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Exosomes' Role in the Early Diagnosis, Progression, and Therapy of Oral and Head and Neck Squamous Cell Carcinoma

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Abbreviations: MSCs: Mesenchymal Stem/Stromal Cells; mRNA: messenger RNA; miRNAs: MicroRNAs, EVs: Extracellular Vesicles; MVBs: Multivesicular Bodies; PDGF: Platelet-Derived Growth Factor; FGF: Fibroblast Growth Factor; EGF: Epidermal Growth Factor.

Abstract

Oral cancer includes about 2% of all malignancies, whereas the most prevalent kind is Oral Squamous Cell Carcinoma (OSCC), which constitutes for 90% of oral cancers with a poor prognosis and a superior local relapse. Since OSCC often spreads to lymph nodes in its advanced stages, the investigation of new treatment methods should be prioritized. Head and Neck Squamous Cell Carcinoma (HNSCC) is one of the predominant malignant tumors influencing human health as a result of late diagnosis and a superior rate of invasion. HNSCC patients have an overall poor survival rate despite recent improvements in treatment techniques. Hence, developing more efficient strategies continues to be a major concern. Small membrane vesicles known as exosomes are found in bodily fluids and are produced by endosomes. They have a range of mRNAs, non-coding RNAs, proteins, circular RNAs, ribosomal RNAs, tsRNAs, and piRNAs. Regulating the tumor microenvironment by exosomes provides the conditions for cancer expansion, and the transfer of their constituents to target cells is associated with the colonization, metastasis, and proliferation of OSCC and HNSCC. In the current study, we examine the part exosomes play in the emergence and identification of OSCC and HNSCC, besides their possible use in the therapy of these cancers.

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Introduction

The most prevalent oral malignancy, Oral Squamous Cell Carcinoma (OSCC), is a genetic condition characterized by a propensity for lymph node metastasis (LNM) [1]. The 5-year endurance rate for OSCC is only 50%, which is still a poor prognosis despite recent enhancements in treatment outcomes. Therefore, late diagnosis leads to the infeasibility of curative resections [2]. The sixth most prevalent kind of cancer globally is head and neck squamous cell carcinoma (HNSCC) [3]. The pharynx, larynx, and oral cavity are all parts of the head and neck region, and because they are all covered in squamous epithelium, most of head and neck tumors are squamous cell carcinomas [4]. The main HNSCC development sources are some genetic alterations and environmental factors like Human Papillomavirus (HPV) infection, alcohol consumption, and tobacco use [5,6,7]. Even though there have been numerous developments in HNSCC treatment approaches and molecularly targeted techniques, the 5-year endurance rate has not yet increased notably and is approximately 60% [8,9]. Exosomes are small (diameter: 30-150 nm), membranous, and extracellular endocytic microvesicles that are created after endocytosis, MVB formation, and finally secretion [10-13]. Extracellular vesicles (EVs), so called microvesicles, exosomes, and apoptotic bodies, are nanoscale lipid bilayer vesicles that are normally discharged from cells into the extracellular matrix (ECM) [14-17]**(Figure 1)**. According to recent studies, EVs can carry proteins, mRNAs, DNA fragments, and non-coding RNAs (ncRNAs) as "cargo", which can be used as diagnostic biomarkers for OSCC and HNSCC [12,13,18]. Exosomes have an important role in inducing long-distance intercellular connection signals by microRNA (miRNAs), mRNAs, and proteins [19,20,21].

Exosomal miRNAs regulate the expression of proteins included in the genomic instability, proliferation, apoptosis, and metastasis of tumor cells as well as immune responses, making them valuable diagnostic biomarkers for different malignancies. Serum miRNAs are able to be utilized as OSCC identification markers because research has shown that patients with OSCC have superior rates of exosomal miR-21 compared to healthy people and patients with chronic hepatitis [22]. Furthermore, exosomes are essential for the development of OSCC and HNSCC and their transformation manner should be investigated carefully to be able to obviously describe their role in diagnosis and treatment. This article reviews the recent advances of exosomes in the detection, progression, and treatment of OSCC and HNSCC and highlights the multifaceted functions of tumor micro environmental-derived EVs, exosomes exchanges between normal and cancer cells, and the noninvasive diagnostic/therapeutic applications of exosomes.

Exosome Biogenesis

The formation of early endosomes (EEs), whose membranes bud to cytoplasmic contents and result in the formation of intraluminal vesicles (ILVs), is the beginning biogenesis of exosomes [23,24]. Multivesicular Bodies (MVBs) or "late endosomes" (LEs) are created by dozens of ILVs and can recycle vesicles after transport to the trans-Golgi network [25]. Exosome biogenesis necessitates an ESCRT-dependent and occasionally ESCRT-independent regulated endosomal sorting complex for transfer. ESCRT is made up of associated proteins (VPS4, Tsg101, and ALIX) and four complexes (ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III). The protein ubiquitin (ub) checkpoint determines a crossing point for the delivery of cargo which is located at the beginning part of the ESCRT-dependent route and includes all the ESCRT subunits. ESCRT-0 recognizes ubiquitinated cargo proteins by using STAM1/2 and HRS heterodimer [26,27,28]. The membrane is then deformed by the addition of ESCRT-I and ESCRT-II to ESCRT-0, which stabilizes the membrane neck. ESCRT-III cleaves the vesicle neck using the Vps4 complex, releasing the buds into the endosome [26]. Finally, lysosomes are ready to degrade ILVs whose cargo has not been deubiquitinated by deubiquitylating enzymes (DUBs) [29].

ESCRT-independent pathway in exosome biogenesis occurs in melanosomes and consists of lipids and related proteins such as tetraspanin [30]. Tetraspanin CD63 participates in invagination of melanosome membrane independently from ESCRT and ceramide [31]. A different marker protein called ALIX delivers unubiquitinated cargoes to the ILVs by interacting with ESCRT-III and binding to Tetraspanin CD63 [32]. Pmel17, a melanosomal protein, interacts with lipids and its luminal domains to cause the formation of ILV [33]. According to the latest discoveries, the lipid-rich areas of an endosomal membrane, like sphingomyelin, ceramide, and cholesterol are where the protein PLP (proteolipid protein) is transported to the ILVs independently of ESCRT. A defect in sphingomyelinase (SMase or SMPD2), the enzyme that converts sphingomyelin to ceramide, prevents the formation of ILV because ceramide-rich regions of endosomes are more prone to inward budding [32]. The incoming MVBs reach their ultimate intracellular location after completing all of these steps. There are two possible outcomes for MVBs: either they move toward the plasma membrane and discharge their ILVs into the extracellular space, or their ubiquitinated cargo causes them to fuse with lysosomes and degrade [34.35]. With the aid of the soluble N-ethyl maleimide (NEM)-sensitive factor attachment protein receptor (SNARE) complex, membrane fusion and subsequent exosome secretion are accomplished. The process of membrane fusion is triggered by synaptotagmin, a calcium-sensing protein, interacting with MVBs. The trans-SNARE complex then allows accumulated MVBs to dock with the membrane, releasing exosomes into the extracellular environment [36]. Understanding exosome biogenesis and release **(Figure 1)** is certainly crucial as it can be beneficial in developing new therapeutic strategies.

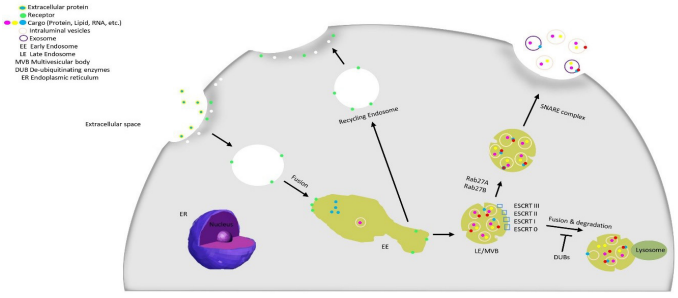

Figure 1: Biogenesis and secretion of exosome inside endosomal system. Early endosomes (EEs) are composed by endodontic vesicles binding. EEs go through two routes as illustrated: Either by getting back to the plasma membrane or being converted into LEs/MVBs by means of sprouting inside of the membrane, resulting in packing of cargos into ILVs. ILVs are later categorized as ESCRT independent or dependent proteins which are composed of four different types including ESCRT-O, ESCRT-1, ESCRT-II and ESCRT-111, constituting ESCRT mechanism that wide spreads the under layers on the part of the concave sprouting endosomal membrane. Afterwards, ILVs either get degraded by lysosomes or rescued by DUBs. Later, MVBs are guided toward the cell periphery by means of Rab27A and Rab27B. After all, MVBs are combined with plasma membrane by getting aid from SNARE complex and ILVs are freed into the extracellular scope, now known as exosomes.

Exosome contents

Exosomes, which are secreted by various cells, contain a collection of biomolecules, like nucleic acids, lipids, and proteins [37,38]. Exosome composition is also influenced by a collection of proteins, containing enzymes, transcription factors, receptors, extracellular matrix proteins, nucleic acids (miRNA, mRNA, and DNA), and lipids [39]. Exosome content also contains different cell-specific lipids, which are crucial for exosome biogenesis, regulating recipient cell homeostasis, and maintaining exosome morphology [40-42]. The internal membrane of MVBs contains the high-density lipid lyosbisphosphatidic acid (LBPA), which promotes the formation of ILV [43]. Many ILVs such as tetraspanins, Tsg101, and Alix constitute exosome content. Moreover, many exosome nucleic acids consisting of mRNAs and non-coding RNAs like lncRNAs, miRNAs, ribosomal RNAs (rRNAs), circRNAs, piwi-interacting RNAs (piRNAs), small nuclear RNAs (snRNAs), transfer RNAs (tRNAs), and small nucleolar RNAs (snoRNAs) have been found recently [44].

The protein content of the exosome is mainly composed of specific and non-specific proteins. Cell adhesion molecules (CAMs), tetraspanins, integrins, MHC class I, and II, and transferrin receptors (TfR) belong to specific protein types. Simultaneously, non-specific exosome proteins are flotillin, Rab2, Rab7, annexin, cytoskeleton proteins including tubulin, actin, myosin, heat shock molecules like Hsc70 and Hsc90, and proteins such as Alix that regulate the genesis of MVBs [39,45].

According to the latest discoveries, exosomes can influence target cells' hemostasis by changing the recipient cells' lipid composition, particularly their levels of cholesterol and sphingomyelin. A database called ExoCarta contains more than 47,000 entries for mRNA, lipid, and protein and contains both unpublished and published information about exosome content. Furthermore, the data saved in ExoCara can be useful for exosome characterization [46].

Exosome characterization

Characterization of exosomes is mainly accomplished using several biophysical, molecular, and microfluidic methods such as dynamic light scattering, nanoparticle tracking analysis, resistive pulse sensing, transmission electron microscopy, atomic force microscopy, and flow cytometry **(Table 1)**.

One of the biophysical techniques named Nanoparticle Tracking Analysis (NTA) is used to ascertain the concentration and size dispensation of exosomes with a size range of 10 nm to 2 μm. In this technique, the movement of exosomes is tracked using image analysis of each particle [47]. NTA has many advantages, such as the ability to measure extremely small particles (30 nm) and to detect various Evs, including exosomes. Another advantage of this method is very quick and easy sample preparation that only takes a few minutes [48]. The application of fluorescently labeled antibodies can also be used to find antigens on Evs [47].

A different method known as photon correlation spectroscopy, also referred to as Dynamic Light Scattering (DLS), is utilized for determining the size of exosomes. DLS operates by a monochromatic coherent laser beam passing through a suspension of particles [49]. Positive and negative interference as well as time-dependent fluctuations in scattering intensity are the results of Brownian motions of the particles in a sample. This technique is not complicated to use, however, it does not involve particle visualization. This method is excellent for being able to measure monodispersed suspension particles with sizes ranging from 1 nm to 6 μm.

When larger vesicles are present, even in trace amounts, they prevent the detection of smaller particles in the suspension [50,51]. Analyzing size and location of Evs in red blood cells has shown the effectiveness of this technique [52].

Exosome concentration and size dispensation are determined by means of Tunable Resistive Pulse Sensing (TRPS), a novel approach for distinguishing colloidal pieces with sizes ranging from about 50 nm in diameter to the size of cells [52]. A collection of nanoparticle suspensions composed of magnetic beads and different biomolecules are measured successfully by TRPS. This method's main drawback, which results in pore blocks from particles, is a stability issue with the system. Another drawback is that the sensitivity problem makes it impossible to identify very small particles opposed to the background noise of the system. It has been discovered that the stability and sensitivity of the system can be increased by optimizing the system's parameters, including accuracy, system noise, and sensitivity cutoff limits [53]. Using this method, the extracellular matrix-binding of leukemia-derived Evs to particles with diameters between 200 and 300 nm was examined [54]. By analyzing the size distributions of Evs made to transport enzymes, TRPS has also been used to treat Alzheimer's disease and deliver anticancer miRNAs to tumor cells [55,56].

By detecting and documenting interactions between a sample's surface and a probing tip, atomic force microscopy (AFM) can be utilized to study exosomes instead of optical and electron diffraction methods. This method is renowned for its capacity to analyze samples in their natural settings while requiring little sample preparation and without compromising the process [57,58]. Characterizing morphology, abundance, biomolecular make-up, and biomechanics of exosome is facilitated by using AFM as a nanoscale tool [57]. Under various experimental conditions, such as temperature, the condition of the AFM tip, varying scan speed, or the power between the probe and the sample, the sample is characterized using external analyses. AFM has been found practical for characterizing Evs derived from saliva [59], synovial fluid [60], and blood [61,62].

Transmission electron microscopy, known as TEM, is an approach that is frequently utilized for describing the dimension, shape, and composition of a wide variety of biological components. TEM generates images by first sending an electron beam through a specimen and then generating a second electron. TEM and cryo-electron microscopy (cryo-EM) are the two primary forms of electron microscopy that are utilized in the majority of biological research settings. Specimens require being fixed in glutaraldehyde and then dehydrated before they can be viewed using TEM. Additionally, the images need to be obtained in a vacuum environment. Numerous drawbacks of TEM include the extensive, destructive, and difficult preparation that may change the morphology of the Evs. Furthermore, biological samples can get damaged by TEM electron beams. Cryo-EM is used for EV analysis to minimize damage from TEM electron beams. Since the samples are submerged in liquid nitrogen during cryo-EM, there are no ultrastructural alterations or element reallocation. Cryo-EM is the most effective technique for generating proteins and nanoparticles free of artifacts caused by dehydration. Finding specific proteins within exosomes, which are often labeled and visualized using particular fluorescent dyes, is the most essential part of investigating the biological roles of exosomes. Exosomes are typically labeled and visualized using

unique fluorescent dyes. However, due to inflated fluorescence signals, exosomes are not able to be distinguished by means of this method [63,64]. Therefore, immunogold EM has been used as a substitute method for figuring out how certain proteins work.

Another technique for quantifying exosome size and structure through a molecular approach is flow cytometry [65]. Ultracentrifugation, followed by western blotting and NTA, is one of the most reliable techniques for this method because the initial samples are so important [66]. Particles smaller than 300 nm are too nanoscopic for conventional flow cytometers to detect [67]. In order for a flow cytometer to perform its intended function, a laser beam with a particular wavelength must be sent via a fluid stream that contains suspended particles. In addition, by employing this technology, fluorescently dyed particles are able to be assessed, which makes it possible for flow cytometry to investigate the granulation and sizes of the particles compared to one another [68]. Because of the limitations imposed by their size on detection, conventional flow cytometers are not able to detect a sizable number of particles. Recent research has resulted in the development of a flow cytometer that possesses high-resolution imaging, fluorescence amplification, and improved sensitivity forward scatter detection. This allows researchers to differentiate labeled exosomes from background pollutants [69,70]. The latest generation of flow cytometers has the ability to identify Evs with a diameter of less than 300 nm, enables the rapid identification of suspended exosomes, and quantifies and categorizes exosomes depending on the amount of antigen expression they display [71].

Table 1: Exosome characterization methods.

Method	Advantages	Disadvantages	Throughput	References
Nanoparticle tracking analysis	minimal sample provision, quick, and reusable samples	Because of the requirement for a high level of sample purity, polydispersed particles cannot be used.	High	[48,72,73,74]
Dynamic light scattering	Quick (minutes), samples are reusable	The bias for larger particles makes it unsuitable for polydispersed particles, and a minimum sample concentration is needed.	High	[72,74,75,76]
Tunable resistive pulse sensing	Appropriate for polydispersed samples	Multiple membranes are required for the various sizes of exosomes, which are ascertained by the size and shape of the membrane pores, the vesicle surface characteristic, and membrane clogging.	High	[72,78,79,80]
Atomic force microscopy	Clear disparity on flat samples, more detailed images	Influenced by vesicle immobilization	No	[74,78,82,83,84]
Flow cytometry	Single particle detection	Influenced by particle aggregates	High	[76,86]
Transmission electron microscopy	High-resolution images	Sample preparation (fixation and staining)	No	[15,87]

The role of exosomes in the pathogenesis of OSCC and HNSCC

Exosome derived miRNAs' role in cancer pathogenesis has been an interesting subject of research in recent years. Exosomal miRNAs are considered important arbitrators in the cross-talk between cancer cells and macrophages. Tumor-related macrophages (TAMs), which be classified into two groups (M1 and M2) according to their task, are vital constituents of the tumor microenvironment and have a crucial part in cancer pathogenesis [88]. Different cells containing immune and tumor cells in TME can transfer nucleotides and proteins during the cancer progression and lead to pathogenesis of tumor growth and metastasis [89,90]. The uptake of exosomes from OSCC cells by monocytes activates the NF-κB route and creates a pro-inflammatory environment [91]. Proteomes vary from on another including compatibility with cisplatin therapy inside exosomes which leads to notable alterations in the secreted exosomes [92].

MiRs can apply both anti- and pro-tumorigenic influences using miR-specific and context-reliant mechanisms [93]. It has been found that the reduction of miR-34a levels leads to the pathogenesis of different kinds of cancer such as HNSCC [94]. Hypoxia induces caveolin-1 secretion in HNSCC cells through trafficking by Evs, which can create a pseudo-hypoxic environment and result in lepathogenesis and tumor development [95,96].

Proliferation

Exosome derived miRNAs are conceivable identifying biomarkers for different malignancies, which aid in adjusting expression of protein in proliferating cell and immune response [97]. Exosomal miR-34a-5p and miR-382-5p from CAFs have a crucial duty in the proliferating, migrating, and invading OSCC [98,99]. MiR-24-3p, miR-891a, miR-106a-5p, miR-2a-5p, and miR-1908 which are derived from OSCC cells suppress the feedback from T-cell in the tumor body and manipulate cell differentiation and proliferation [100]. Furthermore, TGFBR1 activity is diminished by miR-142-3p originated from exosomes which results in proliferating OSCC cell in vitro and in vivo [101]. MiR-101-3p and MiR-223 have been discovered to impede cell proliferation due to their tumor suppressor properties. In addition, miR-34a-5p restrains proliferating SCC-15 and CAL-27 cells [102]. OSCC originated exosomes lead to production of LncRNA FLJ22447 which aids in activation of CAFs and promotes OSCC cell proliferation through IL-33 [103].

Angiogenesis has a crucial part in cancer progression. According to a recent study, TGF-β containing exosomes that are originated from HNSCC encourage angiogenesis in vitro and in vivo [104].

Furthermore, miR-3188 carrying exosomes that are originated from CAF are able to affect the proliferation of HNSCC cells in vitro and in vivo. Therefore, exosome-delivered miR-3188 can have a therapeutic value in prohibiting HNSCC development [105]. As salivary exosomal miRNAs are promising diagnostic biomarkers for OSCC, the proliferation of recipient malignant

cells also increases following the effect of exogenous exosome miR-24-3p by targeting the PER1 protein [106]. HNSCC cell proliferation and resistance to apoptosis occur due to two tumor suppressors named CDKN1B and ING5 [107].

Metastasis

Exosomes induce OSCC metastasis by means of conveying their substance to target cells. Metastatic invasion results in poor OSCC prognosis, with a tendency for local recurrence and distant metastasis [124]. Exosomal miRNAs are capable of being diagnostic biomarkers for different malignancies, tumor metastasis, and immune responses [108]. OSCC develops following increased miRNA expression in exosomes [109].

MiR-342-3p and miR-1246 have an extensive part in metastasis of OSCC and raising the chance of cell motility and invasive ability [110]. Exosomes originated from tumors are able to operate as message carriers in the tumor environment, resulting in tumor enlargement and metastasis [98]. CAFs are one of the most prevalent constituents of the TME and have a crucial part in tumor progression and metastasis [111]. Latest discoveries have shown that metastasis of OSCC cells is improved by exosomes that are originated from CAFs. Tumor growth and metastasis are commonly associated with angiogenesis. OSCC metastasis can be influenced by exosome-derived OSCC cells acting as preventative or promotive agents on angiogenesis [112].

Over 90% of all head and neck cancers are categorized as HNSCC with superior rates of tumor recurrence/metastasis and poor patient endurance [113]. Li et al. showed that cancer cell encroachment, metastasis, and angiogenesis were induced by exosomes originated from HCC transferring LOXL4 between HCC cells and human umbilical vein endothelial cells (HUVECs) [114]. Epithelial To Mesenchymal Conversion (EMT) has a crucial role in tumor progress and induces the encroachment and metastasis of tumor cells into the stroma. Exosome-derived intercellular connection results in EMT, and investigation of this process has resulted in great advances cancer metastasis field [115]. After the plasma from 44 patients with HNSCC and 7 healthy donors were evaluated , superior levels of immunosuppressive proteins in CD44v3+ exosomes were found in comparison with CD44v3(-) exosomes. The relative fluorescence magnitude of the mentioned markers was related to more developed disease phases and lymph node metastasis [116]. Recently, researchers have discovered that tumor cell-derived exosomes are able to produce Heat Shock Proteins (HSP), that are present at superior rates in patients with HNSCC and may be utilized as biomarkers for cancer metastasis [117,118].

Table 2: Exosomes' influence on the tumor microenvironment.

Origin/cell line of exosome	Isolation method	Outcome	Ref.
PCI-13 HNSCC	Differential centrifugation and mini-SEC	Apoptosis of triggered CD8+ T cells by TEX and cell surface signaling used for regulating Treg restrainer purposes.	[119]
PCI-13 HNSCC	SEC and ultracentrifugation	Proliferation and signaling of activated CD8+ T cells were impeded by TEX and led to Treg enlargement.	[120]
PCI-13 HNSCC	SEC and ultracentrifugation	Treg's production, growth, biological activity and abiding against apoptosis was incited by TEX.	[121]
C15 and C17 PDX (originated from patient's xenograft) NPC	Differential centrifugation and sucrose gradient flotation	TEX expedited Treg engagement and enlargement of CD25high FOXP3high Treg.	[122]
PCI-13 HNSCC	Differential centrifugation, SEC, and ultracentrifugation	The expression of genes associated with immune-function in T cell subdivisions interpreting into elevated adenosine generation and lack of CD69 expression on triggered T cells.	[123]
UM-SCC-1 and 96-VU-147T-UP-6 HNSCC	Differential ultracentrifugation and iodixanol gradient centrifugation	TEX and patient-originated exosomes (from both plasma and tumor) induced neurite projection in PC12 neuronal replica cells.	[124]
PCI-13 and UM-SCC47 HNSCC	Differential centrifugation and mini-SEC	Endothelial cells were proliferated and migrated by TEX which induced tube formation and angiogenesis.	[125]
HOC313 OSCC	SEC and ultracentrifugation	TEX induced cell growth of highly metastatic cells and promoted cell motion of cells that are poorly metastasized by means of delivering miR-1246.	[109]
RT-7 OSCC and HSC-3	Differential centrifugation and Total Exosome Isolation Kit (Invitrogen)	Alteration of normal epithelial cells to a mesenchymal phenotype by EGFR-positive TEX that was impeded by cetuximab.	[126]
SVpgC2a, SQCC/Y1, and SVFN8 OSCC	Differential centrifugation and ultracentrifugation	Transcriptome profile in oral keratinocytes was altered by TEX concerning pathways associated with matrix remodeling and immune modulation.	[127]
CAL-27 OSCC and SCC-9	ExoQuick Exosome Precipitation Kit (System Biosciences)	TEX originated from hypoxic cells escalated migration and encroachment of normoxic cells by delivering miR-21.	[128]
HPV(+) UM-SCC-2, HPV(-) PCI-13, and PCI-30 HNSCC	Differential centrifugation and mini-SEC	Immune modulatory proteins were transferred by HPV(+) and HPV(-) TEX and T cell activity was impeded. Dendritic cell activity was restrained only by HPV(-) TEX	[129]
HPV(+) UM-SCC-2, HPV(-) PCI-13, and PCI-30 HNSCC	Differential centrifugation and mini-SEC	HPV(-) and HPV(+) TEX received the proteomic load. CD276 and CD47 were abundantly present in HPV(+) TEX , while more MUC-1, tumor-protective/growth-improving antigens and HLA-DA were found in HPV(-) TEX.	[130]
HPV(+) SCC-90, HPV(-) SAS, and CAL-33 HNSCC	Differential centrifugation and ultracentrifugation	Macrophages were modified into the M1 phenotype by iR-9-improved TEX from HPV(+) HNSCC and resulted in accretion of radiosensitivity of HPV(+) HNSCC patients.	[131]

HSC-3 and SCC-9 OSCC	Differential centrifugation and ultracentrifugation	TEX originated from cisplatin-resistant cells produced chemoresistance in platin-naive cells and resulted in abatement of DNA destruction signaling in reaction to cisplatin.	[108]
Primary, HNSCC patient-derived cancer-associated fibroblasts	Differential centrifugation and ultracentrifugation	TEX originated from cisplatin-resistant cancer-associated fibroblasts awarded chemoresistance and transferring functional miR-196a led to formation of an aggressive phenotype in cancer cells.	[106]
KYSE30 and KYSE180 ESCC	Differential centrifugation and ultracentrifugation	Radioresistant cells revealed different miRNA expression profiles in comparison with normal cells and exosome derived miR-339-5p arbitrated in controlling radiosensitivity.	[132]
UM-SCC-6 HNSCC	Differential centrifugation and SEC	After irradiated cells disengaged TEX, a proteomic analysis showed overexpression of proteins included as a result of reaction to radiation, ROS metabolism, and DNA restoration.	[133]
FaDu HNSCC	Total Exosome Isolation Kit (Invitrogen) and ultracentrifugation	Irradiated cells disengaged the proteomic profile of TEX which was notably changed in comparison with TEX from nonirradiated cells.	[134]
BHY and FaDu HNSCC	Differential centrifugation and ultracentrifugation	TEX originated from irradiated cells aided in endurance and proliferation and awarded a moving phenotype to receiver cancer cells.	[135]

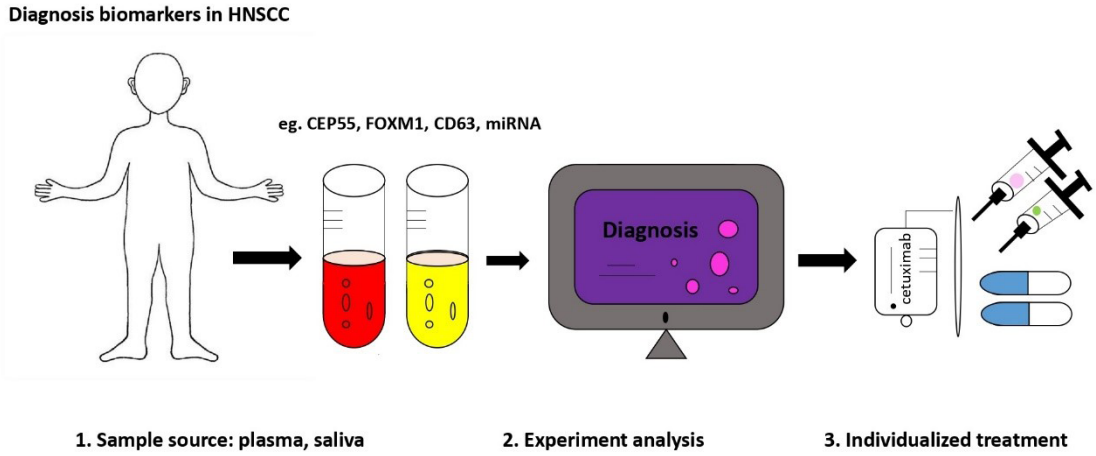

Figure 2: The extracted exosomes from blood or saliva were experimented in the research facility. MiR-10b-5p, miR-486, miR-517b-3p, miR-3-2b-3p, CEP55, FOXM1 and CD63 are carried by exosomes and are able to be utilized as possible biomarkers for HNSCC diagnosis, therapy and prognosis assessment.

Resistance to conventional therapy

Improved resistance against conventional OSCC therapies has contributed to numerous problems in combating malignant OSCC. Exososmes derived from miRNAs have a crucial part in growing, metastasis, and resistance against drugs [108]. Based on studies by Liu et al., exosomes target PDCD4 and PTEN to award the cisplatin abidance of the parental OSCC cells after miR-21 is transferred by cisplatin-resistant OSCC cells[136]. Hence, factors related to resistance are ought to be included as therapeutic targets for the productive therapy of OSCC as exosomes might be a potential vector for abidance shifting in cancer cells. In addition to OSCC, gastrointestinal cancer is also resistant to chemotherapy agents like cisplatin. Exosome derived lncRNAs induce tumor cell's chemo resistance and the progress in TME by various procedures. lncRNA HOTTIP derived from exosomes attaches to miR-218 for triggering HMGA1 and has an important part in awarding cisplatin abidance to impressionable cancer cells[137].

Tumor abidance to conventional chemotherapy drugs is still a substantial challenge in combating HNSCC[138]. Exosomes can promote resistance by sequestering, efflux, or protecting cells from the influence of drugs and inhibiting its intracellular accretion [139], or by external therapeutic resistance by promoting intercellular connection and transferring mRNAs, miRNAs, DNAs, and/or proteins[106].

Targeting CDKN1B and ING5 contributes to HNSCC cell proliferation and resistance to apoptosis [140]. Exosomes provide cellular communications in TME, which aids them in anticancer therapy resistance. A significant amount of information about the effect of exosomes on the TME was obtained following an analysis of TEX originated from supernatants of human tumor cell origins **(Table 2)**. An established theory suggests that upregulation of AKT signaling has an important part in radioresistance in HNSCC cells [141].

The role of exosomes in the diagnosis of OSCC and HNSCC

As late OSCC diagnosis is risky, prevention, early diagnosis, and treatment productiveness require urgent improvement [142]. In this regard, the duty of exosomes and their transport pattern in the diagnosis and therapy of OSCC should be considered. A disruption in miRNAs regulation leads to the progression of miscellaneous kinds of cancers like OSCC. Hence, miRNAs are promising diagnostic biomarkers for OSCC [143-145]. Exosomal protein markers like TRAP1, EGFR, heat shock protein 90 (HSP-90), and MMP-13, that are able to influence the intracellular activity of genes are potential diagnostic markers for early OSCC diagnosis [146,147]. According to Li et al., there are free exosomes in blood with contents associated with OSCC, which include proteins such as PF4V1, CXCL7, F13A1, and ApoA1 and have the potential to be used for diagnosing OSCC [148]. As an alternative method for tissue-based sampling, a more noninvasive technique known as liquid biopsy is used for the diagnosis of OSCC [149]. Liquid biopsies identify exosomes, circulating tumor DNA, and circulating tumor cells for diagnosing oral cancer

using blood and saliva [97].

Evs are potential cancer biomarkers due to being present in body fluids like blood and saliva **(Figure 2)**. Diagnostics based on Evs are the most suitable noninvasive diagnosis candidates [150]. Exosomal Centrosomal protein 55 (CEP55) and forkhead box protein M1 (FOXM1) mRNA carriers in the blood are potential noninvasive cancer biomarkers in the diagnosis and prognosis of HNSCC [127].

The role of exosomes in the treatment of OSCC and HNSCC

Over the past few years, the treatment of OSCC has improved significantly. MiRNAs are potential treatment options for OSCC treatment [105,144,145]. Exosomal miRNAs such as salivary exosome MiR-24-3p have been identified as potential therapeutic targets in the treatment of OSCC [151]. Xie et al. used lenti-miR-138 virus gd T cell-derived exosomes (gdTDEs) as a drug delivery system in the treatment of OSCC [152]. Conflicting effects identified in normal prostatic epithelial cells with treatment of exosome-containing miR-143 have resulted in bio-safety confirmation [153]. According to Rosenberg et al., exosome therapy suppresses angiogenic activity, including vessel density and vascular area as exosomes control VEGF secretion by preventing the angiogenic activity [97]. Liquid biopsy aids in repeated sampling to monitor the treatment response in cancers such as OSCC screening programs [154]. Moreover, the constitution of target-specific exosomes demonstrates an increase in the efficacy of cancer treatment [155].

Even though there have been numerous improvements in HNSCC surgical treatment, chemoradiotherapy, and immunotherapy, there's still no effective way to control more than two-thirds of HNSCC patients' clinical progression [156]. The use of biomarkers in the detection of HNSCC has gained popularity because it increased the effectiveness of treatment in recent years [157]. Disease-associated exosomal miRNAs such as HPV or EGFR, which are overexpressed in 90% of HNSCC, are considered highly valuable, as they have the potential to guide HNSCC therapy [158,159,160]. According to Theodoraki and colleagues, patients' plasma-derived exosomes at defined time points before, during, and after therapy could be reliable noninvasive biomarkers in the treatment of patients cured with surgery/©RT and those with advanced HNSCC relapse [161]. Synthetic exosome-mimics, which have limited bioavailability, are useful for inducing endogenous and exogenous delivery in the treatment of HNSCC [162]. According to Kobayashi et al., soluble inhibitors of UCH-L1 have been seen to be effective in diminishing lymph node metastasis of HNSCC [131]. MiRNA-9-rich EXOs derived from HPV (+) HNSCC result in polarization of macrophage M1 by downregulating peroxisome proliferator-activated receptor δ (PPARδ) and developing the radiosensitivity of tumors, making miRNA-9 a useful potential treatment for HNSCC [163]. The first-line treatment for HNSCC is Cisplatin-based chemotherapy regimens, which are mostly used in combination with 5-Fluorouracil (5-FU) or taxane.

Conclusion

In this review, we highlighted exosome biogenesis and the main mechanisms for exosome-derived metastasis and chemoresistance. Understanding the role of exosomes in influencing tumor phenotype, angiogenesis, immune modulation, metastasis, and drug resistance is an essential point that should not be ignored. There are still numerous challenges in separating, expanding, and identifying clinically relevant exosomes, especially in heterogeneous cancers such as OSCC and HNSCC. On the other hand, cancer-derived specific exosomes seem to be promising for early cancer diagnosis and therapy. Moreover, accurate identification of exosomes and their cargos provides a more opportunistic future as efforts to evolve new exosome-based therapeutics and diagnostics are necessary to increase the survival of cancer patients.

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All authors contributed to the conception and the main idea of the work. Y.A, M.T.A and T.A.M.M drafted the main text, figures, and tables. S.KH supervised the work and provided the comments and additional scientific information. M.S.C and Y.P also reviewed and revised the text. All authors read and approved the final version of the work to be published.

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References

1. Bu J, Bu X, Liu B, Chen F, Chen P. Increased expression of tissue/salivary transgelin mrna predicts poor prognosis in patients with oral squamous cell carcinoma (OSCC) surgery. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research. 2015; 21: 2275.

2. Huang W-C, Jang T-H, Tung S-L, Yen T-C, Chan S-H, et al. A novel miR-365-3p/EHF/keratin 16 axis promotes oral squamous cell carcinoma metastasis, cancer stemness and drug resistance via enhancing β5-integrin/c-met signaling pathway. Journal of Experimental & Clinical Cancer Research. 2019; 38: 1-17.

3. Leemans CR, Snijders PJ, Brakenhoff RH. The molecular landscape of head and neck cancer. Nature Reviews Cancer. 2018; 18: 269-282.

4. Castellsagué X, Quintana MJ, Martínez MC, Nieto A, Sanchez MJ, Juan A, et al. The role of type of tobacco and type of alcoholic beverage in oral carcinogenesis. International journal of cancer. 2004; 108: 741-749.

5. Carlander A-LF, Larsen CG, Jensen DH, Garnæs E, Kiss K, Andersen L, et al. Continuing rise in oropharyngeal cancer in a high HPV prevalence area: A Danish population-based study from 2011 to 2014. European Journal of Cancer. 2017; 70: 75-82.

6. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, et al. Case–control study of human papillomavirus and oropharyngeal cancer. New England Journal of Medicine. 2007; 356: 1944-1956.

7. Mork J, Lie AK, Glattre E, Clark S, Hallmans G, Jellum E, et al. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. New England Journal of Medicine. 2001; 44: 1125-1131.

8. Chi AC, Day TA, Neville BW. Oral cavity and oropharyngeal squamous cell carcinoma—an update. CA: a cancer journal for clinicians. 2015; 65: 401-421.

9. Epstein JB, Thariat J, Bensadoun RJ, Barasch A, Murphy BA, et al. Oral complications of cancer and cancer therapy: from cancer treatment to survivorship. CA: a cancer journal for clinicians.

2012; 62: 400-422.

10. Mellman I. Endocytosis and molecular sorting. Annual review of cell and developmental biology. 1996; 12: 575-625.

11. Keller S, Sanderson MP, Stoeck A, Altevogt P. Exosomes: from biogenesis and secretion to biological function. Immunology letters. 2006; 107: 102-108.

12. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. Journal of Cell Biology. 2013; 200: 373-383.

13. Abak A, Abhari A, Rahimzadeh S. Exosomes in cancer: small vesicular transporters for cancer progsion and metastasis, biomarkers in cancer therapeutics. Peer J. 2018; 6: e4763.

14. Kalra H, Simpson RJ, Ji H, Aikawa E, Altevogt P, Askenase P, et al. Vesiclepedia: a compendium for extracellular vesicles with continuous community annotation. PLoS biology. 2012; 10: e1001450.

15. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. Journal of extracellular vesicles. 2018; 7: 1535750.

16. Babushkina EA, Belokopytova LV, Grachev AM, Meko DM, Vaganov EA. Variation of the hydrological regime of Bele-Shira closed basin in Southern Siberia and its reflection in the radial growth of Larix sibirica. Regional Environmental Change. 2017; 17: 1725-1737.

17. Ahmadi M, Rezaie J. Tumor cells derived-exosomes as angiogenenic agents: possible therapeutic implications. Journal of translational medicine. 2020; 18: 1-17.

18. Gai C, Camussi F, Broccoletti R, Gambino A, Cabras M, Molinaro L, et al. Salivary extracellular vesicle-associated miRNAs as potential biomarkers in oral squamous cell carcinoma. BMC cancer. 2018; 18: 1-11.

19. Pourhanifeh MH, Mahjoubin-Tehran M, Shafiee A, Hajighadimi S, Moradizarmehri S, Mirzaei H, et al. MicroRNAs and exosomes: Small molecules with big actions in multiple myeloma pathogenesis. IUBMB life. 2020; 72: 314-333.

20. Ohno S-I, Ishikawa A, Kuroda M. Roles of exosomes and microvesicles in disease pathogenesis. Advanced drug delivery reviews. 2013; 65: 398-401.

21. Zhang J, Li S, Li L, Li M, Guo C, Yao J, et al. Exosome and exosomal microRNA: trafficking, sorting, and function. Genomics Proteomics Bioinform. 2015; 13: 17-24.

22. Wang H, Hou L, Li A, Duan Y, Gao H, Song X. Expression of serum exosomal microRNA-21 in human hepatocellular carcinoma. BioMed research international. 2014; 2014.

23. Huotari J, Helenius A. Endosome maturation. The EMBO journal. 2011; 30: 3481-500.

24. McAndrews KM, Kalluri R. Mechanisms associated with biogenesis of exosomes in cancer. Molecular cancer. 2019; 18: 1-11.

25. Williams RL, Urbé S. The emerging shape of the ESCRT machinery. Nature reviews Molecular cell biology. 2007; 8: 355-368.

26. Juan T, Fürthauer M, editors. Biogenesis and function of ESCRT-dependent extracellular vesicles. Seminars in cell & developmental biology. 2018.

27. Ren X, Hurley JH. VHS domains of ESCRT-0 cooperate in high-avidity binding to polyubiquitinated cargo. The EMBO journal. 2010; 29: 1045-1054.

28. Kobayashi H, Tanaka N, Asao H, Miura S, Kyuuma M, Semura K, et al. Hrs, a mammalian master molecule in vesicular transport and protein sorting, suppresses the degradation of ESCRT proteins signal transducing adaptor molecule 1 and 2. Journal of Biological Chemistry. 2005; 280: 10468-10477.

29. Yeates EFA, Tesco G. The endosome-associated deubiquitinating enzyme USP8 regulates BACE1 enzyme ubiquitination and degradation. Journal of Biological Chemistry. 2016; 291: 15753-15766.

30. Babst M. MVB vesicle formation: ESCRT-dependent, ESCRT-independent and everything in between. Current opinion in cell biology. 2011; 23: 452-457.

31. Van Niel G, Charrin S, Simoes S, Romao M, Rochin L, Saftig P, et al. The tetraspanin CD63 regulates ESCRT-independent and-dependent endosomal sorting during melanogenesis. Developmental cell. 2011; 21: 7087-21.

32. McGough IJ, Vincent J-P. Exosomes in developmental signalling. Development. 2016; 143: 2482-2493.

33. Theos AC, Truschel ST, Tenza D, Hurbain I, Harper DC, Berson JF, et al. A lumenal domain-dependent pathway for sorting to intralumenal vesicles of multivesicular endosomes involved in organelle morphogenesis. Developmental cell. 2006; 10: 343-354.

34. Baietti MF, Zhang Z, Mortier E, Melchior A, Degeest G, Geeraerts A, et al. Syndecan–syntenin–ALIX regulates the biogenesis of exosomes. Nature cell biology. 2012; 14: 677-685.

35. Kumar B, Garcia M, Murakami JL, Chen C-C. Exosome-mediated microenvironment dysregulation in leukemia. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2016; 1863: 464-470.

36. Kennedy MJ, Ehlers MD. Mechanisms and function of dendritic exocytosis. Neuron. 2011; 69: 856-875.

37. Azmi AS, Bao B, Sarkar FH. Exosomes in cancer development, metastasis, and drug resistance: a comprehensive review. Cancer and Metastasis Reviews. 2013; 32: 623-642.

38. Subra C, Laulagnier K, Perret B, Record M. Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. Biochimie. 2007; 89: 205-212.

39. Van Niel G, Porto-Carreiro I, Simoes S, Raposo G. Exosomes: a common pathway for a specialized function. Journal of biochemistry. 2006; 140: 13-21.

40. Vidal M, Sainte-Marie J, Philippot JR, Bienvenue A. Asymmetric distribution of phospholipids in the membrane of vesicles released during in vitro maturation of guinea pig reticulocytes: evidence precluding a role for "aminophospholipid translocase". Journal of cellular physiology. 1989; 140: 455-462.

41. Chu Z, Witte DP, Qi X. Saposin C–LBPA interaction in late-endosomes/lysosomes. Experimental cell research. 2005; 303: 300-307.

42. Minciacchi VR, Freeman MR, Di Vizio D, editors. Extracellular vesicles in cancer: exosomes, microvesicles and the emerging role of large oncosomes. Seminars in cell & developmental biology. 2015.

43. Bissig C, Lenoir M, Velluz M-C, Kufareva I, Abagyan R, Overduin M, et al. Viral infection controlled by a calcium-dependent lipid-binding module in ALIX. Developmental cell. 2013; 25: 364-373.

44. van den Boorn JG, Daßler J, Coch C, Schlee M, Hartmann G. Exosomes as nucleic acid nanocarriers. Advanced drug delivery re-

views. 2013; 65: 331-335.

45. Poliakov A, Spilman M, Dokland T, Amling CL, Mobley JA. Structural heterogeneity and protein composition of exosome-like vesicles (prostasomes) in human semen. The Prostate. 2009; 69: 159-167.

46. Mathivanan S, Fahner CJ, Reid GE, Simpson RJ. ExoCarta 2012: database of exosomal proteins, RNA and lipids. Nucleic acids research. 2012; 40: D1241-D4.

47. Szatanek R, Baj-Krzyworzeka M, Zimoch J, Lekka M, Siedlar M, Baran J. The methods of choice for extracellular vesicles (EVs) characterization. International journal of molecular sciences. 2017; 18: 1153.

48. Dragovic RA, Gardiner C, Brooks AS, Tannetta DS, Ferguson DJ, Hole P, et al. Sizing and phenotyping of cellular vesicles using Nanoparticle Tracking Analysis. Nanomedicine: Nanotechnology, Biology and Medicine. 2011; 7: 780-788.

49. Bryant G, Abeynayake C, Thomas JC. Improved particle size distribution measurements using multiangle dynamic light scattering. 2. Refinements and applications. Langmuir. 1996; 12: 6224-6228.

50. Hoo CM, Starostin N, West P, Mecartney ML. A comparison of atomic force microscopy (AFM) and dynamic light scattering (DLS) methods to characterize nanoparticle size distributions. Journal of Nanoparticle Research. 2008; 10: 89-96.

51. Lawrie A, Albanyan A, Cardigan R, Mackie I, Harrison P. Microparticle sizing by dynamic light scattering in fresh-frozen plasma. Vox sanguinis. 2009; 96: 206-212.

52. Anderson W, Lane R, Korbie D, Trau M. Observations of tunable resistive pulse sensing for exosome analysis: improving system sensitivity and stability. Langmuir. 2015; 31: 6577-6587.

53. Patko D, Gyorgy B, Nemeth A, Szabó-Taylor K, Kittel A, Buzás EI, et al. Label-free optical monitoring of surface adhesion of extracellular vesicles by grating coupled interferometry. Sensors and Actuators B: Chemical. 2013; 188: 697-701.

54. Shimbo K, Miyaki S, Ishitobi H, Kato Y, Kubo T, Shimose S, et al. Exosome-formed synthetic microRNA-143 is transferred to osteosarcoma cells and inhibits their migration. Biochemical and biophysical research communications. 2014; 445: 381-387.

55. Katsuda T, Kosaka N, Takeshita F, Ochiya T. The therapeutic potential of mesenchymal stem cell-derived extracellular vesicles. Proteomics. 2013; 13: 1637-1653.

56. Binnig G, Quate CF, Gerber C. Atomic force microscope. Physical review letters. 1986; 56: 930-933.

57. Yuana Y, Oosterkamp TH, Bahatyrova S, Ashcroft B, Garcia Rodriguez P, Bertina RM, et al. Atomic force microscopy: a novel approach to the detection of nanosized blood microparticles. Journal of thrombosis and haemostasis: JTH. 2010; 8: 315-323.

58. Sharma S, Zuñiga F, Rice GE, Perrin LC, Hooper JD, Salomon C. Tumor-derived exosomes in ovarian cancer - liquid biopsies for early detection and real-time monitoring of cancer progression. Oncotarget. 2017; 8: 104687-104703.

59. György B, Módos K, Pállinger E, Pálóczi K, Pásztói M, et al. Detection and isolation of cell-derived microparticles are compromised by protein complexes resulting from shared biophysical parameters. Blood. 2011; 117: e39-48.

60. Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol. 2014; 30: 255-289.

61. Hardij J, Cecchet F, Berquand A, Gheldof D, Chatelain C, Mullier F, et al. Characterisation of tissue factor-bearing extracellular vesicles with AFM: comparison of air-tapping-mode AFM and liquid Peak Force AFM. J Extracell Vesicles. 2013; 2.

62. Sharma S, Rasool HI, Palanisamy V, Mathisen C, Schmidt M, Wong DT, et al. Structural-mechanical characterization of nanoparticle exosomes in human saliva, using correlative AFM, FESEM, and force spectroscopy. ACS nano. 2010; 4: 1921-1926.

63. Zaborowski MP, Balaj L, Breakefield XO, Lai CP. Extracellular Vesicles: Composition, Biological Relevance, and Methods of Study. Bioscience. 2015; 65: 783-797.

64. Pospichalova V, Svoboda J, Dave Z, Kotrbova A, Kaiser K, et al. Simplified protocol for flow cytometry analysis of fluorescently labeled exosomes and microvesicles using dedicated flow cytometer. J Extracell Vesicles. 2015; 4: 25530.

65. Dragovic RA, Gardiner C, Brooks AS, Tannetta DS, Ferguson DJ, Hole P, et al. Sizing and phenotyping of cellular vesicles using Nanoparticle Tracking Analysis. Nanomedicine : nanotechnology, biology, and medicine. 2011; 7: 780-788.

66. Suárez H, Gámez-Valero A, Reyes R, López-Martín S, Rodríguez MJ, et al. A bead-assisted flow cytometry method for the semi-quantitative analysis of Extracellular Vesicles. Sci Rep. 2017; 7: 11271.

67. Szatanek R, Baj-Krzyworzeka M, Zimoch J, Lekka M, Siedlar M, et al. The Methods of Choice for Extracellular Vesicles (EVs) Characterization. Int J Mol Sci. 2017; 18.

68. van der Vlist EJ, Nolte-'t Hoen EN, Stoorvogel W, Arkesteijn GJ, Wauben MH. Fluorescent labeling of nano-sized vesicles released by cells and subsequent quantitative and qualitative analysis by high-resolution flow cytometry. Nature protocols. 2012; 7: 1311-1326.

69. Erdbrügger U, Rudy CK, Etter ME, Dryden KA, Yeager M, Klibanov AL, et al. Imaging flow cytometry elucidates limitations of microparticle analysis by conventional flow cytometry. Cytometry Part A: the journal of the International Society for Analytical Cytology. 2014; 85: 756-770.

70. Orozco AF, Lewis DE. Flow cytometric analysis of circulating microparticles in plasma. Cytometry Part A : the journal of the International Society for Analytical Cytology. 2010; 77: 502-514.

71. Rőszer T. Understanding the Mysterious M2 Macrophage through Activation Markers and Effector Mechanisms. Mediators of inflammation. 2015; 2015: 816460.

72. Patel GK, Khan MA, Zubair H, Srivastava SK, Khushman M, Singh S, et al. Comparative analysis of exosome isolation methods using culture supernatant for optimum yield, purity and downstream applications. Scientific reports. 2019; 9: 1-10.

73. Kanwar SS, Dunlay CJ, Simeone DM, Nagrath S. Microfluidic device (ExoChip) for on-chip isolation, quantification and characterization of circulating exosomes. Lab on a Chip. 2014; 14: 1891-900.

74. Yang F, Liao X, Tian Y, Li G. Exosome separation using microfluidic systems: Size-based, immunoaffinity-based and dynamic methodologies. Biotechnology Journal. 2017; 12: 1600699.

75. Kowal J, Arras G, Colombo M, Jouve M, Morath JP, Primdal-Bengtson B, et al. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. Proceedings of the National Academy of Sciences. 2016; 113: E968-E77.

76. Rupert DL, Claudio V, Lässer C, Bally M. Methods for the physical characterization and quantification of extracellular vesicles in

biological samples. Biochimica et Biophysica Acta (BBA)-General Subjects. 2017; 1861: 3164-3179.

77. Lässer C, Eldh M, Lötvall J. Isolation and characterization of RNA-containing exosomes. JoVE (Journal of Visualized Experiments). 2012; e3037.

78. Wang Z, Wu H-j, Fine D, Schmulen J, Hu Y, Godin B, et al. Ciliated micropillars for the microfluidic-based isolation of nanoscale lipid vesicles. Lab on a Chip. 2013; 13: 2879-2882.

79. Benedikter BJ, Bouwman FG, Vajen T, Heinzmann AC, Grauls G, et al. Ultrafiltration combined with size exclusion chromatography efficiently isolates extracellular vesicles from cell culture media for compositional and functional studies. Scientific reports. 2017; 7: 1-13.

80. Lai JJ, Chau ZL, Chen SY, Hill JJ, Korpany KV, Liang NW, et al. Exosome Processing and Characterization Approaches for Research and Technology Development. Advanced Science. 2022; 2103222.

81. Soung Y, Ford S, Zhang V, Chung J. Exosomes in Cancer Diagnostics. Cancers (Basel). 2017; 9.

82. Coumans FA, Brisson AR, Buzas EI, Dignat-George F, Drees EE, El-Andaloussi S, et al. Methodological guidelines to study extracellular vesicles. Circulation research. 2017; 120: 1632-1648.

83. Shin H, Oh S, Hong S, Kang M, Kang D, Ji Y-g, et al. ACS Nano. 2020; 14: 5435-5444.

84. Parisse P, Rago I, Ulloa Severino L, Perissinotto F, Ambrosetti E, et al. Atomic force microscopy analysis of extracellular vesicles. European biophysics journal. 2017; 46: 813-820.

85. Barile L, Lionetti V, Cervio E, Matteucci M, Gherghiceanu M, et al. Extracellular vesicles from human cardiac progenitor cells inhibit cardiomyocyte apoptosis and improve cardiac function after myocardial infarction. Cardiovascular research. 2014; 103: 530-541.

86. Smith ZJ, Lee C, Rojalin T, Carney RP, Hazari S, et al. Single exosome study reveals subpopulations distributed among cell lines with variability related to membrane content. Journal of extracellular vesicles. 2015; 4: 28533.

87. Skotland T, Sandvig K, Llorente A. Lipids in exosomes: Current knowledge and the way forward. Progress in lipid research. 2017; 66: 30-41.

88. Ridder K, Keller S, Dams M, Rupp AK, Schlaudraff J, et al. Extracellular vesicle-mediated transfer of genetic information between the hematopoietic system and the brain in response to inflammation. PLoS Biol. 2014; 12: e1001874.

89. Nazimek K, Bryniarski K, Askenase PW. Functions of Exosomes and Microbial Extracellular Vesicles in Allergy and Contact and Delayed-Type Hypersensitivity. International archives of allergy and immunology. 2016; 171: 1-26.

90. Momen-Heravi F, Bala S. Extracellular vesicles in oral squamous carcinoma carry oncogenic miRNA profile and reprogram monocytes via NF-κB pathway. Oncotarget. 2018; 9: 34838-34854.

91. Khoo XH, Paterson IC, Goh BH, Lee WL. Cisplatin-Resistance in Oral Squamous Cell Carcinoma: Regulation by Tumor Cell-Derived Extracellular Vesicles. Cancers (Basel). 2019; 11.

92. Welponer H, Tsibulak I, Wieser V, Degasper C, Shivalingaiah G, Wenzel S, et al. The miR-34 family and its clinical significance in ovarian cancer. Journal of Cancer. 2020; 11: 1446-1456.

93. Zhu M, Wu J, Ma X, Huang C, Wu R, Zhu W, et al. Butyl benzyl phthalate promotes prostate cancer cell proliferation through miR-34a downregulation. Toxicology in vitro: an international journal published in association with BIBRA. 2019; 54: 82-88.

94. Shan Y, You B, Shi S, Shi W, Zhang Z, Zhang Q, et al. Hypoxia-Induced Matrix Metalloproteinase-13 Expression in Exosomes from Nasopharyngeal Carcinoma Enhances Metastases. Cell death & disease. 2018; 9: 382.

95. Huang CH, Yang WH, Chang SY, Tai SK, Tzeng CH, et al. Regulation of membrane-type 4 matrix metalloproteinase by SLUG contributes to hypoxia-mediated metastasis. Neoplasia (New York, NY). 2009; 11: 1371-1382.

96. Wang H, Hou L, Li A, Duan Y, Gao H, Song X. Expression of serum exosomal microRNA-21 in human hepatocellular carcinoma. Biomed Res Int. 2014; 2014: 864894.

97. Lousada-Fernandez F, Rapado-Gonzalez O, Lopez-Cedrun JL, Lopez-Lopez R, Muinelo-Romay L, et al. Liquid Biopsy in Oral Cancer. Int J Mol Sci. 2018; 19.

98. Vu LT, Peng B, Zhang DX, Ma V, Mathey-Andrews CA, et al. Tumor-secreted extracellular vesicles promote the activation of cancer-associated fibroblasts via the transfer of microRNA-125b. J Extracell Vesicles. 2019; 8: 1599680.

99. Ye SB, Li ZL, Luo DH, Huang BJ, Chen YS, et al. Tumor-derived exosomes promote tumor progression and T-cell dysfunction through the regulation of enriched exosomal microRNAs in human nasopharyngeal carcinoma. Oncotarget. 2014; 5: 5439-5452.

100. Dickman CT, Lawson J, Jabalee J, MacLellan SA, LePard NE, et al. Selective extracellular vesicle exclusion of miR-142-3p by oral cancer cells promotes both internal and extracellular malignant phenotypes. Oncotarget. 2017; 8: 15252-15266.

101. Rabinowits G, Bowden M, Flores LM, Verselis S, Vergara V, et al. Comparative Analysis of MicroRNA Expression among Benign and Malignant Tongue Tissue and Plasma of Patients with Tongue Cancer. Frontiers in oncology. 2017; 7: 191.

102. Ding L, Ren J, Zhang D, Li Y, Huang X, Hu Q, 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: 397-406.

103. Teng Y, Gao L, Loveless R, Rodrigo JP, Strojan P, et al. The Hidden Link of Exosomes to Head and Neck Cancer. Cancers (Basel). 2021; 13.

104. Wang X, Qin X, Yan M, Shi J, Xu Q, et al. Loss of exosomal miR-3188 in cancer-associated fibroblasts contributes to HNC progression. Journal of experimental & clinical cancer research: CR. 2019; 38: 151.

105. He L, Ping F, Fan Z, Zhang C, Deng M, et al. Salivary exosomal miR-24-3p serves as a potential detective biomarker for oral squamous cell carcinoma screening. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2020; 121: 109553.

106. Qin X, Guo H, Wang X, Zhu X, Yan M, et al. Exosomal miR-196a derived from cancer-associated fibroblasts confers cisplatin resistance in head and neck cancer through targeting CDKN1B and ING5. Genome biology. 2019; 20: 12.

107. Huang WC, Jang TH, Tung SL, Yen TC, Chan SH, et al. A novel miR-365-3p/EHF/keratin 16 axis promotes oral squamous cell carcinoma metastasis, cancer stemness and drug resistance via enhancing β5-integrin/c-met signaling pathway. Journal of experimental & clinical cancer research: CR. 2019; 38: 89.

108. Liu T, Chen G, Sun D, Lei M, Li Y, Zhou C, 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: 808-816.

109. Sakha S, Muramatsu T, Ueda K, Inazawa J. Exosomal microRNA miR-1246 induces cell motility and invasion through the regulation of DENND2D in oral squamous cell carcinoma. Sci Rep. 2016; 6: 38750.

110. Bae S, Brumbaugh J, Bonavida B. Exosomes derived from cancerous and non-cancerous cells regulate the anti-tumor response in the tumor microenvironment. Genes & cancer. 2018; 9: 87-100.

111. Bovy N, Blomme B, Frères P, Dederen S, Nivelles O, Lion M, et al. Endothelial exosomes contribute to the antitumor response during breast cancer neoadjuvant chemotherapy via microRNA transfer. Oncotarget. 2015; 6: 10253-10266.

112. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin. 2017 ; 67: 7-30.

113. Li R, Wang Y, Zhang X, Feng M, Ma J, Li J, et al. Exosome-mediated secretion of LOXL4 promotes hepatocellular carcinoma cell invasion and metastasis. Mol Cancer. 2019; 18: 18.

114. Wee I, Syn N, Sethi G, Goh BC, Wang L. Role of tumor-derived exosomes in cancer metastasis. Biochimica et biophysica acta Reviews on cancer. 2019; 1871: 12-19.

115. Theodoraki MN, Matsumoto A, Beccard I, Hoffmann TK, Whiteside TL. CD44v3 protein-carrying tumor-derived exosomes in HNSCC patients' plasma as potential noninvasive biomarkers of disease activity. Oncoimmunology. 2020; 9: 1747732.

116. Regimbeau M, Abrey J, Vautrot V, Causse S, Gobbo J, Garrido C. Heat shock proteins and exosomes in cancer theranostics. Seminars in cancer biology. 2021.

117. Gehrmann M, Specht HM, Bayer C, Brandstetter M, Chizzali B, et al. Hsp70--a biomarker for tumor detection and monitoring of outcome of radiation therapy in patients with squamous cell carcinoma of the head and neck. Radiation oncology (London, England). 2014; 9: 131.

118. Li YY, Tao YW, Gao S, Li P, Zheng JM, et al. Cancer-associated fibroblasts contribute to oral cancer cells proliferation and metastasis via exosome-mediated paracrine miR-34a-5p. EBioMedicine. 2018 ; 36: 209-220.

119. Muller L, Simms P, Hong CS, Nishimura MI, Jackson EK, et al. Human tumor-derived exosomes (TEX) regulate Treg functions via cell surface signaling rather than uptake mechanisms. Oncoimmunology. 2017; 6: e1261243.

120. Wieckowski EU, Visus C, Szajnik M, Szczepanski MJ, Storkus WJ, et al. Tumor-derived microvesicles promote regulatory T cell expansion and induce apoptosis in tumor-reactive activated CD8+ T lymphocytes. Journal of immunology (Baltimore, Md : 1950). 2009; 183: 3720-3730.

121. Szajnik M, Czystowska M, Szczepanski MJ, Mandapathil M, Whiteside TL. Tumor-derived microvesicles induce, expand and up-regulate biological activities of human regulatory T cells (Treg). PloS one. 2010; 5: e11469.

122. Mrizak D, Martin N, Barjon C, Jimenez-Pailhes AS, Mustapha R, et al. Effect of nasopharyngeal carcinoma-derived exosomes on human regulatory T cells. Journal of the National Cancer Institute. 2015; 107: 363.

123. Muller L, Mitsuhashi M, Simms P, Gooding WE, Whiteside TL. Tumor-derived exosomes regulate expression of immune function-related genes in human T cell subsets. Sci Rep. 2016; 6: 20254.

124. Madeo M, Colbert PL, Vermeer DW, Lucido CT, Cain JT, et al. Cancer exosomes induce tumor innervation. Nature communications. 2018; 9: 4284.

125. Ludwig N, Yerneni SS, Razzo BM, Whiteside TL. Exosomes from HNSCC Promote Angiogenesis through Reprogramming of Endothelial Cells. Molecular cancer research : MCR. 2018; 16: 1798-1808.

126. Fujiwara T, Eguchi T, Sogawa C, Ono K, Murakami J, Ibaragi S, et al. Carcinogenic epithelial-mesenchymal transition initiated by oral cancer exosomes is inhibited by anti-EGFR antibody cetuximab. Oral oncology. 2018; 86: 251-257.

127. Qadir F, Aziz MA, Sari CP, Ma H, Dai H, Wang X, et al. Transcriptome reprogramming by cancer exosomes: identification of novel molecular targets in matrix and immune modulation. Mol Cancer. 2018; 17: 97.

128. Li L, Li C, Wang S, Wang Z, Jiang J, 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: 1770-1780.

129. Ludwig S, Sharma P, Theodoraki MN, Pietrowska M, Yerneni SS, et al. Molecular and Functional Profiles of Exosomes From HPV(+) and HPV(-) Head and Neck Cancer Cell Lines. Frontiers in oncology. 2018; 8: 445.

130. Ludwig S, Marczak L, Sharma P, Abramowicz A, Gawin M, et al. Proteomes of exosomes from HPV(+) or HPV(-) head and neck cancer cells: differential enrichment in immunoregulatory proteins. Oncoimmunology. 2019; 8: 1593808.

131. Tong F, Mao X, Zhang S, Xie H, Yan B, et al. HPV + HNSCC-derived exosomal miR-9 induces macrophage M1 polarization and increases tumor radiosensitivity. Cancer letters. 2020; 478: 34-44.

132. Luo A, Zhou X, Shi X, Zhao Y, Men Y, et al. Exosome-derived miR-339-5p mediates radiosensitivity by targeting Cdc25A in locally advanced esophageal squamous cell carcinoma. Oncogene. 2019; 38: 4990-5006.

133. Abramowicz A, Wojakowska A, Marczak L, Lysek-Gladysinska M, Smolarz M, et al. Ionizing radiation affects the composition of the proteome of extracellular vesicles released by head-and-neck cancer cells in vitro. Journal of radiation research. 2019; 60: 289-297.

134. Jelonek K, Wojakowska A, Marczak L, Muer A, Tinhofer-Keilholz I, et al. Ionizing radiation affects protein composition of exosomes secreted in vitro from head and neck squamous cell carcinoma. Acta biochimica Polonica. 2015; 62: 265-272.

135. Mutschelknaus L, Azimzadeh O, Heider T, Winkler K, Vetter M, et al. Radiation alters the cargo of exosomes released from squamous head and neck cancer cells to promote migration of recipient cells. Sci Rep. 2017; 7: 12423.

136. Wang J, Lv B, Su Y, Wang X, Bu J, Yao L. Exosome-Mediated Transfer of lncRNA HOTTIP Promotes Cisplatin Resistance in Gastric Cancer Cells by Regulating HMGA1/miR-218 Axis. OncoTargets and therapy. 2019; 12: 11325-11338.

137. Vasan N, Baselga J, Hyman DM. A view on drug resistance in cancer. Nature. 2019; 575: 299-309.

138. Dong X, Bai X, Ni J, Zhang H, Duan W, Graham P, et al. Exosomes and breast cancer drug resistance. Cell death & disease. 2020; 11: 987.

139. Steinbichler TB, Dudás J, Skvortsov S, Ganswindt U, Riechelmann H, et al. Therapy resistance mediated by exosomes. Mol Cancer. 2019; 18: 58.

140. Lang L, Lam T, Chen A, Jensen C, Duncan L, et al. Circumventing AKT-Associated Radioresistance in Oral Cancer by Novel

Nanoparticle-Encapsulated Capivasertib. Cells. 2020; 9.

141. Csősz É, Lábiscsák P, Kalló G, Márkus B, Emri M, et al. Proteomics investigation of OSCC-specific salivary biomarkers in a Hungarian population highlights the importance of identification of population-tailored biomarkers. PloS one. 2017; 12: e0177282.

142. Tahiri A, Leivonen SK, Lüders T, Steinfeld I, Ragle Aure M, et al. Deregulation of cancer-related miRNAs is a common event in both benign and malignant human breast tumors. Carcinogenesis. 2014; 35: 76-85.

143. Xu H, Yang Y, Zhao H, Yang X, Luo Y, et al. Serum miR-483-5p: a novel diagnostic and prognostic biomarker for patients with oral squamous cell carcinoma. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2016; 37: 447-453.

144. Božinović K, Sabol I, Dediol E, Milutin Gašperov N, Manojlović S, et al. Genome-wide miRNA profiling reinforces the importance of miR-9 in human papillomavirus associated oral and oropharyngeal head and neck cancer. Sci Rep. 2019; 9: 2306.

145. Kirave P, Gondaliya P, Kulkarni B, Rawal R, Garg R, et al. Exosome mediated miR-155 delivery confers cisplatin chemoresistance in oral cancer cells via epithelial-mesenchymal transition. Oncotarget. 2020; 11: 1157-1171.

146. Kaskas NM, Moore-Medlin T, McClure GB, Ekshyyan O, Vanchiere JA, Nathan CA. Serum biomarkers in head and neck squamous cell cancer. JAMA otolaryngology-- head & neck surgery. 2014; 140: 5-11.

147. Xie C, Ji N, Tang Z, Li J, Chen Q. The role of extracellular vesicles from different origin in the microenvironment of head and neck cancers. Mol Cancer. 2019; 18: 83.

148. Li L, Cao B, Liang X, Lu S, Luo H, et al. Microenvironmental oxygen pressure orchestrates an anti- and pro-tumoral γδ T cell equilibrium via tumor-derived exosomes. Oncogene. 2019; 38: 2830-2843.

149. Xue VW, Wong CSC, Cho WCS. Early detection and monitoring of cancer in liquid biopsy: advances and challenges. Expert review of molecular diagnostics. 2019; 19: 273-276.

150. Becker A, Thakur BK, Weiss JM, Kim HS, Peinado H, et al. Extracellular Vesicles in Cancer: Cell-to-Cell Mediators of Metastasis. Cancer Cell. 2016; 30: 836-848.

151. Xie C, Du LY, Guo F, Li X, Cheng B. Exosomes derived from microRNA-101-3p-overexpressing human bone marrow mesenchymal stem cells suppress oral cancer cell proliferation, invasion, and migration. Molecular and cellular biochemistry. 2019; 458: 11-26.

152. Kosaka N, Iguchi H, Yoshioka Y, Hagiwara K, Takeshita F, et al. Competitive interactions of cancer cells and normal cells via secretory microRNAs. The Journal of biological chemistry. 2012; 2: 1397-1405.

153. Rosenberger L, Ezquer M, Lillo-Vera F, Pedraza PL, Ortúzar MI, et al. Stem cell exosomes inhibit angiogenesis and tumor growth of oral squamous cell carcinoma. Sci Rep. 2019; 9: 663.

154. Wang J, Zheng Y, Zhao M. Exosome-Based Cancer Therapy: Implication for Targeting Cancer Stem Cells. Front Pharmacol. 2016; 7: 533.

155. Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, et al. Head and neck squamous cell carcinoma. Nature reviews Disease primers. 2020; 6: 92.

156. Qu X, Li JW, Chan J, Meehan K. Extracellular Vesicles in Head and Neck Cancer: A Potential New Trend in Diagnosis, Prognosis, and Treatment. Int J Mol Sci. 2020; 21.

157. Nonaka T, Wong DTW. Liquid Biopsy in Head and Neck Cancer: Promises and Challenges. Journal of dental research. 2018; 97: 701-708.

158. Hess J, Unger K, Maihoefer C, Schüttrumpf L, Wintergerst L, Heider T, et al. A Five-MicroRNA Signature Predicts Survival and Disease Control of Patients with Head and Neck Cancer Negative for HPV Infection. Clinical cancer research: an official journal of the American Association for Cancer Research. 2019; 25: 1505-1516.

159. Xu MJ, Johnson DE, Grandis JR. EGFR-targeted therapies in the post-genomic era. Cancer metastasis reviews. 2017; 36: 463-473.

160. Theodoraki MN, Laban S, Jackson EK, Lotfi R, Schuler PJ, Brunner C, et al. Changes in circulating exosome molecular profiles following surgery/(chemo)radiotherapy: early detection of response in head and neck cancer patients. British journal of cancer. 2021; 125: 1677-1686.

161. Li SP, Lin ZX, Jiang XY, Yu XY. Exosomal cargo-loading and synthetic exosome-mimics as potential therapeutic tools. Acta Pharmacol Sin. 2018; 39: 542-551.

162. Kobayashi E, Hwang D, Bheda-Malge A, Whitehurst CB, Kabanov AV, et al. Inhibition of UCH-L1 Deubiquitinating Activity with Two Forms of LDN-57444 Has Anti-Invasive Effects in Metastatic Carcinoma Cells. Int J Mol Sci. 2019; 20.

163. Gosepath EM, Eckstein N, Hamacher A, Servan K, von Jonquieres G, et al. Acquired cisplatin resistance in the head-neck cancer cell line Cal27 is associated with decreased DKK1 expression and can partially be reversed by overexpression of DKK1. Int J Cancer. 2008; 123: 2013-2019.