

Current Status, Opportunities, and Challenges of Exosomes in Oral Cancer Diagnosis and Treatment

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Abstract: Oral cancer is one of the most common cancers in the world, with more than 300,000 cases diagnosed each year, of which oral squamous cell carcinoma accounts for more than 90%, with a 5-year survival rate of only 40–60%, and poor prognosis. Exploring new strategies for the early diagnosis and treatment of oral cancer is key to improving the survival rate. Exosomes are nanoscale lipid bilayer membrane vesicles that are secreted by almost all cell types. During the development of oral cancer, exosomes can transport their contents (DNA, RNA, proteins, etc) to target cells and promote or inhibit the proliferation, invasion, and metastasis of oral cancer cells by influencing the host immune response, drug-resistant metastasis, and tumour angiogenesis. Therefore, exosomes have great potential and advantages as biomarkers for oral cancer diagnosis, and as drug delivery vehicles or targets for oral cancer therapy. In this review, we first describe the biogenesis, biological functions, and isolation methods of exosomes, followed by their relationship with oral cancer. Here, we focused on the potential of exosomes as oral cancer biomarkers, drug carriers, and therapeutic targets. Finally, we provide an insightful discussion of the opportunities and challenges of exosome application in oral cancer diagnosis and treatment, intending to offer new ideas for the clinical management of oral cancer.

Keywords: exosomes, oral cancer, biomarkers, drug delivery vehicles, therapeutic target

Introduction

As the first part of the upper gastrointestinal tract, the oral cavity is responsible for food intake, mastication, articulation, swallowing, sensation and expression.¹ The normal performance of these physiological functions is inevitably affected to a greater or lesser extent by oral cancer, and the quality of life of most clinical patients is severely reduced in the late stage due to postoperative cosmetic defects and loss of oral and maxillofacial functions.² Oral cancer, according to the International Classification of Diseases, Tenth Revision [ICD-10], includes cancers of the lip, cheek, tongue, gingiva, floor of the mouth, hard palate, and other unspecified parts of the oral cavity.³ Oral squamous cell carcinoma (OSCC) accounts for more than 90% of these cancers^{4–6} and is the most common malignancy of the head and neck. It has a high prevalence worldwide and is a major public health concern. In 2020, the number of new cases of OSCC worldwide was approximately 377,000, and the number of deaths in the same year was approximately 177,000.⁷ Early detection, even at the preclinical stage, is essential to reduce the morbidity and mortality of OSCC. Some studies have shown that patients with oral cancer can have a 5-year survival rate of more than 80% if diagnosed early (stages I and II). Unfortunately, up to 50% of oral cancers are diagnosed at an advanced stage (stages III and IV) after the onset of symptoms, such as pain, bleeding, or oral and maxillofacial masses. The risk of developing advanced oral cancer is significantly higher when the diagnosis is delayed for more than one month, and treatment for patients with advanced oral cancer is not only costly but also has a poor prognosis.^{8,9} Therefore, the early diagnosis of oral cancer is closely related to the prognosis, and it is urgent to develop methods for the early diagnosis thereof. OSCC has a high potential for local infiltration and lymph node metastasis,^{4–6} and once metastasis is present, conventional treatment approaches fail to achieve satisfactory results. Despite the

development of multidisciplinary and comprehensive sequential treatment strategies and levels for OSCC, the 5-year survival rate has not improved significantly in the past decades (remaining around 50%).^{10–13} Therefore, in addition to focusing on early diagnosis of oral cancer, finding emerging strategies for targeted treatment is an effective way to reduce mortality and improve patients' quality of life. The discovery of early diagnostic markers and targets for oral cancer treatment is the focus of current research.¹⁴

Extracellular vesicles are small membrane-derived vesicles secreted into the extracellular space. Based on the size of extracellular vesicles, they are classified into three main types: (1) apoptotic vesicles, (2) microvesicles, and (3) exosomes.¹⁵ Exosomes are lipid bilayer membrane vesicles with a diameter of approximately 30–150 nm¹⁶ and can be secreted by almost all cells (such as immune cells,^{17–19} tumour cells,²⁰ and nerve cells)²¹. Exosomes contain a variety of molecules present in various body fluids, including saliva, blood, and urine. Exosomes have specific surface proteins that distinguish these nanoparticles from other microvesicles or apoptotic vesicles.²² These surface proteins (eg CD63, CD81, or CD9) allow for the screening, selective recruitment and analysis of exosomes of cancer cell origin.²³ Previous studies have shown that the “cargo” loaded in exosomes can be used as a novel diagnostic biomarker for OSCC.^{24–27} Exosomal surface proteins or contents are diverse and dynamically altered, with significant differences between patients with oral cancer and healthy individuals.^{28,29} This provides a basis for circulating exosomes as a biomarker for the early diagnosis of oral cancer. In addition, exosomes are the “messengers” of intercellular communication,^{30,31} which can participate in intercellular information transfer, transmitting biological information, such as proteins, lipids and nucleic acids, from the parent cell to the recipient cell in a stable manner,^{27,32} causing a series of relevant reactions in the recipient cell, thus playing a regulatory role.³³ Many studies have shown that after delivering exosomal contents to target cells, exosomes can influence the host immune response, drug-resistant metastasis, tumour angiogenesis and other processes by inducing phenotypic and functional changes in the recipient cells,^{34–36} thereby promoting or inhibiting the proliferation, invasion and metastasis of oral cancer. Moreover, exosomes have the characteristics required for easy uptake by cancer cells, small size, good biocompatibility, easy access, and high stability.^{37–39} This makes it possible to deliver drugs through exosomes or use engineered exosomes for targeted treatment of oral cancer. Therefore, exosomes have great potential and advantages for the diagnosis and treatment of oral cancer.

Research on exosomes for various cancers is increasing annually.³⁷ The research progress and clinical application of exosomes in oral cancer diagnosis and treatment have also attracted much attention.^{40–42} This review takes a fresh perspective on the impact of exosomes on the development of oral cancer. It highlights the current status, opportunities, and challenges of exosomes in the diagnosis and treatment of oral cancer, providing new insights into the search for emerging biomarkers and potential targets for the diagnosis and treatment of oral cancer.

Biogenesis, Biological Functions, and Isolation Methods of Exosomes

The biogenesis of exosomes consists of different stages, including three main steps: biogenesis, loading and transport of related substances, and release.⁴³ First, the plasma membrane bud inwards to form endocytic vesicles (ie early endosomes), and in some cases, early endosomes can be created by the trans-Golgi network (TGN). Early endosomes then form late endosomes through cargo selection and fusion, followed by invagination of the endosomal membrane to form intraluminal vesicles (ILVs) that promote the formation of multivesicular bodies (MVBs). Eventually, MVBs fuse with the plasma membrane, leading to the release of vesicle contents called exosomes.^{44–47} The biogenesis of exosomes is shown in [Figure 1](#). In addition, MVBs can be degraded by lysosomes or autophagosomes.^{48,49} The released exosomes interact with target cells in three ways: (1) fusion of the plasma membrane between target cells and exosomes, (2) the binding of target cell membrane receptors with proteins on the membrane surface of exosomes, and (3) the *in vivo* transformation of exosomes by target cell endocytosis.⁵⁰ The origin and biogenesis of exosomes are mediated by tumour susceptibility gene 101 (TSG101), apoptosis-linked gene 2-interacting protein X (ALIX), endosomal sorting complexes required for transport (ESCRT) protein, soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (SNARE) complex proteins, and other related substances. However, the specific mechanism underlying the role and function of these related substances in exosome biogenesis requires further investigation.^{31,49,51,52}

Exosome release can be achieved by plasma membrane fusion, extracellular action of MVB, or by small cytoplasmic protrusions directly from the cell surface.^{37,53} Exosome uptake is achieved by interacting with proteins on the surface of exosomes (such as CD9, CD63, and CD81)^{54–56} and recipient cells. Simultaneously, exosomes can transfer their contents from parent cells to

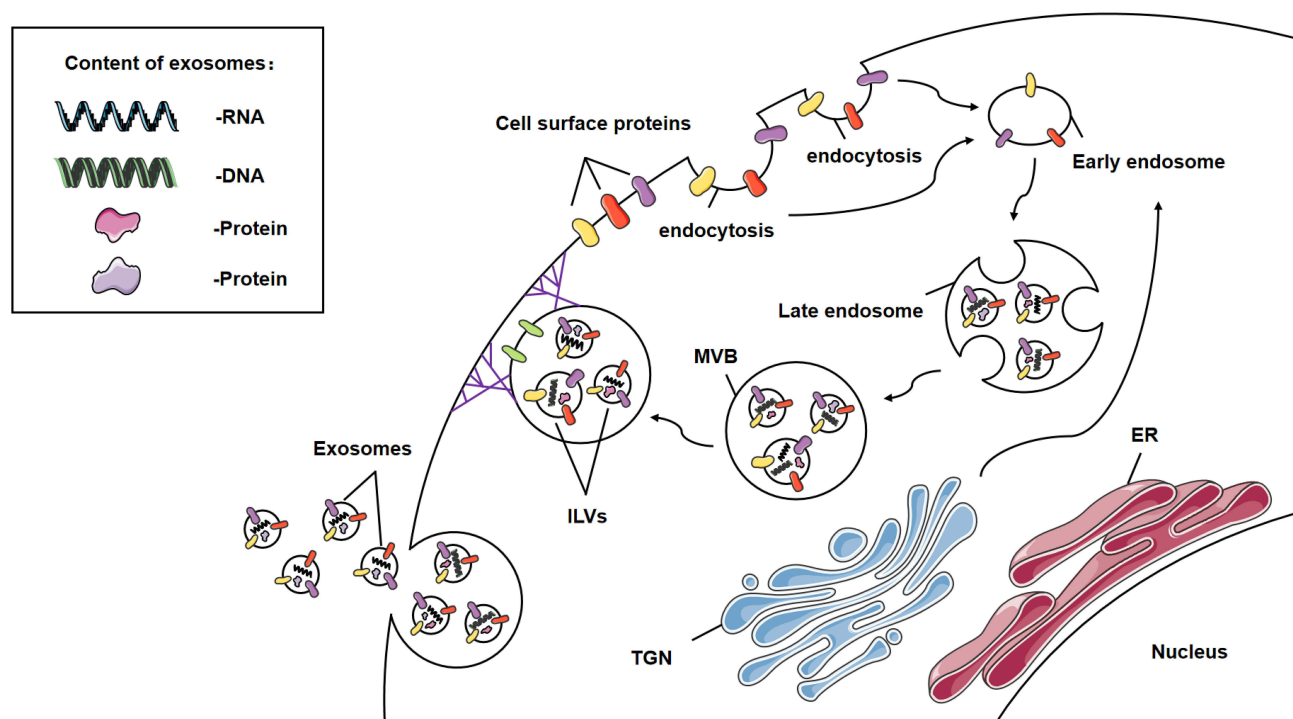


Figure 1 Exosome biogenesis. Exosomes contain DNA, RNA, and other proteins. The release of exosomes into the extracellular environment involves three distinct steps: exosome biogenesis, loading and transporting of related substances, and release. The plasma membrane buds inward to form early endosomes, which can also be created by the trans-Golgi network. Early endosomes are loaded and fused to form late endosomes, and the endosomal membrane invaginates to form intraluminal vesicles. Exosomes are released outside the cell when multivesicular bodies merge with the plasma membrane.

nearby and distant recipient cells, thus mediating intercellular communication.^{27,32} Under physiological conditions, exosomes are important mediators of intercellular communication. Under pathological conditions (eg cancer, neurodegenerative diseases, cardiovascular diseases, infectious diseases, and respiratory diseases), exosomes play an essential role in regulating cellular activity.^{57–62} During oral cancer progression, exosomes produced by different cells (eg macrophages) can transfer nucleotides and proteins between cells and participate in the complex pathogenesis of oral cancer development and metastasis.^{63–67} Therefore, it is essential to explore the influence of exosomes on oral carcinogenesis and progression.

Therefore, obtaining high-purity and -quality exosomes is an essential condition and foundation for clinical and experimental purposes. Currently, based on the size, morphological structure, and specific surface proteins of exosomes, different methods of exosome isolation have been discovered, including ultra-high-speed centrifugation,^{56,68–71} polymer precipitation,^{70,72,73} chromatography including molecular sieve chromatography,⁵⁶ density gradient centrifugation,⁷⁴ ultrafiltration,⁷⁵ immunoaffinity capture,^{56,75} and microfluidics-based separation techniques,^{76–78} among others. Because exosomes can be obtained in many ways and isolated by many methods, it provides the basis and feasibility to study the application of exosomes in oral cancer diagnosis and treatment. However, each of these methods has its own advantages and disadvantages, and further improvements are needed to promote the research and application of exosomes in oral cancer diagnosis and treatment (Table 1).

Exosomes Associated with Oral Cancer

Tumour-derived exosomes can transmit their own biogenetic information to the surrounding normal or tumour cells^{79,80} to produce various substances that favour tumour proliferation, invasion, and metastasis, thus forming a microenvironment conducive to tumour metastasis.⁸¹ This microenvironment can induce the transformation of normal cells around the tumour to tumour cells,^{82–84} thus regulating the behaviour of recipient cells.^{32,85,86} Research related to exosomes and oral cancer is currently underway. Current studies on the effect of exosomes on oral cancer have focused

Table I Exosome Isolation Methods

Isolation Method	Principle	Advantages	Disadvantages	Ref
Ultracentrifugation	Based on ultrahigh-speed centrifugation	Simple operation Wide application Low cost	Low purity Low integrity	[68–71]
Polymer precipitation technology	Exosomes combine with polymers and agglomerate in the solution	High integrity High yield	Low purity More mixture	[70,72,73]
Molecular sieve chromatography	Based on the size difference between molecules	High purity High integrity	High cost	[56]
Density gradient centrifugation	Ultracentrifugation combined with sucrose density gradient	High purity	Complicated operation Time-consuming	[74]
Ultrafiltration	Based on membrane ultrafiltration	High yield	Low purity Low efficiency Pore size clogging	[75]
Immunoaffinity capture	Antibody-coated magnetic beads bind specifically to exosomal membrane proteins	High purity	High cost	[56,75]
Separation technology based on microfluidics	Based on size	High purity High Quality Efficient	High cost Hard to popularize	[76–78]

on salivary exosomes from patients with oral cancer, exosomes of oral cancer cell origin, and exosomes obtained from circulating plasma or purified serum from patients with oral cancer.

The difference between oral cancer and other tumours is that the relationship between oral cancer and exosomes can be studied using salivary exosomes, in addition to tumour cell-, plasma-, or serum-derived exosomes. Therefore, it is hypothesized that there is still much room for discovering exosomes associated with oral cancer development as a possible way to find emerging oral cancer diagnostic methods and alternative treatment strategies.

Salivary Exosomes in Oral Cancer Patients

Different components of the salivary exosomes of patients with oral cancer may be directly related to oral cancer progression. The most studied oral cancer target is RNA (miRNAs account for the vast majority). miRNAs are small, non-coding RNA molecules consisting of 19–25 nucleotides that can regulate gene expression by degrading or inhibiting translation through complementary binding to a portion of the target mRNA sequence.⁸⁷ Langevin et al⁸⁸ performed miRNA microarray analysis of salivary exosomes from head and neck squamous cell carcinomas and the corresponding healthy controls. Compared with the corresponding controls, differences in miRNA expression levels were found in salivary exosomes from patients with head and neck squamous cell carcinoma. To obtain more specific results, He et al⁸⁹ evaluated the miRNA microarray analysis of salivary exosomes from patients with OSCC compared to the corresponding control saliva samples, and the differences in miRNA expression levels were determined. In addition, Momen-Heravi et al⁹⁰ provided a detailed review of the emerging role of non-coding RNAs in oral cancer. The RNAs associated with salivary exosomes in oral cancer and their differentially expressed salivary exosomal proteins suggest a link between exosomes and oral cancer. Some investigators have experimentally found a significant increase in the number and irregular morphology of salivary exosomes in oral cancer. These suggest that there are differences in morphology, number, surface markers and contents of saliva-derived exosomes between oral cancer patients and healthy controls and that exosomes may be the “key” to the early diagnosis of oral cancer.

Oral Cancer Cell-Derived Exosomes

Oral cancer cell-derived exosomes can promote oral cancer progression by modulating the immune response, promoting angiogenesis, and promoting aggressive metastasis.^{34–36,91} It has been shown that polarized tumour-associated macrophages (TAMs) can promote tumour growth and metastasis by producing large amounts of growth factors and cytokines,

or by inhibiting the proliferation of effector T cells and directly suppressing the T cell immune response to cancer.^{63–65} Oral cancer cell-derived exosomes can influence oral cancer progression by affecting the phenotype of the host's macrophages. It has been found that OSCC cell-derived exosomes can polarize macrophages into M1-like TAMs⁹² or modulate, enhance, and induce macrophage transition to the M2 phenotype^{93–96} which in turn affects the proliferation, invasion and migration of OSCC.

Blood supply plays a crucial role in cancer progression by providing nutrients for tumour growth and metabolism.⁹⁷ The expression levels of exosomal miRNAs in OSCC cells were different compared to the corresponding controls, and differentially expressed exosomal miRNAs were associated with angiogenesis, migration of vascular endothelial cells, and microvessel density.^{98,99} It is hypothesized that OSCC cell-derived exosomes could also promote oral cancer progression by promoting angiogenesis.

In addition, highly invasive OSCC cell-derived exosomes can induce a phenotypic shift from low-invasive OSCC cells to highly invasive or promote the motility of low-invasive OSCC cells, promoting OSCC invasion and migration. Exosomes isolated from the highly metastatic human oral cancer cell line HOC313-LM can promote tumour cell growth by activating ERK and AKT, transferring biomarkers with highly aggressive characteristics to less aggressive cells, increasing the motility of low invasive OSCC cells, and leading to increased cancer cell motility and invasiveness.¹⁰⁰ In contrast, exosomes from highly invasive OSCC cell lines can induce a shift in non-invasive OSCC cell lines to a highly invasive phenotype, which in turn promotes OSCC invasion and migration.¹⁰¹

Exosomes Obtained by Isolation and Purification of Circulating Plasma or Serum from Oral Cancer Patients

There is a large amount of research data on the relationship between exosomes and diseases related to liver,¹⁰² kidneys,¹⁰³ lungs,¹⁰⁴ cardiovascular diseases,^{105,106} and diseases affecting the central nervous system.¹⁰⁷ In contrast, research data related to the relationship between circulating plasma and purified exosomes isolated from patients with oral cancer are scarce and need to be explored through continued research. It has been shown that upregulation of circ_0000199 in circulating plasma exosomes from patients with OSCC promotes cell proliferation and inhibits apoptosis, and is positively associated with poorer survival outcomes, whereas in knockdown experiments the opposite was noted.¹⁰⁸ In addition, Guo et al¹⁰⁹ identified several differentially expressed proteins in serum exosomes of patients with OSCC, including C-reactive protein, vascular haemophilia factor, and leucine-rich alpha-2-glycoprotein, as potential biomarkers for the specific diagnosis of OSCC. They also found that OSCC-associated serum exosomes might promote the migration of oral cancer cells.

Current Status of Research on Exosomes in the Diagnosis and Treatment of Oral Cancer

Current Status of Exosomes in the Diagnosis of Oral Cancer

Surgical tissue biopsy is considered the gold standard for diagnosing solid tumours. Morphology-based biopsy is often physically invasive and time-consuming, adding excessive stress and pain to the patient. In addition, different sites of the primary tumour exhibit extensive inter- and intra-tumour variability, this tumour heterogeneity makes it difficult to determine an exact and effective treatment plan based on a single biopsy resulting in the need for another invasive biopsy.^{110–113} In these situations, this diagnostic approach not only raises the cost of treatment but may also result in potential complications, leading to reduced patient compliance. Over the past few decades, exosome-based liquid biopsies have received much attention from clinicians and scientists, with the advantages of easy access, convenience, non-invasiveness, time and effort savings, high reliability, reproducibility, ease of early detection, low cost, and high benefit.³⁷ They can also track and monitor tumour progression and drug resistance in real time allowing for continuous sampling to provide information on tumour heterogeneity,¹¹⁴ making it an ideal method for early screening and diagnosis of oral cancer. Figure 2 provides a summary of exosomes as a source of biomarkers for oral cancer diagnosis.

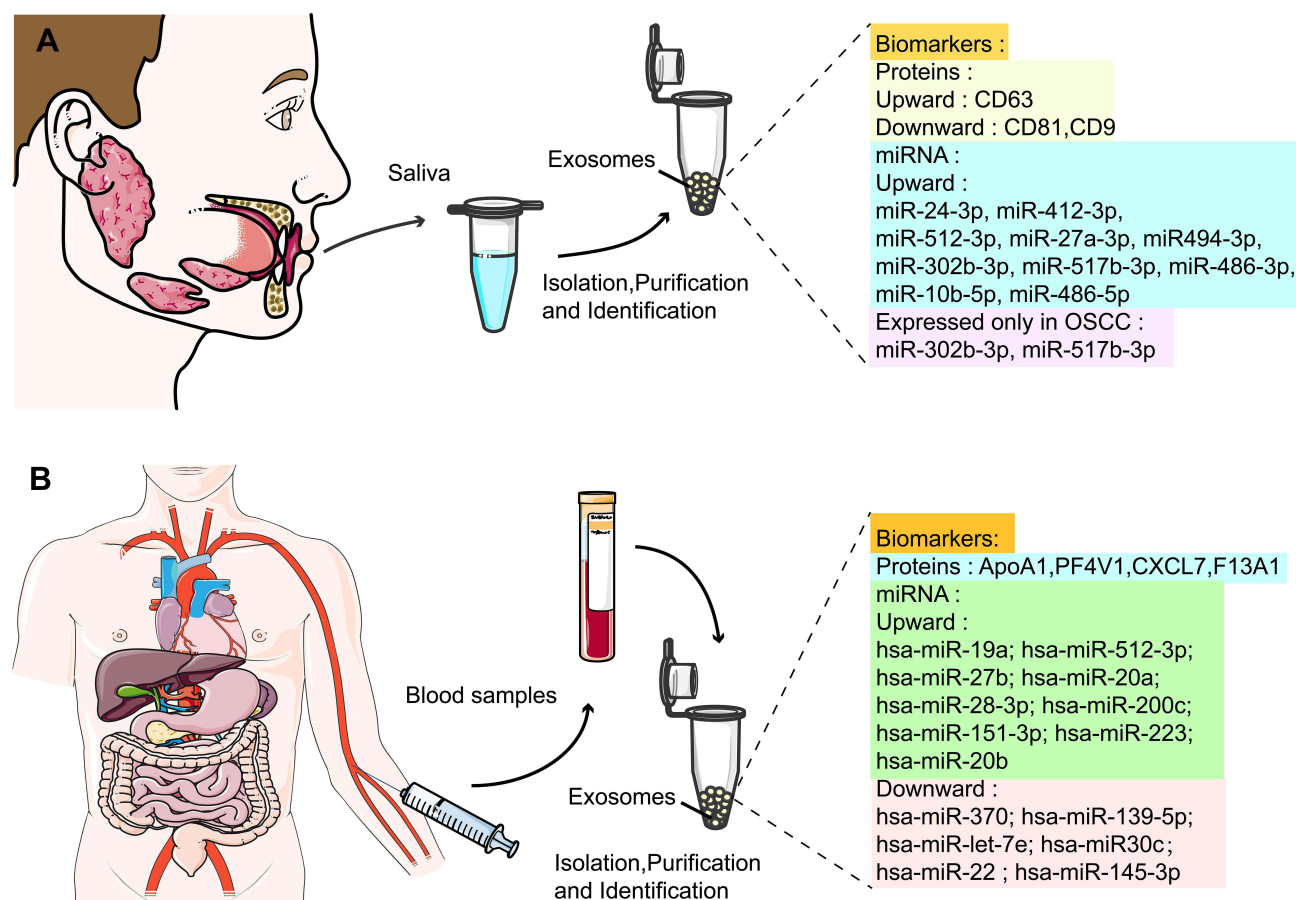


Figure 2 Exosomes as biomarker sources for oral cancer diagnosis. **(A)** Salivary exosomes in patients with oral cancer as potential biomarkers for the diagnosis of oral cancer. **(B)** Plasma or serum purified exosomes in patients with oral cancer as potential biomarkers for the diagnosis of oral cancer.

Salivary Exosomes from Oral Cancer Patients as a Potential Tool for Oral Cancer Diagnosis

Saliva is an emerging biofluid for the early detection and diagnosis of diseases, reflecting the relevant conditions of oral and systemic health, and is a valuable source of clinically relevant information of these diseases.¹¹⁵ Saliva is secreted by three pairs of major salivary glands (parotid, submandibular, and sublingual) and numerous minor salivary glands throughout the oral cavity.^{116,117} The acquisition of salivary exosomes is not only painless, easy to obtain, cost-effective, and efficient, but also avoids unnecessary complications caused by invasive biopsies or blood collection, thus increasing patient compliance and motivation for treatment. The identification of alterations in salivary exosome production and abnormal expression of its contents may provide a new direction for oral cancer diagnosis. Recent studies have demonstrated that miRNAs in human salivary exosomes are potential biomarkers for oral cancer diagnosis. One researcher collected miRNA sequencing results from salivary exosomes of head and neck squamous cell carcinoma (HNSCC) patients and their corresponding healthy controls, and found that miR-486-3p, miR-10b-5p, and miR-486-5p were expressed at significantly higher levels in the salivary exosomes of HNSCC patients.⁸⁸ The miRNA sequencing results of salivary exosome samples from OSCC patients showed that miR-24-3p, miR-412-3p, miR-512-3p, miR-27a-3p, and miR-494-3p expressions were significantly upregulated in salivary exosomes from patients with OSCC compared to that in their corresponding healthy controls, whereas miR-302b-3p, miR-517b-3p were only expressed in salivary exosomes from patients with OSCC.^{25,89} These studies suggest that miRNAs in salivary exosomes could be a potential resource for the diagnosis and differential diagnosis of oral cancer and may become a new oral cancer biomarker. However, more practical clinical data are required to confirm their diagnostic accuracy. Nevertheless, many differentially expressed RNAs still exist in the saliva of patients with oral cancer, and these RNAs hold great promise for research. These findings may aid in the diagnosis of oral cancer. In addition, exosomal protein markers are expected to be a new way to diagnose oral cancer by

specifically identifying signature proteins that are differentially expressed on the surface of salivary exosomes in oral cancer patients to distinguish oral cancer patients from healthy individuals. In 2011, Sharma et al⁹⁰ used high-resolution atomic force microscopy to show the differences between salivary exosomes from healthy individuals and patients with oral cancer. They found that exosomes in the saliva of oral cancer patients were irregular in shape and increased in size, number, and aggregation, especially oral cancer exosomes with a significantly higher surface density of CD63. Later, Zlotogorski-Hurvitz et al⁴² used nanoparticle tracking analysis to evaluate saliva samples from 36 patients with oral cancer and 25 healthy individuals, validating the claims of Sharma et al. They showed differences in the expression of exosome markers by ELISA and Western blotting between patients with oral cancer and healthy individuals showing higher expression of CD63 and lower expression of CD81 and CD9. Therefore, according to the morphological, molecular, and surface marker expression differences of salivary exosomes between patients with oral cancer and healthy individuals, salivary exosomes can be used as a potential tool for the early screening and auxiliary diagnosis of oral cancer patients. However, saliva is a complex fluid that is rich in a variety of proteins,¹¹⁸ and some salivary impurity protein components may be mixed with the salivary exosomes obtained by isolation. This may mask the presence of low levels of salivary exosomal proteins that may be important biomarkers¹¹⁹ and thus interfere with the accuracy of salivary exosome-based diagnosis of oral cancer. Therefore, studies on specific exosomal proteins that are directly related to oral cancer progression are scarce. At present, some scholars have obtained the proteome map of human parotid exosomes using multidimensional protein identification technology,¹²⁰ which may be an auxiliary means to discover exosome protein markers related to oral cancer.¹²¹

Purified Exosomes from Plasma or Serum of Oral Cancer Patients as Potential Substances for Oral Cancer Diagnosis

The acquisition of salivary exosomes has significant advantages (eg the body cannot tolerate frequent blood collection) and provides a positive patient experience. Compared to salivary exosomes, exosomes obtained from plasma or serum purification may be more stable and less susceptible to interference. These may provide more accurate information on relevant biomarkers with differential expression. Therefore, differential expression of plasma or serum-purified exosome-associated biomarkers in oral cancer patients may be more representative, accurate, and suitable for real-time tracking and assessment of oral cancer progression. It has been shown that ApoA1, PF4V1, CXCL7, and F13A1 in serum-purified exosomes are valuable as potential markers for detecting metastasis of oral squamous cell carcinoma. Combining these biomarkers can improve the sensitivity of OSCC diagnosis and reduce clinical misdiagnosis in patients with OSCC with lymph node metastasis.¹²² Many differentially expressed miRNAs were also present in plasma or serum purified exosomes from patients with oral cancer compared to healthy individuals.

It was recently shown that circulating plasma exosome circ_0000199 expression levels were significantly higher in OSCC patients than healthy controls,¹⁰⁸ and may be a potential biomarker for the diagnosis of oral cancer. Similarly, Rabinowits et al¹²³ screened several exosomal miRNAs from tumour tissue samples and the plasma of patients with squamous cell carcinoma of the tongue. Sixteen of these miRNAs were differentially expressed between tumour tissues and matched benign tissues, while 15 of them had the same expression profile in plasma exosomes: nine were upregulated (hsa-miR-19a, hsa-miR-512-3p, hsa-miR-27b, hsa-miR-20a, hsa-miR-28-3p, hsa-miR-200c, hsa-miR-151-3p, hsa-miR-223, and hsa-miR-20b), and six were downregulated (hsa-miR-370, hsa-miR-139-5p, hsa-miR-let-7e, hsa-miR-30c, hsa-miR-22, and hsa-miR-145-3p). These differentially expressed miRNAs can be used as non-specific biomarkers of oral cancer. The combined application of these markers may aid in improving the sensitivity of oral cancer diagnosis and tracking dynamic changes in oral cancer. The application of relevant biomarkers in exosomes for oral cancer diagnosis has considerable research prospects and room for further exploration.

Current Status of Exosomes in Oral Cancer Treatment

The combination of surgical excision of lesions and radiotherapy remains the main treatment option for oral cancer; however, the results are unsatisfactory. Currently, the search for emerging therapeutic modalities to reduce the chemotherapy resistance rate, improve drug targeting, and improve patient survival rate remains a direction for future efforts. Recently, exosomes have received considerable attention as drug carriers and targets for the treatment of oral cancer (Table 2).

Table 2 Status of Exosomes in Oral Cancer Treatment

Key Cargo	Effect	Biological Function	Intervention Measures and Potential Effects	Proposed Mechanism	Exosomes from	Ref
miR-1294	Inhibits Proliferation	Inhibit OSCC cell proliferation and migration	Up-regulated of exosomes miR-1294/inhibit the growth and migration of OSCC cells	Regulation of c-Myc pathway	OSCC	[152]
miR-6887-5p	Inhibits Proliferation	Inhibit tumour growth and OSCC cell colony formation	Up-regulated of exosomes miR-6887-5p /inhibit the progression of oral cancer	/	OSCC	[153]
miR-101-3p	Inhibits Proliferation, Metastasis	Inhibit the progression of oral cancer	Up-regulated of exosomes miR-101-3p /inhibits the progression of oral cancer	Downgrade COL10A1	HBMSCs	[154]
miR-221	Angiogenesis	Promote HUVEC migration and angiogenesis	Down-regulated of exosomes miR-221 /inhibits the progression of oral cancer	Downgrade PIK3R1	OSCC	[98]
miR-210-3p	Angiogenesis	Promoting angiogenesis in OSCC	Down-regulated of exosomes miR-210-3p/inhibit-s the progression of oral cancer	Activation of PI3K/AKT pathways and Downgrade ephrinA3	OSCC	[99]
miR-21	Promoting progression	Induction of cisplatin resistance	Down-regulated expression of exosomes miR-21 / Enhance chemotherapy sensitivity	/	Cisplatin-resistant OSCC cells	[163]
miR-200c	Inhibiting progression	Combining TUBB3and PPP2R1B makes HSC-3DR more sensitive to DTX	Up-regulated the expression of exosomes miR-200c/ Enhance chemotherapy sensitivity	Combined with TUBB3 and PPP2R1B	NTECs	[91]
miR-23a-3p	Promoting proliferation and invasion	Promote the transformation of macrophages to M2 phenotype, thereby promoting the proliferation and invasion of OSCC	Down-regulated the expression of exosomes miR-23a-3p/ inhibit proliferation and invasion of oral cancer	Activation of SOCS1/STAT1 pathways	OSCC	[93,94,96]
miR-29a-3p	Promoting proliferation and invasion	Promote M2-like macrophage polarization, promoting OSCC proliferation and invasion	Down-regulated the expression of exosomes miR-29a-3p/inhibit proliferation and invasion of oral cancer	Activation of ERK1/2 pathway	OSCC	[93,94]
lncRNA FLJ22447	Promoting Proliferation	Activation of Lnc-CAF by IL-33 induces OSCC cell proliferation	Lnc-CAF knockdown or IL-33 knockdown can reduce tumour cell proliferation	/	OSCC	[172]
EGFR	Promoting invasion and migration	EGFR-rich exosomes can enter epithelial cells and promote EMT, facilitating the invasion and migration of OSCC	Therapeutic antibodies against EGFR/ inhibit OSCC invasion and migration	/	OSCC cells stimulated by EGF	[160]
THBS1	Promoting migration	Promoting macrophage polarization to M1-like tumour-associated macrophages and promoting OSCC migration	Down-regulated the expression of exosomes THBS1/ inhibit migration of oral cancer	/	OSCC	[92]
CMTM6	Promoting proliferation and invasion	Promote M2-like macrophage polarization, promoting OSCC	Down-regulated the expression of exosomes CMTM6/ inhibit proliferation and invasion of oral cancer	Activation of ERK1/2 pathway	OSCC	[94,95]

(Continued)

Table 2 (Continued).

Key Cargo	Effect	Biological Function	Intervention Measures and Potential Effects	Proposed Mechanism	Exosomes from	Ref
PD-L1	Promoting invasion and migration	Drive immunosuppression	Down-regulated the expression of exosomes PD-L1/inhibit invasion and migration of oral cancer	/	Oral cancer	[167,168]
NAPI	Inhibiting progression	NAPI enhances cytotoxicity of natural killer cells	Up-regulating the expression of NAPI-rich exosomes/inhibit OSCC progression	Activation of IRF-3 pathway	Oral cancer	[171]
primary human macrophage (PHM)-derived exosomes	Inhibiting progression	Reduced sensitivity of OSCC cells to chemotherapeutic agents	Inhibition of interaction between OSCC cells and macrophage-derived exosomes/ Enhance chemotherapy sensitivity	Activation of AKT/ GSK-3 β pathways	Macrophages	[164]
OSCC-derived exosomes	Promoting proliferation invasion and migration	/	Down-regulating the expression of exosomes from OSCC/inhibit OSCC progression	Activation of PI3K/ Akt, MAPK/ERK, JNK-1/2 pathways	OSCC	[175]

Exosomes as Natural Drug Delivery Vehicles for Oral Cancer

The interaction of therapeutic agents with non-targeted sites in the body (ie, off-target effects) can lead to harmful side effects, such as those observed in cancer chemotherapy. Tumours and health tissue can experience significant toxic side effects during chemotherapy, which is a major problem in oncology. The delivery of drugs to target organs and the prevention of toxic side effects remain the current focus of researchers. Precise targeted delivery of chemotherapeutic drugs may be a potential way to reduce the toxic side effects of chemotherapy.¹²⁴ Recent advances in drug delivery methods aim to enhance targeting strategies, improve drug delivery rates, control drug release, and prolong drug action. The disadvantages of large materials, such as instability, low absorption rate, and low degradability in vivo, make them unsuitable for drug delivery applications. Based on the concept of targeted drug delivery, researchers have also worked on the development of non-toxic, stable, and biocompatible drug carriers.¹²⁵ Therefore, nanomaterial-based drug delivery methods have been favoured. Nanoparticle carriers can be classified as inorganic materials (eg metal nanoparticles) or organic materials (eg liposomes and exosomes).¹²⁶ Liposomes have been used as carriers for cancer therapy.¹²⁷ Compared with liposomes, exosomes have superior drug delivery properties, such as good stability, allowing them to travel to distant target organs, a hydrophilic core that can encapsulate water-soluble drug molecules, and high biosafety, which does not induce an immune response in the body.^{128,129} In addition, exosomes can easily deliver their contents to targets by fusing with cell membranes, binding to membrane surface receptors, and cytotocytosis. The nature of exosomes allows them to be loaded with many types of cargo (eg DNA, RNA, and proteins), and the loading of these cargoes can be achieved in vivo or in vitro.¹³⁰ Therefore, exosomes can be used as natural drug delivery vehicles for oral cancer.

According to the different methods of exosomal drug loading, the two main types are passive and active drug loading. We next describe the characteristics, advantages, and disadvantages related to these two drug loading methods of exosomes. The passive drug loading method of exosomes includes three forms: the first is to separate and purify the exosomes from the donor cell and mix them with the drug to be loaded.¹³¹ This method can ensure the integrity of the exosomal membrane, but the drug loading rate is low. The second form is to transfect the drugs to be loaded into the donor cells, and then encapsulate these drugs into the exosomes inside the donor cells.¹³² This approach is safe and effective for immunotherapy and cancer treatment, but the drugs may have toxic effects on the donor cells. The third form is the fusion of plasmid DNA containing the therapeutic protein code with the coding peptide capable of targeting the therapeutic protein to the exosomes, followed by transfection into the nucleus of the donor cell. Plasmid DNA is then transcribed and translated to produce a drug that can

be localized to the exosomes, ultimately allowing the drug to be integrated into the exosomes.¹³³ This is a way to consistently produce exosomal drugs, but it is mechanistically complex, time-consuming, and costly. Because passive drug loading of exosomes does not use any external physical or chemical factors, this method does not affect the integrity of exosomes and is a technique that can be considered. Although the passive loading method for exosomes has the above advantages, this method has a low loading rate and is time-consuming. To increase the loading rate and shorten the loading time in case of exosomes, researchers normally use the active drug loading method.

There are four main approaches to active drug loading in exosomes. The first is the electroporation method, in which the exosomal membrane is pored by an electric field, allowing the therapeutic drug to penetrate the exosomes.¹³⁴ This is a widely used method for transporting hydrophilic molecules to the hydrophilic core of exosomes. The second method is the extrusion method, in which isolated and purified exosomes are mixed with therapeutic drugs and passed simultaneously with an extruder through a multi-pore membrane with a pore size of approximately 100–400 nm.¹³⁵ The drug loading rate of exosomes induced by the extrusion method is affected by the number of extrusions, type of exosomes, and type of transfected cells.¹³⁶ This method has good drug loading capacity, but affects the integrity of the exosomal membrane, and the end product may be cytotoxic. The third form is the sonication method, in which the ultrasound energy provided by the probe sonicator stimulates the exosome and affects its integrity, allowing the therapeutic drug to penetrate the exosome during exosome deformation.¹³⁷ This method increases the drug-carrying capacity in the hydrophobic bilayer, but increases the size of the exosome. The fourth method is the freeze-thaw cycle method, which involves freezing and then thawing a mixture of exosomes and drugs.¹³⁸ However, both the electroporation and freeze-thaw cycling methods can cause aggregation of exosomes. There are reviews detailing the methods of exosomal drug loading and the use of exosomes in drug delivery and therapy.¹³⁹ Based on the unique advantages of drug loading by exosomes and their diverse drug loading modes, a solid theoretical and methodological basis has been established for their use as natural drug delivery carriers for oral cancer.

In addition, studies have reported exosomes as carriers of therapeutic small molecules, proteins, and nucleic acids for therapeutic application in diseases. For example, a study reported that exosomes loaded with small molecules, such as adriamycin or paclitaxel, could easily cross the blood-brain barrier for the treatment of brain tumours.¹⁴⁰ Similarly, exosomes loaded with curcumin, a small molecule drug, have inhibited the proliferation of breast cancer cells.¹⁴¹ Exosomes can also deliver therapeutic proteins (such as enzymes, cytoskeletal proteins, and transmembrane proteins) or therapeutic nucleic acids (such as miRNAs) to the corresponding recipient cells, resulting in phenotypic changes in the recipient cells and ultimately inhibiting tumour development as detailed in another review.⁶² For example, some researchers have successfully loaded proteins into the lumen of exosomes, which then efficiently deliver the loaded proteins to the target cells, leading to a significant increase in intracellular protein levels and their functions.¹⁴² Similarly, Zhang et al found that exosomes collected from transfected cells enriched with anti-c-Met siRNA significantly inhibited tumour growth in a mouse transplant tumour model and reversed cisplatin resistance in gastric cancer cells in vitro.¹⁴³ Meanwhile, there are also reviews detailing new advances in exosome-based targeted drug delivery systems. Therefore, exosomes loaded with therapeutic small molecules, proteins, and nucleic acids are also a promising modality for the treatment of oral cancer (this will be explored in detail in later chapters).¹²⁶ Using flow cytometry and in vivo uptake analysis, Wang et al¹⁴⁴ confirmed that the uptake of exosomes by tumour cells was higher than that of macrophages and other immune cells at the single-cell level, suggesting that exosomes can inhibit oral cancer progression by loading drugs or carrying related substances (eg DNA, RNA, or protein), targeted delivery to the tumour site, and accumulation of anticancer treatment. In addition, exosomes can be modified through gene editing or protein modifications to increase their anti-tumour targeting.¹⁴⁵ Several studies have shown that exosomes derived from bone marrow mesenchymal stem cells (MSC) and epithelial cells do not induce toxicity when repeatedly injected into mice.^{146,147} Repeated injections of MSC-derived exosomes are well tolerated without adverse or significant side effects. They can be used for their own treatment¹²⁹ and for treating patients with immune rejection after transplantation.¹⁴⁸ This indicates that exosomes have the advantage of good biocompatibility, almost nontoxic side effects, and can be used as a sound drug delivery system. In addition, the lipid bilayer membrane of exosomes protects their contents from degradation and destruction, making them highly stable in the circulation. Moreover, the small size of exosomes can easily cross various biological barriers (eg blood-brain barrier) and migrate to target cells or organs. This allows

exosomes to maintain their potency and to target distant locations. The use of exosome-loaded drugs for targeted cancer treatment has been reported,¹⁴⁹ revealing a new direction for using exosomes to load and deliver medicines to treat oral cancer.

Exosomes as a Target for Oral Cancer Treatment

Currently, the common treatment options for malignancies include surgery, radiotherapy, chemotherapy, targeted therapy, and adjuvant therapy,¹⁵⁰ most of which can cause significant side effects and financial burden. The development of therapies with low side effects, high benefits, low cost, and high specificity is the goal of researchers. In recent years, there have been an increasing number of studies related to exosomal proteins, miRNAs, lncRNAs, and circRNAs as potential targets for treating oral cancer.⁶²

Since exosomes contain numerous bioinformatic molecules related to the development of oral cancer, it may be possible to consider inhibiting the progression of oral cancer by suppressing the expression of these associated molecules in exosomes. To date, the field of oral cancer has been most studied for exosomal miRNAs, which can control cell division, differentiation, and death, and play an essential role in determining cell fate.¹⁵¹ The role of exosomal miRNAs in the proliferation, invasion, and migration of oral cancer cells has also been extensively studied. For example, the expression level of exosomal miR-1294 is low in OSCC tissue samples, and exosomal miR-1294 inhibits OSCC cell growth by regulating the c-Myc pathway. Upregulation of exosomal miR-1294 expression inhibited the proliferation and migration of OSCC cells, while inhibition of exosomal miR-1294 expression promoted the growth and migration of OSCC cells. Therefore, the expression of exosomal miR-1294 was negatively correlated with oral cancer progression and had an inhibitory effect on OSCC.¹⁵² Similarly, exosomal miR-6887-5p inhibited tumour growth and OSCC cell colony formation in vitro and in vivo. OSCC progression can be inhibited by upregulating the expression of exosomal miR-6887-5p in OSCC patients or by introducing miR-6887-5p-rich exosomes in vitro.¹⁵³ In addition, exosomes from human bone marrow mesenchymal stem cells (HBMSCs) also contain miRNAs associated with oral cancer development. miR-101-3p overexpression in HBMSC-derived exosomes inhibits the proliferation, invasion, and migration of oral cancer cells by downregulating COL10A1, thus inhibiting oral cancer progression. Moreover, a tumorigenic assay in nude mice further confirmed the inhibitory effect of HBMSC-derived exosomes carrying miR-101-3p on oral cancer.¹⁵⁴ This provides a basis for exosome-based targeted therapy for oral cancer, and exosomes are expected to be a potential therapeutic target for OSCC. When exosomes are used as a target for oral cancer therapy, the key is to determine how to increase the exosomes carrying capacity of miR-1294, miR-6887-5p, or miR-101-3p. As described above, exosomes are currently loaded with hydrophilic molecules, such as relevant miRNAs, mainly through the active loading method of electroporation, but this method leads to the aggregation of exosomes and thus a relative decrease in drug loading rate.¹⁵⁵ Therefore, finding new methods to increase the drug loading of exosomes remains the key for efficient treatment of oral cancer. Recently it was reported that Fe₃O₄ nanoparticles and a constant magnetic field can induce exosomal miR-21-5p upregulation in bone marrow MSCs, and this extrinsic induction condition (nanoparticles and constant magnetic field) may be a promising approach to increase exosomes capacity to be loaded with more miRNAs relevant to the treatment of diseases.¹⁵⁶ A related review has also described multifunctional nanomaterials with tunable physical, chemical and biological properties that may play a key role in exosome-based drug delivery.¹⁵⁷ This combination of nanoparticles and exosomes allows for therapeutic implementation on the same platform and has the advantages of providing targeting, improving dispersion, and avoiding clearance by the immune system. This approach of targeted transport of therapeutic exosomes by the combined action of magnetic nanoparticles and external magnetic fields may not only improve the precise localization of the drug in the organism, but also improve drug retention, prolong drug half-life, reduce drug dose, and improve efficacy (even in high blood flow systems).¹⁵⁸ This may be a promising approach to improve the effective drug loading rate and targeting of exosomes, which is another step forward in the clinical application of exosome-loaded drugs for the treatment of oral cancer.

Epithelial-mesenchymal transition (EMT) is closely associated with the invasion and migration of oral cancer cells, a dynamic process in which the migratory and invasive capacities of epithelial cells are enhanced by the loss of intercellular adhesion and polarity. EMT is considered the initiating step of the invasion-metastasis cascade.¹⁵⁹ Inhibition of the EMT process in oral cancer is a new direction for the treatment of oral cancer. It can inhibit the

progression of oral cancer by suppressing any response associated with the promotion of EMT. Fujiwara et al¹⁶⁰ showed that OSCC cells secrete large amounts of exosomes highly expressing epidermal growth factor receptor (EGFR) in response to epidermal growth factor stimulation. Subsequently, EGFR-rich exosomes enter epithelial cells and promote EMT, thereby promoting OSCC invasion and migration. The internalization and pro-EMT effects of OSCC exosomes were blocked by cetuximab. The therapeutic anti-EGFR antibody cetuximab inhibits OSCC cell invasion and migration. Therefore, it is hypothesized that targeted inhibition of exosomal EGFR expression or other biomarkers that promote the EMT process could be a new therapeutic strategy to inhibit oral cancer progression.

The blood supply plays a crucial role in cancer progression by providing nutrients for tumour growth and metabolism. Therefore, angiogenesis is associated with tumour growth and metastasis, and OSCC cell-derived exosomes may have an inhibitory or proangiogenic effect on angiogenesis, thus affecting OSCC progression.¹⁶¹ Angiogenesis is a critical step in tumour development and metastasis, and blocking angiogenesis is a way to discover new therapeutic approaches for oral cancer. In recent years, many studies have elucidated the important role of OSCC cell-derived exosomes in oral cancer angiogenesis. For example, He et al⁹⁸ found that OSCC-derived exosome miR-221 promotes human umbilical vein endothelial cell (HUVEC) migration and angiogenesis by targeting and negatively regulating PIK3R1. Similarly, OSCC-derived exosomal miR-210-3p levels were positively correlated with microvessel density. The expression of miR-210-3p could be upregulated by the PI3K/AKT pathway and downregulated by ephrinA3 expression to promote oral cancer angiogenesis.⁹⁹ Therefore, exosomal miR-221 and miR-210-3p may be closely related to angiogenesis during OSCC proliferation and may become a new target for the clinical treatment of OSCC. Therefore, it is hypothesized that inhibiting angiogenesis-related functions in oral cancer may be a new strategy for the effective clinical treatment of oral cancer.

Resistance to chemotherapy remains a major obstacle in the effective treatment of OSCC and enhancing the specificity and targeting of chemotherapeutic agents remains a critical problem. By establishing cisplatin-resistant cell lines (HSC-3-R and SCC-9-R), Liu et al¹⁶² found that the conditioned medium of cisplatin-resistant OSCC cells enhanced drug resistance in parental OSCC cells. Further studies have revealed that exosomes are involved in resistance transfer. Cisplatin-resistant OSCC cell-derived exosomes can induce cisplatin resistance by targeting phosphatase and tensin homologue (PTEN) and programmed cell death 4 (PDCD4) and transferring miR-21 into OSCC parental cells. Additionally, they established a subcutaneous transplantation mouse model to confirm the role of cisplatin-resistant OSCC cell-derived exosomes in vivo. They concluded that exosomes released from cisplatin-resistant OSCC cells induce cisplatin resistance in OSCC cells through delivery. Therefore, inhibiting exosome miR-21 expression by targeting can enhance chemotherapy sensitivity in OSCC patients and may be a new therapeutic approach for OSCC cisplatin-resistant patients. In contrast, under hypoxic conditions, OSCC cell-derived exosome miR-21 is directly regulated by HIF-1 α and HIF-2 α and its expression level is upregulated, which increases the migration and invasion of OSCC cells.¹⁶³ Therefore, blocking the pathway of exosome activation-related pathways, controlling in vivo environmental factors, or inhibiting the effect of related pathways by targeting may be a new approach to inhibit oral cancer progression. In addition, THP-1 cells and primary human macrophage (PHM)-derived exosomes can reduce the sensitivity of OSCC cells to chemotherapeutic drugs by activating the AKT/ GSK-3 β signalling pathway.¹⁶⁴ Therefore, targeted inhibition of the relevant signalling pathways may be a way to improve the sensitivity of oral cancer chemotherapy. In addition, Cui et al⁹¹ transfected docetaxel (DTX)-resistant HSC-3 cells (HSC-3DR) with lentivirus and co-cultured them with exosomes from normal tongue epithelial cells (NTECs) overexpressing miR-200c. Further studies revealed that NTECs transferred exosomal miR-200c into HSC-3DR cells and made HSC-3DR more sensitive to DTX by binding to TUBB3 and PPP2R1B. This suggests that exosomal miR-200c can enhance the sensitivity of patients with OSCC to chemotherapy, and exosomal miR-200c delivery may be a promising and effective strategy for treating drug resistance in tongue squamous cell carcinoma.

Exosomes also play important roles in regulating immune responses and coordinating immune system-related responses. Tumour-derived exosomes can activate or suppress the host immune system, suggesting that exosomes have potential therapeutic value in modulating immune responses against tumour progression and are promising candidates for immunomodulation in oral cancer.^{165,166} Recent studies have identified that THBS1 from OSCC cell-derived exosomes is involved in the polarization of macrophages to an M1-like phenotype, which polarizes macrophages to M1-like TAMs,⁹² and significantly promotes the migration of OSCC cells. In contrast, the OSCC cell-derived

exosomes miR-23a-3p,^{93,94,96} CMTM6,^{94,95} and miR-29a-3p^{93,94} promote macrophage polarization toward the M2-like phenotype by activating related signalling pathways, which in turn promotes OSCC proliferation and invasion. Therefore, THBS1, miR-23a-3p, CMTM6, and miR-29a-3p may be potential immunomodulatory targets for the treatment of oral cancer. Additionally, exosomal PD-L1 in the plasma of patients with oral cancer can drive immunosuppression and contribute to the immune escape of oral cancer cells.^{167,168} Moreover, PD-L1-driven immunosuppression can be reversed by anti-PD-L1.¹⁶⁹ Therefore, exosomal PD-L1 is a potential target for the treatment of oral cancer. Tumour-derived exosomes can not only mediate immunosuppression and thus promote tumour progression, but also interfere with immunotherapy.¹⁷⁰ In addition, exosomes can also act as immunosuppressive “immune enhancers”. Wang et al¹⁷¹ analysed the effects of oral cancer-derived exosomes on natural killer (NK) cells and found that oral cancer-derived exosomes (NF- κ B-activating kinase-associated protein 1) NAP1 enhanced the cytotoxicity of NK cells through the IRF-3 pathway and enhanced the tumour suppressor function of NK cells. Therefore, exosomes with high NAP1 expression can inhibit OSCC progression. In addition, oral cancer exosomal lncRNA can interact with the tumour microenvironment (TME) through a related mechanism, promoting oral cancer progression, and is a potential target for targeted treatment of oral cancer. Ding et al¹⁷² found that lncRNA FLJ22447 from OSCC exosomes significantly upregulated lncRNA-caf (Lnc-CAF) in tumour-associated fibroblasts. Lnc-CAFs activated by IL-33 can induce OSCC cell proliferation, and high Lnc-CAF/IL-33 expression correlates with high TNM (tumour, node, metastasis) stage, and high Lnc-CAF expression predicts poor prognosis. In vivo, Lnc-CAF or IL-33 knockdown reduced tumour cell proliferation and inhibited tumour growth. However, data from studies related to exosomal lncRNAs associated with oral cancer are lacking and need to be explored further.

Exosomes regulate intercellular communication between cancer cells and act in conjunction with the TME.^{173,174} Exosomes are important signalling mediators that regulate the TME and are closely related to its formation. Interfering with intercellular communication and TME formation by inhibiting the synthesis and release of exosomes may be an effective way to inhibit oral cancer progression. Sento et al¹⁷⁵ showed that OSCC-derived exosomes are taken up by OSCC cells and significantly promote OSCC proliferation, migration, and invasion by activating the PI3K/Akt, MAPK/ERK, and JNK-1/2 pathways. The pro-tumorigenic effect of OSCC-derived exosomes can be blocked by inhibiting the uptake of OSCC-derived exosomes by OSCC cells. Figure 3 provides a comprehensive summary of the progress of research on exosomes as potential targets for the treatment of oral cancer.

Opportunities and Challenges of Exosomes in the Diagnosis and Treatment of Oral Cancer

Opportunities of Exosomes in the Diagnosis and Treatment of Oral Cancer

Opportunities for Exosomes in Oral Cancer Diagnosis

Exosomes have great potential for clinical application as biomarkers for early diagnosis of oral cancer. Momen-Heravi et al¹⁷⁶ used a genome-wide high-throughput miRNA microarray assay and found that miRNA-27b was significantly upregulated in the saliva of patients with OSCC. Moreover, miRNA-27b had higher sensitivity and specificity for the detection of OSCC compared to the detection of other miRNAs. Similarly, miRNA-21 and miRNA-184 were significantly increased in the saliva of patients with OSCC, and miRNA-184 could distinguish OSCC from potential other oral malignant diseases and aid in the differential diagnosis.¹⁷⁷ These studies suggest that the saliva of patients with oral cancer is rich in biomarkers related to oral cancer progression. Exosomes as intercellular messengers may be loaded with these related substances, revealing that there may be more biomarkers in salivary exosomes of patients with oral cancer that are worth exploring. Exosomes may be a potential aid for oral cancer diagnosis.

Several studies have shown that DNA mutations associated with cancer development can characterize the genetic material carried in circulating blood exosomes, suggesting that the detection of serum exosome-associated DNA is a potential method for diagnosing cancer.^{178–181} It is hypothesized that if exosomal DNA provides a larger fragment of DNA than circulating free DNA, this may help detect relevant DNA mutations in circulating serum exosomes of oral cancer patients to diagnose oral cancer, thus avoiding the trauma and pain associated with cancer tissue biopsy. The expression of carcinogenic and tumour suppressor miRNAs in exosomes differs between cancer and normal cells,¹⁸² and

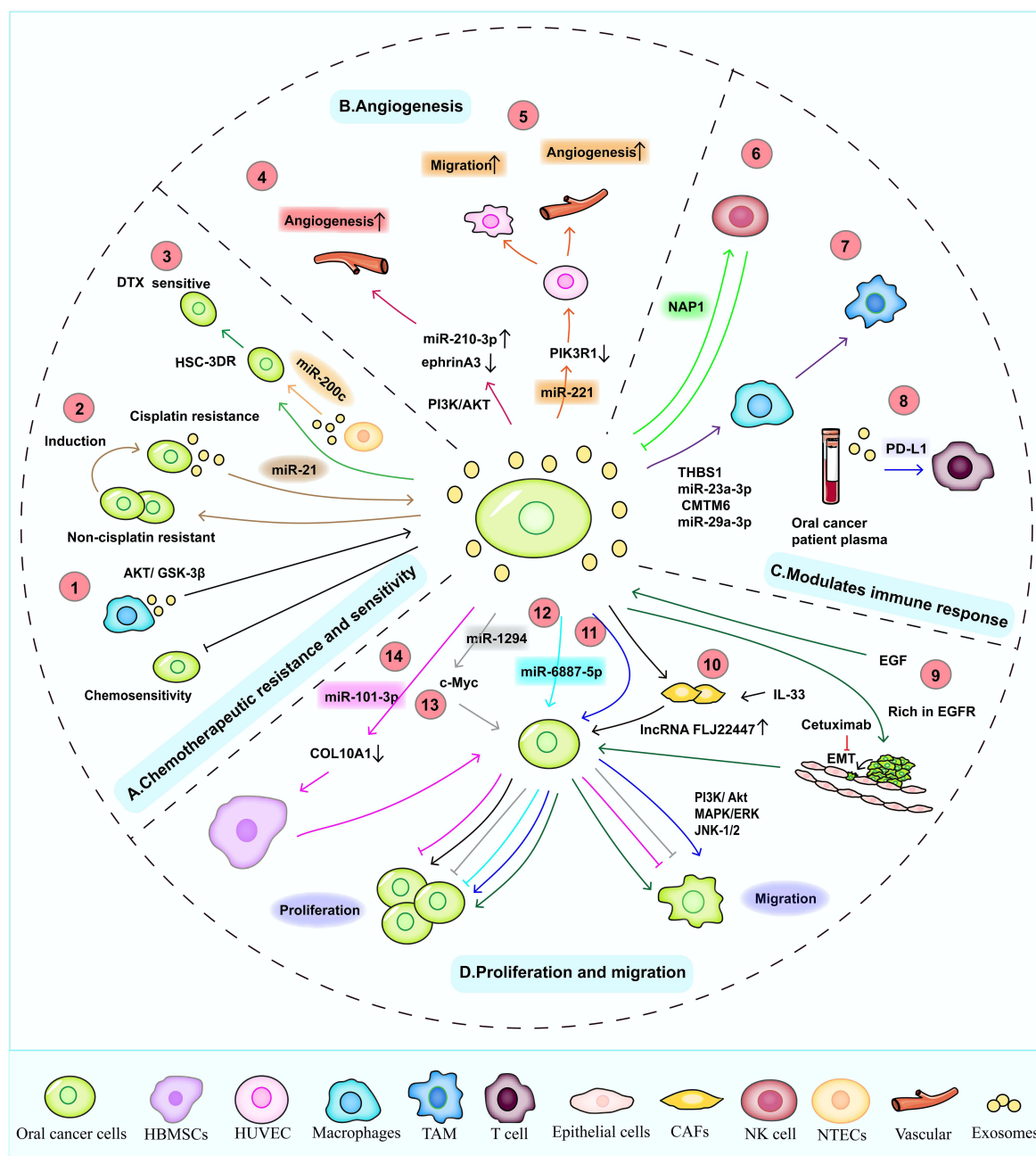


Figure 3 Summary of exosomes as potential targets for oral cancer therapy. **(A)** Chemotherapeutic resistance and sensitivity: ① THP-I and PHM-derived exosomes reduce the susceptibility of oral squamous cell carcinoma (OSCC) cells to chemotherapeutics via the activation of the AKT/GSK-3 β signalling pathway. ② Exosomes produced from cisplatin-resistant OSCC cells transmit miR-21 to OSCC parent cells to induce cisplatin resistance. ③ Normal tongue epithelial cells transfer exosomal miR-200c to HSC-3DR cells and combine with TUBB3 and PPP2R1B to increase the sensitivity of HSC-3DR cells to docetaxel. **(B)** Angiogenesis: ④ Exosomal miR-221 promotes the angiogenesis and migration of human umbilical vein endothelial cells by negatively regulating PIK3R1. ⑤ Additionally, the expression of exosomal miR-210-3p and ephrinA3 can be upregulated by the PI3K/AKT pathway to promote oral cancer angiogenesis. **(C)** Modulation of immune responses: ⑥ Oral carcinoma-derived exosome NAP1 increases the cytotoxicity of natural killer (NK) cells through the IRF-3 pathway and enhances the tumour suppressive function of NK cells. ⑦ However, OSCC cell-derived exosomes can induce the polarization or transformation of macrophages to the M1- or M2-like phenotype by activating related pathway signals, increasing the invasion and proliferation of OSCC. ⑧ Exosomal PD-L1 in the plasma of patients with oral cancer can drive immunosuppression and contribute to immune escape of oral cancer cells. **(D)** Proliferation and migration: ⑨ OSCC cells secrete many epidermal growth factor receptor (EGFR)-expressing exosomes in response to EGF stimulation. Subsequently, EGFR-rich exosomes can enter epithelial cells and promote epithelial-mesenchymal transformation, thereby promoting the invasion and migration of OSCC. ⑩ Activation of Inc-CAF by IL-33 can induce OSCC cell proliferation. ⑪ OSCC-derived exosomes are absorbed by OSCC cells and considerably boost the invasion, propagation, and transplantation of OSCC by activating the JNK-1/2, PI3K/Akt and MAPK/ERK pathways. ⑫ Exosomal miR-6887-5p inhibits tumour growth and OSCC cell colony formation. ⑬ Exosomal miR-1294 can suppress the growth of OSCC cells by regulating the c-Myc pathway. ⑭ HBMSC-derived exosomal miR-101-3p can suppress the invasion, propagation, and transplantation of oral cancer cells by downregulating COL10A1, thus inhibiting the progression of oral cancer. In conclusion, exosomal miR-21, miR-200c, miR-221, miR-210-3p, NAP1, THBS1, miR-23a-3p, CMTM6, miR-29a-3p, PD-L1, EGFR, Inc-CAF, miR-6887-5p, miR-1294, miR-101-3p, OSCC-derived exosomes and macrophage-derived exosomes are potential targets for oral cancer treatment.

there is an increasing number of studies related to cancer diagnosis based on exosomal miRNAs^{183–186} as the detection of specific miRNAs or miRNA groups in exosomes may be a new approach for oral cancer diagnosis. It has been suggested that combining multiple miRNAs may improve the diagnostic potential of exosomal miRNAs,¹⁸⁷ and the related mechanisms of occurrence need to be further investigated. In addition, there are many specific marker proteins on the surface of exosomes, which can specifically bond to these surface proteins by immunocapture for early diagnosis of cancer.^{187–190} According to the idea of previous studies, combining exosomal DNA, RNA and proteins for early diagnosis of cancer may improve the sensitivity and accuracy of exosomal marker-based diagnosis of oral cancer. In addition, more undiscovered or unstudied exosomal DNA, exosomal RNAs (such as miRNAs, lncRNAs, or circRNAs), and exosomal surface proteins can be explored for oral cancer progression, laying the foundation for emerging biomarkers for the clinical diagnosis of oral cancer. For example, Lu et al⁶² established a mouse model of chemically induced OSCC and found that some piRNAs were significantly altered. Recently, Wang et al¹⁹¹ identified piRNAs in exosomes of stem cells. However, the function of exosomal piRNAs in human oral cancer and their relationship with oral cancer need to be further investigated. In addition, because exosomes are widely found in various body fluids, exosome-based liquid biopsies can be used to monitor and track disease progression. This non-invasive method of monitoring disease progression not only benefits the development of optimal treatment strategies for the disease, but also reduces patient suffering. Based on exosomal liquid biopsy, it is also possible to develop intelligent devices for exosomal liquid biopsy to obtain test results and perform preliminary assessments in real time, making the diagnostic process more manageable, and less time-consuming and laborious.

Opportunities for Exosomes in Oral Cancer Treatment

Exosomes have considerable clinical potential for the treatment of oral cancers. The exosomes are small, biocompatible, almost non-toxic, highly permeable and induce less immune rejection,^{146,147} allowing them to be used as carriers for delivering drugs and related genetic material.^{192,193} Compared to conventional chemotherapeutic drugs, exosomes have the advantages of easy avoidance of first-pass metabolic effects, good stability and high targeting, making them an effective drug delivery system.^{128,194,195} Compared with the traditional, more desirable liposome or polymeric nanoparticle systems, exosomes have superior degradability, biosafety, and easy accessibility, effectively avoiding the degradation of autophagosomes and lysosomes, allowing direct delivery of cargo to the cytoplasm, which makes exosome-based targeted drug delivery possible for oral cancer treatment.^{128,195} In addition, CD47 on exosomes produce a signal that protects them from phagocytosis, and blood-derived ribonucleases may protect exosomal RNA from damage. These protective mechanisms may prolong the time for exosomes to remain and function in vivo, as well as allow exosomes to deliver cargo to more distant targets and perform their functional roles.^{196,197} Therefore, exosomes could be used as drug delivery vehicles to effectively deliver therapeutic agents to oral cancer lesions to inhibit the progression of oral cancer, which is good news in the search for new strategies to treat oral cancer.⁹⁶ Kandimalla et al¹⁴⁹ demonstrated that the use of colostrum-derived exosomes as a vehicle for the tumour-targeted oral agent paclitaxel not only improved the therapeutic efficacy against lung cancer in situ, but also reduced the systemic and immunotoxicity of conventional intravenous injections, providing a solid theoretical basis for the use of oral exosomal agents of paclitaxel as an alternative to current traditional lung cancer treatment regimens. On this basis, it is also important for researchers to find alternative options for oral cancer treatment using exosomes as carriers of oral cancer-targeted oral agents.

Since oral cancer-derived exosomes transport DNA, RNA, and surface proteins related to the development of oral cancer, their expression can be regulated by artificial intervention and targeting to inhibit the progression and metastasis of oral cancer. And they can exert a series of relevant effects on the receptor cells of the body.¹⁹⁸ Wang et al¹⁹⁹ found that the expression of miRNA-655-3p was significantly downregulated in OSCC tissues and cell lines. Moreover, miR-655-3p was found to inhibit OSCC proliferation and invasion by regulating the PTEN/AKT signalling pathway. Therefore, targeting specific receptor cells and acting through exosomes as messengers loaded with miR-655-3p or other relevant substances capable of inhibiting the development of oral cancer may be a new therapeutic strategy for patients with OSCC. The effective delivery of specific miRNAs or siRNAs by exosomes has been developed to treat central nervous system diseases and cancers.^{146,196,200,201} Therefore, delivering specific DNA, RNA, or proteins via exosomes has considerable research value as an entry point for treating oral cancer.

However, we can artificially modify or edit the expression of exosome-related genes or surface proteins to specifically target the surface receptors of the target cells and achieve precise blockage of the occurrence of related responses or activation of interconnected signalling pathways after binding, thus achieving the purpose of precise cancer treatment.²⁰² For example, some researchers have used programmable genotypic fusion vesicles with high affinity for SIRP α variants and PD-1 to block the effects of both CD47 and PD-L1 mechanisms, significantly enhancing the phagocytosis of cancer cells by macrophages and promoting antigen presentation to activate anti-tumour T-cell immunity. This bispecific targeting design better targets tumour cells and improves therapeutic efficacy, while reducing systemic side effects. It has also been demonstrated in model experiments of malignant melanoma and breast cancer.¹⁴⁵ Therefore, engineered exosomes through gene editing or protein modification are also very promising for research in the targeted treatment of oral cancer (eg through targeted inhibition of angiogenesis, invasive metastasis, and chemoresistant metastasis, enhancement of chemotherapy sensitivity, and modulation of the immune response).

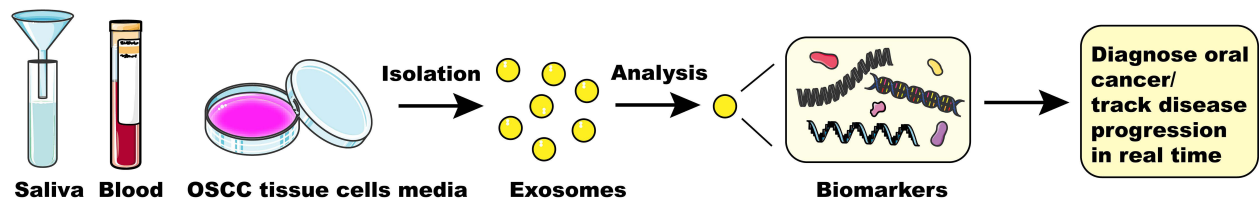
In addition, specific aptamers (also known as chemoantibodies) designed on the surface of drug-laden exosomes can guide drug-loaded exosomes to the appropriate cell surface and bind to the corresponding target site for action.^{202,203} RNA aptamers targeting vascular endothelial growth factor were the first to enter the clinic as therapeutic agents,²⁰⁴ heralding the great potential of such drug-loaded exosomes with aptamers designed on their surface for targeted treatment of oral cancer. The aptamer binds to the target with high affinity through the same mechanism as antibody-antigen binding.^{205,206} Compared to antibodies, aptamers have advantages, such as higher stability, lower toxicity, more remarkable tissue penetration properties, and absence of immunogenicity, making them more promising for application in precision medicine.^{207,208} Because aptamers are chemically synthesized without animals or cultured cells, they are not limited to expansion or batch differences.²⁰⁹ Therefore, novel artificially designed affinity ligands combined with exosomes loaded with specific drugs are expected to be the next generation of intelligently engineered exosomes for precision medicine. Aptamer-mediated exosome drug delivery has the advantages of low cost, high benefit, simple operation, and a good safety profile and is a research hotspot for intelligent nano-drug delivery systems. This heralds a new era of targeted delivery of nanomedicine for oral cancer treatment.

In addition to targeted therapy for oral cancer, exosomes may be used as vaccines to prevent oral cancer. Ovarian cancer ascites-derived exosomes in combination with granulocyte-macrophage colony-stimulating factor for colorectal cancer and autologous dendritic cell derived exosomes for the treatment of patients with metastatic melanoma have been previously suggested in Phase I clinical trials to be feasible, safe, and well-tolerated. These two types of subcutaneous immunotherapies may be options for treating advanced colorectal cancer and metastatic melanoma.^{210,211} Many scholars have developed vaccines by applying tumour-derived exosomes to restore tumour-specific immunity and promote tumour clearance.²¹² Therefore, a thorough understanding of the boundaries between oral cancer-derived exosomes and specific and intrinsic immunity can help explore the pathways by which oral cancer-derived exosomes affect the immune system to facilitate tumour clearance. However, most studies are still in the preclinical stage, and more prospective clinical trials are needed to support the application of exosomes in oral cancer treatment practice. Figure 4 outlines the opportunities for exosomes to be used in the diagnosis and treatment of oral cancer.

The Challenge of Exosomes in the Diagnosis and Treatment of Oral Cancer

There are still many problems to overcome before exosomes can be successfully used in clinical diagnosis and treatment. The study of exosomes in oral cancer diagnosis focuses on saliva, cells, and exosomes isolated from circulating plasma or serum. First, saliva has a complex composition and ensuring the stability of samples during transportation and storage remains challenging. Ensuring the stability of exosomes during long-term storage is an issue that needs to be studied. Because of their low content of specific proteins, serum-derived exosomes require large amounts of serum or culture medium to obtain sufficient exosomes.¹⁹⁸ At present, although there are many methods to extract exosomes, the purity and quality of the obtained exosomes are still not high. Obtaining a large number of ideal exosomes is time-consuming, laborious, costly, and inefficient. Combined application of multiple exosome extraction methods may solve this problem. In addition, some investigators have proposed the application of exosomes mixed with inorganic/organic nanoparticles to address the shortcomings of inefficient exosome isolation, low drug loading rate, difficulty in characterisation, and lack of specific biomarkers.¹⁵⁸ Exosomal hybrid inorganic/organic nanoparticles may provide good diagnostic and therapeutic

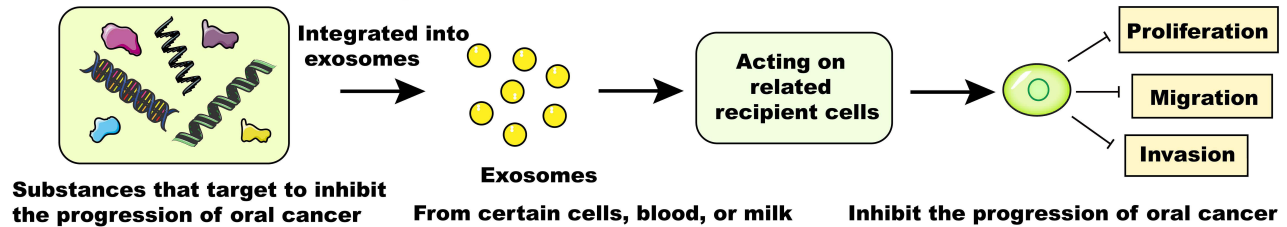
A Application of diagnosis



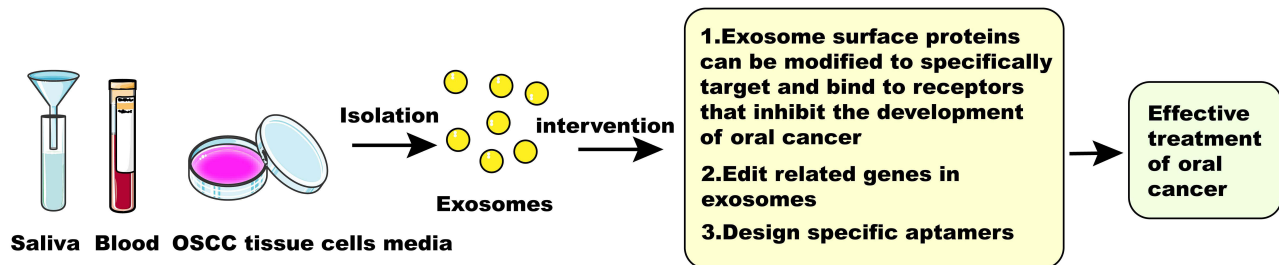
B Application of treatment

a.Exosome as drug delivery system

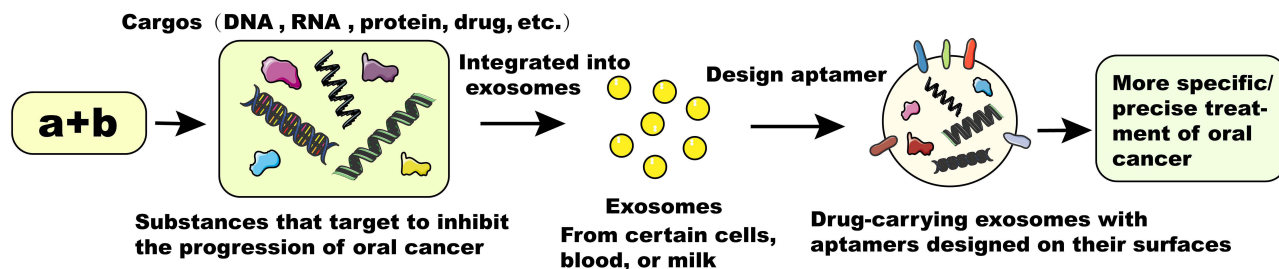
Cargos (DNA, RNA, protein, drug, etc.)



b.Artificial modification, editing exosome genes or surface proteins



c.Drug-loaded exosomes are combined with artificially designed aptamers



d.Development of oral cancer vaccine using exosomes

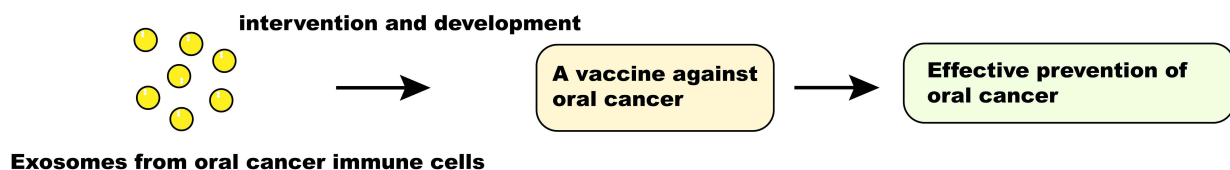


Figure 4 Opportunities of exosomes in diagnosis and treatment of oral cancer: (A) Application of diagnosis. (B) Application of treatment. A. Exosome as drug delivery system. B. Artificial modification, editing exosome genes or surface proteins. c. Drug-loaded exosomes are combined with artificially designed aptamers. d. Development of oral cancer vaccine using exosomes.

functions; however, more attention and evaluation of their safety, biocompatibility, and biodegradability are needed to ensure that inorganic/organic materials are safe, non-toxic, and do not accumulate in vivo.

Second, there is still a lack of unified standards for the acquisition, separation, and purification methods of exosomes, and the reference range of diagnostic content. The performance of exosomes is affected by many pre-analysis factors,²¹³ which reduces the reliability of exosome-based diagnosis of oral cancer. To overcome these obstacles, some researchers have established novel, highly sensitive, and specific exosome assays,^{214–216} but these assays are costly, complex, and time-consuming. Therefore, there is still a need to develop more economical, efficient, time-saving, and feasible exosome-based assays for clinical use of such a method for oral cancer diagnosis.

The application of exosomes in oral cancer treatment includes two aspects: drug delivery and targeted therapy. Before applying exosome-targeted drug delivery in clinical trials, the shortcomings of existing exosome delivery methods (such as low drug delivery rate) must be overcome to achieve clinical efficacy with a small number of exosomes, thus reducing their toxicity and cost.¹⁹⁴ Another important challenge for exosome-based targeted drug delivery for oral cancer is the need to meet the requirements of national regulatory agencies and obtain approval for exosome-based therapeutics. At present, some studies have shown that targeted treatment of oral cancer can be achieved by modifying exosome surface proteins or their contents.¹⁴⁵ However, these research results are still in the stage of animal experiments. Much clinical research support is still needed before these research results can be stably and reliably applied to clinical patients with oral cancer. However, clinical studies on exosomes in oral cancer treatment are usually time-consuming, laborious, and expensive. At present, although we have made great achievements in the application of exosome drug delivery, many challenges still need to be overcome (such as improving pharmacokinetic effects, optimizing targeting, and clinical application of mass production). Some investigators have suggested that it is highly unlikely that high-quality exosomes can be produced on a large scale for sustained clinical administration to achieve therapeutic effects. Therefore, premature clearance of exosomes in vivo remains one of the current challenges in the use of exosome-loaded drugs for oral cancer treatment.²¹⁷ The use of exosome-targeted therapy for oral cancer still needs to address the following issues (including determining the route of exosome delivery, the optimal dose of exosomes to be administered, the frequency of treatment, and the time interval between exosome deliveries) to ultimately achieve optimal clinical efficacy of exosomes without any adverse effects.¹⁹⁴

The development of an exosome-based vaccine for oral cancer seems promising.^{218,219} However, due to the potential of oral cancer-derived exosomes to inhibit anti-tumour responses and promote metastasis, extensive studies are needed to address safety issues related to oral cancer-derived exosomes. The heterogeneous origin and differential effects of oral cancer-derived exosomes may be a major challenge in future vaccine development. Therefore, a better understanding of oral cancer-derived exosomes and their effects on cancer progression is a challenging new research area. A significant amount of basic and clinical research is required before the currently proposed exosome-based vaccine for oral cancer prevention can be translated into clinical practice.

Despite these challenges, exosomes hold revolutionary implications and great promise for the diagnosis and treatment of oral cancer. As research continues, we expect the application of exosomes in oral cancer practice to arrive in the near future. Figure 5 summarizes the challenges of exosomes in the diagnostic and therapeutic applications related to oral cancer.

Summary

Exosomes are “messengers” that communicate between cells, transport their contents to recipient cells, and influence the progression of oral cancer by modulating host-related immune responses, tumour angiogenesis, drug resistance, or aggressive metastasis. The close association between exosomes and oral cancer has led to extensive studies of exosomes as oral cancer biomarkers, drug carriers, and therapeutic targets. Overall, exosome samples are obtained in a simple, low-cost, highly beneficial, less time-consuming, and non-invasive manner, which makes exosomes highly advantageous as biomarkers for oral cancer diagnosis and tracking disease progression, but the benefits and methods of exosome isolation and purification need to be improved. Exosomes have shown promise for clinical applications in cancer diagnosis.^{220,221} Based on the differentially expressed exosomal biomarkers between patients with oral cancer and healthy individuals and the biological functions and characteristics of exosomes, the use of exosomes to

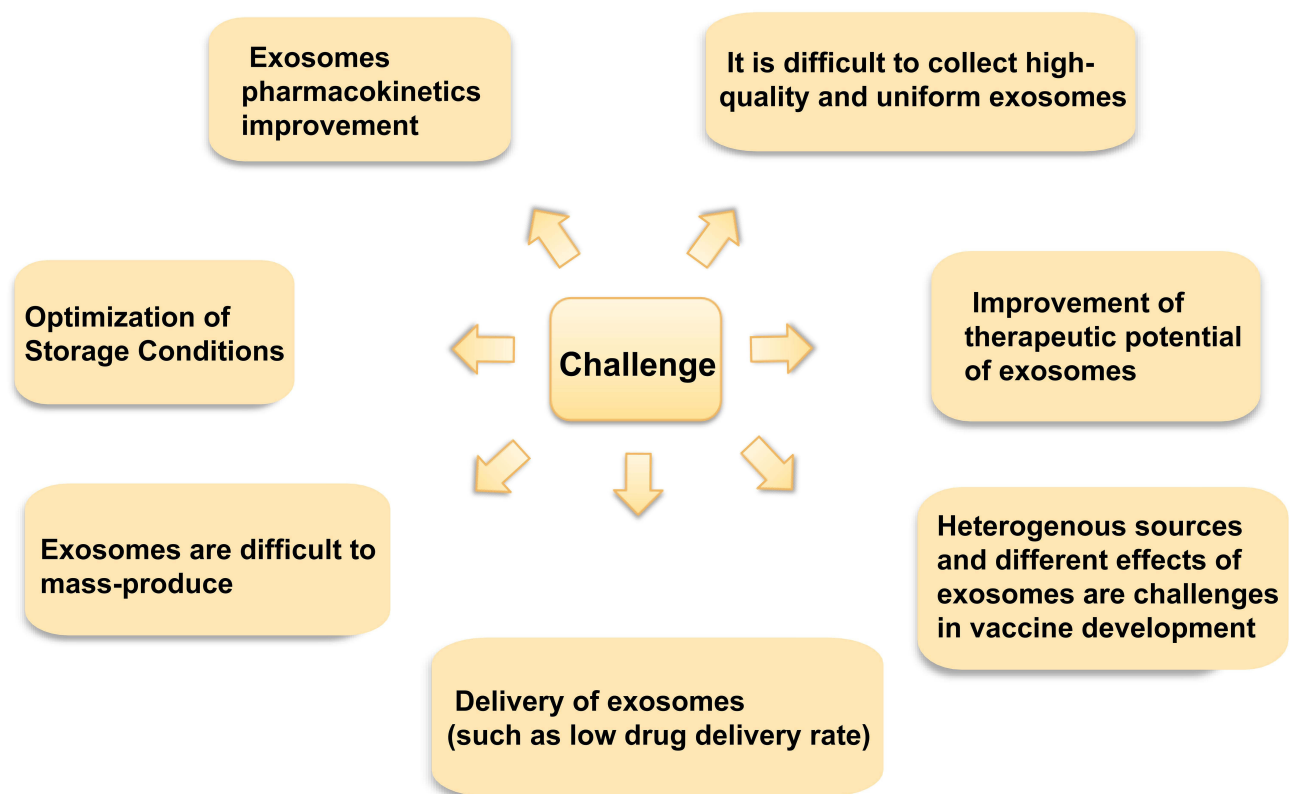


Figure 5 Challenges in the application of exosomes in the diagnosis and treatment of oral cancer.

specifically diagnose oral cancer is almost within reach. Second, the advantages of exosomes, such as good biocompatibility, high cancer uptake, high stability, and ease of crossing various biological barriers, make them a suitable drug delivery system for targeted treatment of oral cancer. Currently, exosomal drug delivery has been reported in the treatment of cancer.¹⁴⁹ However, there is a lack of research on exosomal drug delivery for oral cancer treatment, so it is worthy of further study in future work. Finally, initial results have been achieved in exosome-targeted therapy of oral cancer, and we can try to inhibit the progression of oral cancer by artificially integrating and wrapping relevant DNA, RNA, or proteins that can target and inhibit the progression of oral cancer into exosomes, so that they can interact with specific receptor cells of the body. Moreover, it can also influence the progression of oral cancer by regulating the expression of exosome-related DNA, RNA, and proteins, and intervene in the interaction between exosomes and receptor cells. Engineering smart exosomes to deliver drugs for precise targeting of oral cancer is currently a hot research topic that is receiving much attention. By artificially modifying the surface proteins of exosomes, the original biological functions of exosomes can be altered to specifically target oral cancer cells, thus achieving targeted drug delivery, or indirectly blocking the mechanism pathways related to oral cancer progression through aptamers, thereby inhibiting the progression of oral cancer. Notably, the development of exosome-based liquid biopsy diagnostic devices and vaccines for oral cancer prevention has also become a direction of research at this stage. Research on the role and mechanism of action of exosomes in oral cancer and the clinical application of diagnosis and treatment is still in its early stages. There are still many issues to be resolved, such as the timing of exosome collection, the quantity of sample required, the mode of obtaining exosomes, the preservation, isolation, and identification methods, the criteria of diagnosis, as well as the mode of administration, dose, frequency, and time window for drug administration. Therefore, further experimental studies and prospective clinical trials are needed to support the application of exosomes in clinical oral cancer diagnosis and treatment practice. Exosomes have shown application prospects in the diagnosis and treatment of oral cancer and further research on exosomes will provide new

directions for these applications. We speculate that exosomes could be used to diagnose and treat patients with oral cancer in the future.

Abbreviations

ALIX, apoptosis-linked gene 2-interacting protein X; DTX, docetaxel; EGFR, epidermal growth factor receptor; EMT, Epithelial-mesenchymal transition; ESCRT, endosomal sorting complexes required for transport; HBMSCs, human bone marrow mesenchymal stem cells; HNSCC, head and neck squamous cell carcinoma; HSC-3DR, docetaxel resistant HSC-3 cells; HUVEC, human umbilical vein endothelial cell; ILVs, intraluminal vesicles; MSC, mesenchymal stem cells; MVBs, multivesicular bodies; NK, natural killer; NSF, N-ethylmaleimide-sensitive factor; NTECs, normal tongue epithelial cells; OSCC, oral squamous cell carcinoma; PDCD4, programmed cell death 4; PHM, primary human macrophage; PTEN, phosphatase and tensin homologue; SNARE, soluble N-ethylmaleimide-sensitive factor NSF attachment protein receptor; TAMs, tumour-associated macrophages; TGN, trans-Golgi network; TME, tumour micro-environment; TSG101, tumour susceptibility gene 101.

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Disclosure

The authors report no conflicts of interest in this work.

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