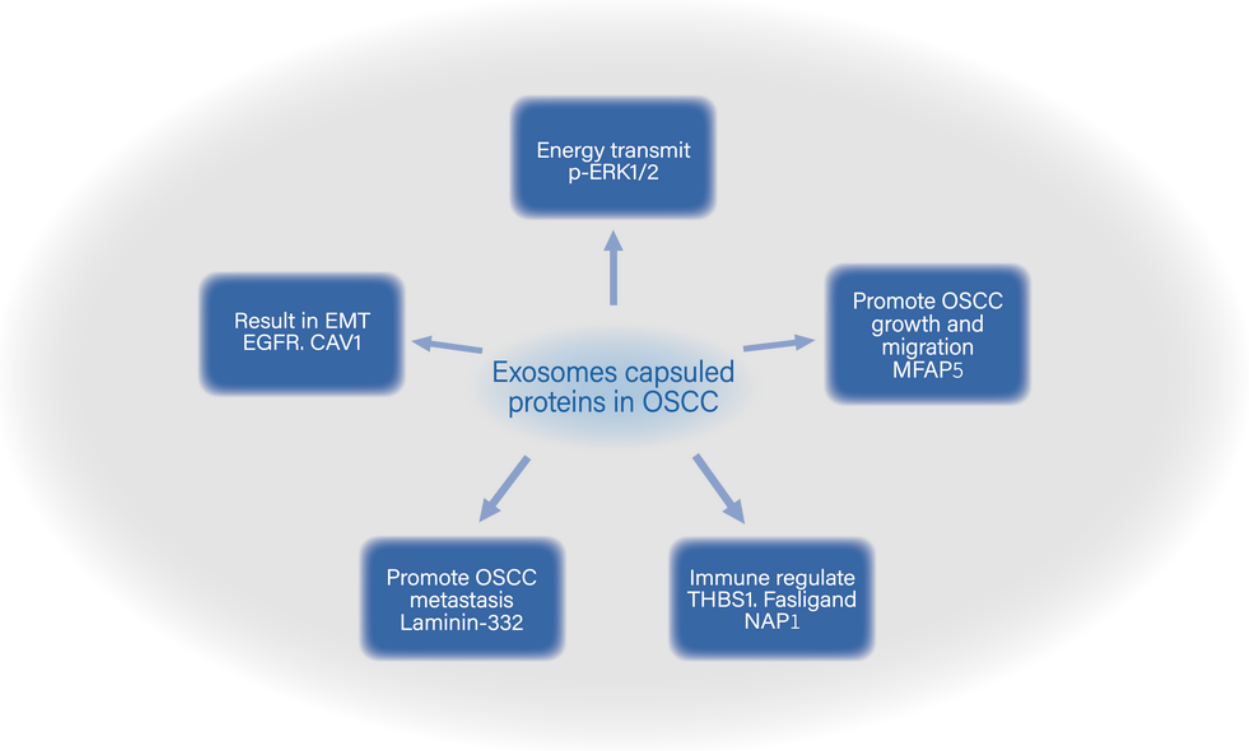

Figure 4

Exosomes' contents and potential functions in the developing process of OSCC