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RNA cargo in motion: the exosomal connection to head and neck cancers

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Highlights:

- ExRNAs are biomarkers for early detection and prognosis of HNCs.
- They regulate NF-κB, EGFR, PI3K/AKT/mTOR, and TP53 in HNCs.
- Salivary and blood ExRNAs enable non-invasive HNC monitoring.
- ExRNAs shape the tumor microenvironment, aiding HNC progression.
- ExRNAs can serve as novel therapeutic targets in the treatment of HNC.

Abstract: Head and neck cancers (HNCs) are malignant tumors that differ from carcinomas in their biological behaviour and require a different diagnostic and treatment approach, especially in cancers like lung, breast, and prostate. Exosomal RNA (exRNA), particularly miRNA, mRNA, and lncRNA in blood or other body fluids through liquid biopsy, is emerging as a non-invasive biomarker for early cancer detection, prognosis, and treatment monitoring. Exosomal RNA modulates key signalling pathways like NF-κB, EGFR, PI3K/AKT/mTOR, and TP53 and contributes to the development, progression and therapeutic resistance of cancers. In this review, we focus on the roles of exosomal RNA in the growth and evolution of head and neck squamous cell carcinoma (HNSCC) as well as the emerging therapeutic strategies targeting exosomal RNA to improve clinical outcomes in HNC patients.

Keywords: head and neck cancers; HNCs; exRNA; miRNA; mRNA; lncRNA; cancer diagnosis

1. Introduction

1.1. The biogenesis of exosomes

The biogenesis of extracellular vesicles (EVs) involves distinct pathways that give rise to different types of vesicles, including exosomes, microvesicles, and apoptotic bodies. Exosomes are formed through the endosomal pathway, beginning with the inward budding of the plasma membrane to create early endosomes. As these mature into multivesicular bodies (MVBs), intraluminal vesicles (ILVs) are formed by further invagination of the endosomal membrane [1]. The sorting of cargo into ILVs is mediated by either the ESCRT (Endosomal Sorting Complexes Required for Transport) machinery or ESCRT-independent mechanisms, involving lipids such as ceramides and proteins like tetraspanins. Once MVBs are formed, they either fuse with lysosomes for degradation or with the plasma membrane to release ILVs as exosomes.

In contrast, microvesicles are directly shed from the plasma membrane through outward budding, while apoptotic bodies form during programmed cell death as the cell fragments. EVs share a common structure: a lipid bilayer that encloses a diverse cargo of proteins, lipids, and nucleic acids, including mRNAs, miRNAs, and lncRNAs. Exosomes, typically 30–150 nm in size, are enriched in tetraspanins (CD9, CD63, CD81), heat shock proteins, and lipid rafts, contributing to their stability and cellular targeting. Microvesicles are generally larger, ranging from 100–1000 nm, and carry surface proteins reflective of their parent cell membrane.

EVs play pivotal roles in intercellular communication, mediating processes such as immune response, tissue repair, and cancer progression. Their ability to transfer bioactive molecules to distant cells makes them potential biomarkers and therapeutic agents. The specific cargo and surface molecules of EVs determine their function and target specificity, highlighting their significance in both physiological and pathological contexts [2].

The biogenesis of exosomes is a complex and tightly regulated process that occurs within the endosomal system. It begins with the inward budding of the plasma membrane, forming early endosomes, which serve as sorting hubs for various cellular components. As these early endosomes mature into late endosomes, they undergo a second inward budding of their limiting membrane, resulting in the formation of small vesicles known as intraluminal vesicles (ILVs) within a larger structure called multivesicular bodies (MVBs [3]). The sorting of cargo, including proteins, lipids, and RNAs, into ILVs is facilitated by two main mechanisms. The first is the ESCRT-dependent pathway, involving a series of protein complexes called the Endosomal Sorting Complexes Required for Transport (ESCRT), which orchestrate cargo selection, membrane budding, and vesicle scission. Alternatively, the ESCRT-independent pathway relies on lipid raft-associated processes, utilizing molecules such as ceramides and tetraspanins like CD63 and CD81 to mediate vesicle formation [4].

MVBs have two possible fates: fusion with lysosomes for degradation or fusion with the plasma membrane for exosome release. The latter involves the coordinated action of Rab GTPases, particularly Rab27a and Rab27b, and SNARE proteins, which facilitate the docking and fusion of MVBs with the plasma membrane. Upon fusion, the ILVs are released into the extracellular environment as exosomes. These secreted exosomes, encapsulated within a lipid bilayer, can then interact with recipient cells through various mechanisms, including direct membrane fusion, receptor-ligand interactions, or endocytosis,

enabling the transfer of their bioactive cargo and influencing a wide range of physiological and pathological processes [5].

1.2. Roles and significance of exosomal RNA in cancer

Exosomal RNA plays a crucial part in cancer research and therapeutic applications as it offers valuable insights into tumor behaviour and serves as a potential therapeutic target. Exosomes, small extracellular vesicles secreted by cancer and other cells, along with a variety of other substances like DNA, RNA, lipids, metabolites, and cytosolic and cell-surface proteins, carry RNA molecules such as mRNA, miRNA, and lncRNA, which can be transferred to recipient cells, influencing processes like metastasis and immune evasion. The profiling of exosomal RNA, particularly miRNA, mRNA, and lncRNA in blood or other body fluids through liquid biopsy, is emerging as a non-surgical biomarker for initial cancer detection, prognosis, and treatment monitoring, notably in cancers like lung, breast, and prostate. By reflecting the genetic changes occurring within tumors, exosomal RNA helps assess tumor burden, progression, and patient-specific responses to therapies, allowing clinicians to personalize treatment strategies. Moreover, targeting exosome formation, release, or modifying exosomal RNA content is being explored as a therapeutic approach to curb cancer progression.

1.3. Head and neck cancers A brief overview

Head and neck cancers (HNCs) are the type of cancers that affect the tissue and organs of the head and neck region of the body. These cancers are usually typically categorized based on their anatomical location, such as the oral cavity, pharynx (throat), larynx (voice box), nasal cavity, paranasal sinuses, and salivary glands [6]. They are primarily made of squamous cell carcinomas that evolve out of the squamous epithelial cells within the mucosae of these areas. HNC risks are closely linked with life habits, environment, and specific viruses The risks of HNC are strongly related directly with the way an individual lives, and the environment he or she is exposed to [7].

Types and classification of HNC and their causes

Anatomical location defines head and neck cancer classification. Malignancies of the lip, tongue, gingiva, mouth floor, hard and soft palates, and buccal mucosa include oral cavity cancers. Affecting the upper, middle, and lower sections of the throat, pharyngeal tumors are further classified as nasopharyngeal, oropharyngeal, and hypopharyngeal tumors. The larynx—including the vocal cord, supraglottis, and subglottic tissues—is affected in laryngeal cancer. While salivary gland tumors affect the parotid, submandibular, and sublingual glands, nasal cavity and paranasal sinus malignancies grow in the nose and sinus areas [8,9].

These malignancies in the head and neck area develop histologically from several cell types. Typically linked with tobacco and alcohol use, squamous cell carcinoma (SCC) is the one of the most typically occurring histological category and develops from the mucosal epithelial cells of the head and neck. Adenocarcinomas grow from glandular cells in the salivary glands; symptoms depend on the gland affected. Beginning from the lymphatic system, lymphomas—both Hodgkin and non-Hodgkin varieties—can also affect the head and neck [10]. While glandular and lymphatic tissues are the sources of adenocarcinomas and lymphomas respectively, SCC remains the most common variety throughout

most areas of the head and neck, especially in mucosal sites. Sarcomas, which develop from connective tissues like bone, cartilage, or muscle, are rare tumors that differ from carcinomas in their biological behaviour and require a different diagnostic and treatment approach. Melanocytes that produce melanin and are found on the head and neck mucosa may require a unique treatment. Mucoepidermoid carcinomas, which mostly affect salivary glands, are uncommon in that they have both mucus-secreting and squamous cells. To effectively treat and prognose head and neck cancers, one must understand their histological types.

HNC are caused by factors both genetic and environmental. The use of tobacco is the most evident risk. Smoking cigars, cigarettes, vapes, pipes, and smokeless tobacco products like tobacco or snuff expose mucosal cell DNA to carcinogens. Alcohol damages DNA and hinders DNA repair, making smoking more cancerous [11]. Targeting tumor suppressor proteins helps HPV oncoproteins E6 and E7 target oropharyngeal carcinoma. E6 binds to p53 via E6AP, causing p53 degradation via ubiquitination, therefore rendering the cell unable to react to DNA damage and so fostering unchecked development. E7 hooks itself to RB1, therefore upsetting its interaction with E2F transcription factors and accelerating DNA synthesis in cells. RB1's inactivity removes a crucial cell cycle checkpoint, therefore enabling unrestrained cell division. Strongly connected to these processes, high-risk HPV—especially HPV-16—drives the development of oropharyngeal cancer [6].

Periodontal disease, poor dental care, and chronic denture discomfort are other risks. By disrupting DNA or generating chronic inflammation, asbestos, wood dust, and formaldehyde increase cancer risk [12]. Cancer risk rises without antioxidant-rich fruits and vegetables, which impede DNA repair and carcinogen removal. Genetic risk is present because HNC is caused by hereditary DNA repair and cell cycle aberrations. Head and neck radiation patients are more likely to acquire localized secondary malignancies [13]. Epstein-Barr Virus (EBV) infection produces genetic and epigenetic changes that promote nasopharyngeal cancer [14]. Cultured betel quid and areca nut consumption increases oral cancer risk [15].

HNC symptoms depend on tumor location. Oral symptoms include red or white spots, jaw enlargement, unexplained bleeding, pain, and trouble chewing or swallowing. Pharyngeal carcinoma can cause throat pain, coughing, voice changes, ear pain, and neck lumps [16]. Laryngeal cancer produces hoarseness, throat pain, coughing, swallowing issues, and ear pain. Epistaxis, recurrent sinus infections, face pain or swelling, and anosmia may suggest nasal cavity and paranasal sinus cancer [17]. Rashes, soreness, and face swelling can develop from salivary gland tumors.

Epigenetic and inherited changes induce HNCCs. Cancer genes along with EGFR, MYC, and RAS are activated by tobacco and alcohol carcinogens, promoting cell proliferation and deactivating tumor suppressor genes like TP53 and CDKN2a. By means of its latent membrane protein 1 (LMP1), EBV induces inflammation, cell survival, and genomic instability via reactive oxygen species (ROS), hence activating pathways such as NF-κB and JAK/STAT. Damage of DNA and cancer follow from this. Cancer proteins E6 and E7, that inactivate p53 and RB1, hence driving unregulated cell proliferation, show different molecular profiles in HPV-driven malignancies, especially from HPV-16. Because of their distinct viral antigens, HPV-positive tumors frequently lack TP53 mutations and react better to less aggressive treatments than tobacco-induced malignancies. Immunotherapy has shown great results [18]. Table 1 shows that bacterial, periodontal, and asbestos and formaldehyde inflammation cause DNA damage and mutations, fueling cancer.

Table 1. Different cancer types affecting the head and neck region, specifying the anatomical areas involved, common causes such as lifestyle factors, infections, and environmental exposures, as well as detailing both early-stage (acute) symptoms and long-term (chronic) symptoms that indicate disease progression.

Cancer type	Region affected/found	Majorly known causes	Common acute symptoms	Common chronic symptoms
Oral cavity cancer	Lips, tongue, gingiva, floor of the mouth, hard and soft palates, buccal mucosa	Tobacco usage, alcohol drinking, HPV infection, poor oral hygiene, chronic irritation from ill-fitting dentures, gum disease, food habits lacking in vegetables and fruits	Persistent sore or ulcer, red/white patches, unexplained bleeding	Swelling in the jaw, pain or difficulty while chewing or swallowing
Pharyngeal cancer	Nasopharynx, oropharynx, hypopharynx	Tobacco use, alcohol consumption, HPV infection, poor oral hygiene, diet low in fruits and vegetables	Sore throat, persistent cough, ear pain	Difficulty swallowing, changes in voice, presence of neck lump
Laryngeal cancer	Glottis (vocal cords), supraglottis (tissues above the cords), sub glottis (tissues beneath the cords)	Tobacco use, alcohol consumption, HPV infection, poor oral hygiene, diet low in fruits and vegetables	Hoarseness, sore throat, coughing	Difficulty swallowing food, pain in the ears
Nasal cavity and paranasal sinus cancer	Nasal cavity, paranasal sinuses	Tobacco use, exposure to wood dust, asbestos, and formaldehyde	Recurrent sinus infections, frequent epistaxis	Sinister sinusitis, facial pain or swelling, anosmia
Salivary gland cancer	Parotid glands, submandibular glands, sublingual glands	Tobacco use, exposure to radiation, poor oral hygiene	Pain in or about the head or face, swelling of the face, particularly in the region of the jaws	Lump or growth on the face, constant feeling of tenderness or discomfort in the glands, prolonged discomfort in the gland regions, redness in the gland locations
Squamous cell carcinoma (SCC)	Mucosal surfaces of the head and neck	Tobacco use, alcohol consumption, HPV infection	It varies based on location and generally includes sores, lumps, or abnormal growths.	Persistent sores, lumps, or abnormal growths that worsen over time
Adenocarcinoma	Salivary glands	Genetic factors, radiation exposure	Swelling, pain in the gland	Difficulty swallowing or moving the jaw
Lymphoma	The lymphatic system in the head and neck	Genetic causes, viral infections (e.g., Epstein-Barr virus)	Inflamed lymph nodes, weariness	Fever, night sweats, weight loss without reason
Sarcoma	Connective tissues like bone, cartilage, muscle	Genetic factors, radiation exposure	Swelling, pain, or tenderness in the affected area	Growing mass or lump, limited movement in the affected area
Melanoma	Melanocytes on the mucosal surface of the head and neck	UV exposure, genetic factors	New or changing pigmented lesions	Lesions that grow, change color or bleed
Mucoepidermoid carcinoma	Salivary glands	Genetic factors, radiation exposure	Painless swelling or mass	Pain or numbness in the affected area, difficulty swallowing or opening the mouth

2. Diverse exosomal RNA types: Shaping tumor behaviour in head and neck cancer

2.1. Diverse forms of extracellular RNAs shape their functional roles

Exosomes are extracellular vesicles arising from all the cells and enclose nucleic acids, transcriptional factors, proteins, and lipids contributing to intercellular communication. The presence of exosomes can be found in many biofluids like saliva, plasma, and urine [19,20]. Exosomal RNA plays many regulating roles through various molecular processes like transcription, translation, and angiogenesis and aids in tumor progression [21,22]. However, genes are extensively transcribed across genomes, and a small segment of it codes for proteins, the coding RNA's-Messenger RNA (mRNA). The complexity of an organism is highly correlated with the abundance of non-coding RNA genes(ncRNA) [23]. ncRNA's can be classified as structural RNA (rRNA, tRNA) and regulatory RNA (siRNA, snRNA, lncRNA, miRNA, YRNA, piRNA, circRNA) as shown in Table 2 [24].

Table 2. Different types of exosomal RNA, their structural features, functional roles, and clinical significance.

Exosomal RNA	Structural and functional description	Clinical application
mRNA	It influences the amount of proteins made. Size varies depending on the gene being transcribed [25].	They enable non-invasive diagnosis by detecting cancer-specific mutations and track tumor dynamics guiding therapy adjustment and resistance mechanisms [26–29].
rRNA	It forms ribosome core, catalyses peptide bond formation, positions mRNA and tRNA, and aids translocation during protein synthesis [30].	rRNA is crucial in pathogen detection, evolutionary studies, and cancer diagnosis, where its levels and modifications serve as potential cancer biomarkers
tRNA	Cloverleaf-shaped molecule, carries specific amino acids to ribosomes via anticodon-codon matching, ensuring accurate protein synthesis. It also proofreads and regulates gene expression [31].	tRNA elucidates genetic and cancer mechanisms, guiding therapeutic developments targeting translation processes [32].
snRNA	snRNA, part of spliceosome, eliminates introns from pre-mRNA, unites exons to form complete mRNA, and regulates alternative splicing, impacting protein diversity and gene expression [33].	snRNA aids pathogen detection via PCR, and informs therapeutic strategies for genetic disorders and cancers by targeting splicing mechanisms, though its clinical application in HNC remains limited [34,35].
siRNA	siRNA, 20–25 nucleotides, binds mRNA, triggers degradation, silences genes, and prevents protein production [36].	siRNA, a gene-silencing tool, holds promise in cancer treatment by targeting and downregulating harmful proteins [37].
YRNA	It forms ribonucleoproteins, modulates cellular stress responses, and regulates RNA quality and immunity. Dysregulation links to autoimmune diseases and cancers [38]	Y-RNA potential for HNC biomarkers and treatment targets under exploration [39].
miRNA	miRNAs, 21 nucleotides, regulate gene expression by binding to mRNA, causing degradation or translation inhibition [40].	It serves as a biomarker, guides therapeutic strategies through miRNA-based therapies like mimics and anti-miRNAs, and offers new avenues in drug development [41].
lncRNA	RNA, and proteins, influencing chromatin structure, RNA processing, and subcellular localization [42].	LncRNAs like HOTAIR and MALAT1 are cancer biomarkers, predict disease progression, and offer therapeutic targets for personalized cancer treatment [43].

Table 2. *Cont.*

Exosomal RNA	Structural and functional description	Clinical application
CircRNA	It is a covalently closed RNA molecule that regulates gene expression through diverse mechanisms, including miRNA sponging, protein binding, and transcriptional modulation.	CircRNAs serve as disease biomarkers and potential therapeutic targets, while viral circRNAs impact immune response, cell growth, and virus replication [44].
piRNA	piRNAs, 24–31 nucleotides, bind Piwi proteins to stifle transposable elements and control gene expression in germ cells. 3' modifications stabilize piRNAs, crucial for genome integrity, epigenetic regulation	piRNAs are potential cancer biomarkers with dysregulated expression. Targeting piRNAs offers promising therapeutic avenues, though requires further research and validation for clinical application [45].
snoRNA	snoRNAs guide rRNA, tRNA, and snRNA modifications, ensuring RNA stability and function. They aid ribosome biogenesis, and spliceosome assembly, and regulate gene expression, critical for protein synthesis and cellular integrity [46].	snoRNAs are potential cancer biomarkers due to altered expression. They offer therapeutic potential by targeting RNA processing pathways for disease treatment [47].

2.2. Coding exosomal RNA: insights into HNC progression and mechanisms

mRNA

Transcriptome analysis of salivary samples identified seven potential biomarkers for oral cancer, which may assist in early diagnosis and treatment planning. Upregulated IL-8, IL-1β, S100P, and H3F3A promote inflammation, cell growth, and metastasis, and disrupt normal gene expression in oral cancer. Downregulated OAZ1 (ornithine decarboxylase antizyme1), DUSP1 (dual specificity phosphatase 1), and SAT (spermine N1-acetyltransferase) impair polyamine control, signaling pathway inhibition, and stress response, contributing to cancer progression [48].

CD44 in pharyngeal cancers promotes tumor evolution by boosting cell adhesion, migration, and invasion compared to other cancers [49].

Exosomal mRNA analysis distinguished HPV-positive and HPV-negative HNCs. The HPV-positive HNCs had HPV16E6 and HPV16E7 proteins which inactivate tumor suppressors, and promote cell growth by evading immune response leading to proliferation [50].

Exosomes from cancer-associated fibroblasts (CAFs) presents a significant part in the progression of oral squamous cell carcinoma (OSCC). Exosomes derived from CAFs (CAFs-Exo) significantly promoted OSCC proliferation and immunosuppression, likely through regulating PIGR (Polymeric Immunoglobulin Receptor), CD81(Cluster of Differentiation 81), UACA (Ubiquitin-Associated and Coiled-Coil Domain Containing Protein), and PTTG1IP (Pituitary Tumor-Transforming Gene 1 Interacting Protein) in Cal-27 cells (Cal-27 cells are a human oral squamous cell carcinoma (OSCC) cell line derived from a primary tumor in the tongue.) CAFs-Exo influences tumor immune control through hsa-miR-139-5p, ACTR2, and EIF6, with potential therapeutic targets identified for OSCC [51].

Rab11 is a protein involved in intracellular trafficking, including exosome secretion. Rab11b expression is linked to better HNSCC survival. Rab11 regulates EGFR and EpCAM exosome secretion, affecting cell migration and invasion. Overexpression of Rab11 suppresses HNSCC, while knockdown promotes tumor progression. Rab11b is a potential prognostic marker and therapeutic target [52].

2.3. Non-coding exosomal RNA: Insights into HNC progression and mechanisms

2.3.1. tRNA

tRFs (tRNA derived fragments) have been found to regulate proliferation, invasion, metastasis, and gene expression in various cancers [53]. In hypopharyngeal carcinoma, tRF-1:30-Lys-CTT-1-M2 was seriously upregulated in the extracellular vesicles of cancer patients compared to healthy controls. tRF-1:30-Lys-CTT-1-M2 can exist as a potential biomarker [54]. Specific tRNA half, 5' tRNA-Val-CAC-2-1, was considerably altered in both serum and tissue samples of the Oral squamous cell carcinoma (OSCC) patients. This tRNA half is associated with key genes participating in cell cycle regulation and differentiation, like FBXO31, WEE1, RB1, and E2F1 [55].

2.3.2. Y-RNA

Higher Y-RNA levels in HNSCC is associated with advanced tumour stage. Y-RNA1 has got association with HPV infection in HNSCC patients. YRNA1 showed potential as a biomarker for cancer detection and prognosis. YRNA1 expression is found in HPV-positive patients with HNSCC [56]. Y RNA-derived small fragments (30–33 nucleotides) show significant dysregulation, indicating alterations in small non-coding RNA networks. Specifically, oral squamous cell carcinoma (OSCC) is associated with reduced levels of 5’-end Y RNA fragments, highlighting distinct Y RNA expression patterns linked to tumor biology [57].

2.3.3. snRNA

U6 small nuclear RNA (snRNA) plays a significant role in breast cancer, particularly in pre-mRNA splicing through its involvement in the spliceosome. Elevated levels of U6 snRNA were found in breast cancer samples, with a 3.6-fold increase compared to controls. Its overexpression correlates with advanced cancer stages and grades, suggesting its role in cancer progression. Additionally, U6 snRNA, when combined with miR-548b-5p, demonstrated strong diagnostic potential, making it a valuable biomarker for detecting breast cancer [58] The role of SnRNA in head and neck cancers remain an area to be explored.

2.3.4. rRNA

Urinary exosomal rRNA holds promise as a non-invasive prostate cancer biomarker [59]. Exosomes derived from human breast cancer cell lines MDA-MB-231 and MDA-MB-436, fragmented 28S and 18S rRNA subunits were predominantly found in the exosomal RNA content. These rRNA fragments made up a significant portion of the exosomal RNA, with only low levels of intact rRNA (5.2% in MDA-MB-231 and 5.6% in MDA-MB-436). The presence of rRNA in these exosomes highlights their potential role as biomarkers for non-invasive cancer diagnostics [60]. While extensively studied in other cancers, the role of exosomal rRNA in head and neck cancers remains largely unexplored.

2.3.5. siRNA

Head and neck tumor cells use exosomes to export PD-L1(programmed death-ligand 1), shielding them from immune attack. Exosomal siRNA is used to target PD-L1 in both immune and cancer cells. This aims

to silence PD-L1, boost immunity, and improve cancer immunotherapy in head and neck cancer [61,62]. siRNA is used to silence specific genes involved in oral cavity cancer pathogenesis. For instance, siRNA can target oncogenes such as EGFR (Epidermal Growth Factor Receptor), that is often upregulated in oral cancers, leading to enhanced cell proliferation and survival [63]. Blocking EGFR signaling has the potential to inhibit the malignant behaviour of OSCC cells. Exosomal delivery of siRNA enhances targeted therapy by improving specificity, stability, and uptake, thus increasing the effectiveness of gene silencing [64,65].

2.3.6. miRNA

There are many types of oral cavity cancer, the predominant one being Oral Squamous Cell Carcinoma (OSCC). Exosomal miRNAs play a major role in tumor microenvironment modification, thus contributing to the growth of tumors, metastasis, angiogenesis, *etc.* Exosomal miRNAs such as miR-21-5p, miR-342-3p, and miR-1246, help in tumor growth. Exosomal miRNAs, including miR-21-5p, miR-342-3p, and miR-1246, play crucial roles in promoting tumor growth [66]. These exosomal miRNAs also inhibit the progression of OSCC. For example, exosomal miRNA-126 suppresses the oncogenic behaviour and signaling of OSCC cells [67]. Exosomal miRNAs play a major role in serving as a molecular marker of HNSCC. For instance, exosomal miR-486 and miR-10b-5p usually help in immune pathway repression and cellular metabolism reprogramming. However, they are noted to be in an upregulated degree in the saliva of HNSCC patients. This indicates that exosomal miRNAs work as major non-invasive salivary biomarkers for HNSCC [68]. Their role in the proliferation and differentiation of Nasopharyngeal cancer cells is paramount, for instance, miR-BART7-3p is found to be a therapeutic target and promising prognostic marker of this cancer. Hsa-miR-24–3p, hsa-miR-891a, hsa-miR106a-5p, hsa-miR20a-5p, and hsa-miR1908 are used to modulate the changes in the differentiation and proliferation of tumor cells [69]. Exosomal miRNA, miR-9 prevents the angiogenesis of Nasopharyngeal cancer cells, by regulation of the PDK/AKT pathway [70].

2.3.7. lncRNA

One of the most predominant types of Oral Cavity Cancer is OSCC. Exosomal lncRNA helps in the chemoresistance and progression of tumor cells, through different mechanisms. Through the research done by Ding *et al.*, it was seen that lncRNA FLJ22447 derived from OSCC exosomes shows upregulation in Cancer-Associated Fibroblasts and proliferation of OSCC is induced by Interleukin-33 (IL-33) [71]. LncRNA has a major role in metastasis, invasion, and maintenance of HNSCC cells. For example, MALAT1 has a significant part in the migration and invasion mediation of tumor cells. Similarly, Linc-ROR exhibits a part in the formation and maintenance of undifferentiated tumor cells [69]. Exosome-derived lncRNA significantly diminishes the radiosensitivity of laryngeal cancer cells. HOTAIR, an exosomal long non-coding RNA, significantly diminishes radiosensitivity in laryngeal cancer cells. This lncRNA acts to negatively influence radiosensitivity in Laryngeal Cancer Cells. It operates as a competitive endogenous RNA (ceRNA) for miR-454-3p and modulates E2F2 [72].

2.3.8. circRNA

CircRNA plays an important role in Oral Cavity Cancer, particularly in OSCC. For example, has_circ_0069313 an exosomal circRNA showed increased PDL1 levels in OSCC, due to the sponging of miR-325-3p. PDL1 helps in the inhibition of effector T-cells. Targeting this can be an important therapeutic strategy. Consequently, has_circ_0069313 presents itself as a viable treatment target for OSCC. Multiple studies indicate that exosomal has_circ_0069313 facilitates the progression of OSCC *in vivo*. They play a major role in the propagation of HNSCC. Through the studies of Chen *et al.*, it was found that there was a difference in the expression of these circRNAs in the HNSCC tissues and non-cancerous control tissues. CircPVT1 is one of the circRNAs studied in HNSCC tissues. Verduci *et al.* demonstrate that the expression of circPVT1 is transcriptionally upregulated by the p53/YAP/TEAD complex, which modulates the expression of miR-497-5p. This alters the malignant characteristics of HNSCC cell lines [69]. Exosomal circRNAs are particularly important due to their tissue specificity and high stability, making them valuable as potential diagnostic and prognostic biomarkers. Therefore, these can be used as a potential biomarker of Nasopharyngeal cancer. It is seen that overexpression of a circRNA called circMYC promoted the proliferation of cells and reduced radio sensitivity [73].

2.3.9. piRNA

piRNA plays a major role in OSCC. For example, increased piR-35462 expression enhanced OSCC cell migration, proliferation, and invasion. This enhanced expression also leads to poor prognosis in OSCC tissues. This piRNA also facilitates the increased expression of fat mass and obesity-associated protein (FTO) in OSCC cells, hence contributing to cancer progression. The overexpression of FTO increases the expression and stability of Twist1 mRNA, resulting in tumor development and epithelial-mesenchymal transition (EMT) [74].

2.3.10. snoRNA

Although snoRNAs from various sources have been linked to cancer, the role of exosomal snoRNAs remains understudied and warrants further investigation.

2.4. Exosomal RNA and their significance in HNC

Exosomal RNAs as mentioned in the above sections, denote the RNAs carried by extracellular vesicles (exosomes). These molecules play crucial cellular processes in tumor cells like its growth, metastasis, angiogenesis, immune modulation, and therapy resistance. HNC particularly comprise a varied array of tumors including conditions in the oral cavity, pharynx, larynx, paranasal sinuses, and nasal cavity. The contributions of exRNAs in HNC have been a topic of active research with growing evidence suggesting their involvement in disease pathogenesis and response to treatment. There are various types of non-coding RNAs like microRNA, lncRNA, circular RNAs, and small nucleolar RNAs (snoRNAs) among others. As Figure 1 illustrates, each of these factors plays a crucial role in HNC, fundamental to the interaction among cancer cells and their environment, which influences the growth and treatment resistance of HNC.

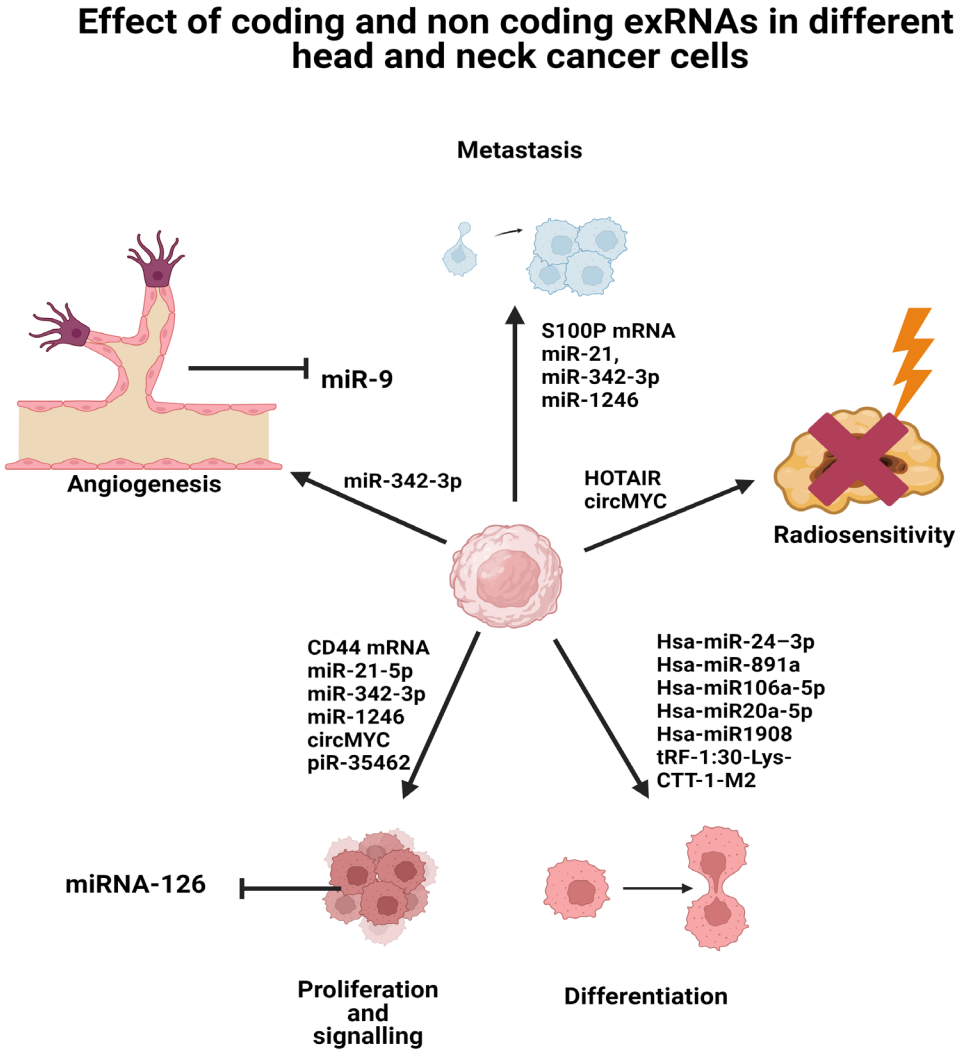

Figure 1. Effects of different exRNAs on head and neck cancer.

MicroRNAs (miRNAs) are one of the most studied exRNAs in the context of HNC. These non-coding RNAs modulate gene expression post the transcription process by target mRNA binding, leading to their degradation and translational inhibition. For instance, miR-31 is associated with resistance to radiation therapy in HNC by targeting HIF-alpha and modulating the hypoxic response. These miRNAs contribute to the aggressive nature of HNC and present potential targets for therapeutic interventions [75,76]. Long non-coding RNAs participate in a variety of regulatory processes including chromatin remodelling, transcriptional and post-transcriptional regulation. Exosomal lncRNAs act as signaling molecules that transform the behaviour of receiving cells. HOTAIR is an exosomal lncRNA that is linked with increased invasiveness and poor outcome in the case of HNC. Small Nuclear RNAs (snRNAs have a part in the splicing of pre-mRNAs. In HNC, aberrant splicing of pre-mRNAs is regulated by altering the expression of oncogenes and tumor suppressors. snRNA also modulates the expression of genes related to EMT and cell migration [77,78].

Circular RNAs behave like miRNA sponges, regulators of gene expression, and interactors with RNA-binding proteins. In the context of HNC, CircPVT1 is the circRNA that promotes cell proliferation

by sponging miR-125b, thereby upregulating MYC expression. CircRNA CircHIPK3 enhances the migratory and invasive capabilities of cancer through its interactions with the various miRNAs. These molecules can also modulate drug resistance by affecting the expression of genes implicated in drug metabolism and apoptosis [79]. Small nucleolar RNAs (snoRNAs) guide chemical modifications of other RNAs such as rRNAs and snRNAs. In HNC, these modulate ribosomal RNA processing and ribosome biogenesis, affecting cell proliferation. For instance, snoRNA SNORD78 is associated with increased metastatic potential by altering the expression of genes involved in cell migration and invasion [80,81].

Small Cajal Body-Specific RNAs (scaRNAs) are involved in the modification of snRNAs within the cajal bodies. These are capable of influencing the splicing of pre-mRNAs and impacting cell proliferation. In HNC, scaRNAs can modulate the expression of genes associated with EMT and cellular migration. They may also modulate therapy resistance by altering the splicing of genes involved in DNA repair and cell death [82]. Piwi-interacting RNAs (piRNAs) are involved in silencing transposons and regulating gene expression in germ cells. In HNC, these influence cancer cell proliferation by regulating gene expression and stability. They are also known to modulate gene expression patterns of the genes involved in cell adhesion and migration, affecting the metastatic potential [83]. Transfer RNAs (tRNAs) and tRNA-derived Fragments (tRFs) play crucial roles in protein synthesis and regulate gene expression, cell proliferation, and apoptosis. These processes are controlled by modulating oncogenes and tumor suppressors. They also affect cell migration and invasion by interacting with various signaling pathways. Ribosomal RNAs (rRNAs) are imperative components of ribosomes and play a key role in protein synthesis. Alterations in rRNA processing and ribosome biogenesis contribute to uncontrolled cell proliferation in HNC. rRNAs also influence the metastatic potential by affecting the translation of proteins participating in cell migration [84].

Exosomal mRNAs are capable of being transported to recipient cells where their codes are translated for various proteins. It is conclusive from Table 3 and the above-mentioned types of exosomal RNAs that most of these RNAs primarily attack the genes that are involved in cell migration and invasion to prevent these tumors from undergoing metastasis. Further research into the mechanisms and pathways affected by the exRNA will be given in the upcoming sessions.

Table 3. Summary of key studies on exosomal RNA in head and neck cancer (HNC) that highlights pivotal studies investigating the role of various exosomal RNA types, including miRNAs, lncRNAs, circRNAs, and piRNAs, in different subtypes of HNCs.

Article title	Author details	Type of HNC	Type of exosomal RNA considered	Findings of the study
Analysis of mRNA-miRNA interaction network reveals the role of CAFs-derived exosomes in the immune regulation of oral squamous cell carcinoma	Wei-Zhou Wang, Xue Cao, Li Bian *et al*. [51]	Oral squamous cell carcinoma (OSCC)	hsa-miR-139-5p, ACTR2 and EIF6	This study finds that the exosomes derived from the Cancer- associated fibroblasts (CAFs) have a regulatory role on cancer by promoting cell migration and proliferation in Cal-27 cells. This is achieved by cellular immunity modification

Table 3. *Cont.*

Name of the paper	Author details	Type of HNC	Type of exosomal RNA considered	Findings of the study
Rab11 suppresses head and neck carcinoma by regulating EGFR and EpCAM exosome secretion	Kunihiro Yoshida, Kaung Htike, Takanori Eguchi *et al*. [52]	Human oral cancers	Rab11	While Rab11 was found to promote breast and lung cancer, researchers find that Rab11 deters HNC by means of EGFR receptor recycling via exosome secretion.
MicroRNAs as Therapeutic Targets in Nasopharyngeal Carcinoma	Sumei Wang, François-Xavier Claret, Wanyin Wu *et al*. [70]	Nasopharyngeal carcinoma (NPC)	miR-21, miR-9, mi-223, miR-24-3p and miR-29a	miR-21 was reported in higher levels in a variety of cancer cell types like oesophageal and breast cancer. In NPC, these miRNAs were found to act as biomarkers as well as initiate the progression of tumor cells. This could also be exacerbated by an initiation of an inflammatory response as a result of binding of the RNAs to the toll-like receptors on immune cells.
The Emerging Role of Exosomes in Oral Squamous Cell Carcinoma	Yanhui Lu, Zhichao Zheng, Yunyi Yuan *et al*. [71]	Oral squamous cell carcinoma (OSCC)	miR-24-3p, miR-382-5p, miR-142-3p and miR-34a-5p,	This study details the ways in which exosomal RNA are able to increase the proliferation and invasion of OSCC. These highly expressed RNAs were capable of acting as biomarkers as well as influencing the cell motility and metastasis in case of cancer.
Loss of exosomal miR-3188 in cancer-associated fibroblasts contributes to HNC progression	Xiaoning Wang, Xing Qin, Ming Yan *et al*. [85]	Human primary fibroblasts isolated from tumors (CAFs) of CAL 27, 293 T and MC-3 T3-E1 cells	miR-3188	Recent studies indicate that exosomal miRNAs, particularly miR-3188, are significantly reduced in cancer-associated fibroblasts (CAFs) compared to normal fibroblasts (NFs) in HNCs, contributing to tumor progression by targeting BCL2 and influencing malignant phenotypes.
Circulating exosomal LncRNAs: A non-invasive liquid biopsy biomarker for real-time monitoring of cancer therapeutic efficacy in HNSCC	Jospin Sindya S, Jeevitha Rajanathadurai, Lakshmi Thangavelu [86]	Oral squamous cell carcinoma (OSCC)	miR-140-5p, miR-143-5p, and miR-145-5p	The paper highlights that exosomes in biological fluids contain diverse RNA types, including lncRNAs, which are crucial for understanding HNSCC. These exosomal lncRNAs serve as non-invasive biomarkers for monitoring therapeutic efficacy in head and neck cancer.
Exosomal non-coding RNAs-Mediated Crosstalk in the Tumor Microenvironment	Qi Chen, Yuefeng Li, Yueqin Liu *et al*. [87]	Oral squamous cell carcinoma (OSCC)	miR-365	Recent studies indicate that exosomal miR-196a from cancer-associated fibroblasts enhances head and neck cancer cell growth and cisplatin resistance, while exosomal miR-365 from tumor-associated macrophages induces gemcitabine resistance by promoting pyrimidine metabolism in pancreatic cancer cells.
Exosome-mediated long noncoding RNA (lncRNA) PART1 suppresses malignant progression of oral squamous cell carcinoma via miR-17-5p/SOCS6 axis.	Yuhen Du, Yanjie Shuai, Hongling Wang [88]	Oral squamous cell carcinoma (OSCC) and tongue squamous cell carcinoma	miR-17-5p/PART1-SOCS6 axis	This study talks about the inhibitory reaction that is brought about by PART1 in various HNCs. It has been suggested that this exosomally- transmitted RNA functions as a tumor suppressor along with SOCS6, a JAK/STAT pathway suppressor.

Table 3. *Cont.*

Name of the paper	Author details	Type of HNC	Type of exosomal RNA considered	Findings of the study
M1 macrophage-derived exosomes and their key molecule lncRNA HOTTIP suppress head and neck squamous cell carcinoma progression by upregulating the TLR5/NF-κB pathway	Huaili Jiang, Lei Zhou, Na Shen *et al.* [89]	Head and neck squamous cell carcinoma (HNSCC)	HOTTIP lncRNA	HOTTIP suppressed HNSCC by the competitive binding to miR-19a-3p and miR-19b-3p and the upregulation of the NF-κB pathway
Exosomal microRNAs Targeting TP53 Gene as Promising Prognostic Markers for Head and Neck Squamous Cell Carcinoma	Vijayashree Priyadharsini Jayaseelan, Paramasivam Arumugam [90]	Head and neck squamous cell carcinoma (HNSCC)	hsa-miR-421, hsa-miR-548f-5p, and hsa-let-7c-5p	The study identified 26 exosomal microRNAs in head and neck cancer (HNC), with hsa-miR-421 and hsa-let-7c-5p showing significant differential expression and prognostic value, particularly in blood and saliva, indicating their potential as biomarkers for HNC.
Salivary exosomal microRNAs as biomarkers for head and neck cancer detection—a literature review	Cosmin Ioan Faur, Horatiu Rotaru, Ciprian Osan [91]	Head and neck squamous cell carcinoma (HNSCC)	miR-486-5p, miR-24-3p and miR-200a	The review identified twelve salivary exosomal microRNAs (miRs) associated with head and neck cancer (HNC), with miR-10b-5p, miR-486-5p, miR-24-3p, and miR-200a highlighted as the most useful biomarkers for early detection in saliva samples.

3. Regulatory impact of exosomal RNA on key cellular pathways

Studying exRNAs like lncRNAs, circRNAs, and miRNAs is significant in understanding more about regulating the different signaling pathways involved in developing the HNSCC and OSCC. LncRNAs can mesh to work with DNA, RNA, and proteins, modulating gene expression by acting as scaffolds, guides, and decoys. miRNA can bind to target mRNAs to downregulate their expression, leading to repression and degradation. These properties allow exRNAs to meddle with various signaling pathways and work in favour of the cancer cells, making them attractive targets for targeted inhibition studies and therapies when dealing with HNSCC.

3.1. NF-kb pathway

The NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling pathway deals with regulating immune and inflammatory responses, cell survival, and proliferation. NF-κB dimers that have entered the nucleus bind to specific DNA sequences called κB sites to recruit coactivators [92].The coactivators induce target gene transcription that deals with immune response, inflammation, and proliferation. The dysregulation of NF-κB signaling can potentially cause diseases like autoimmune disorders, inflammatory conditions, and cancer.

Studies regarding the exosomal version of the long non-coding RNA (lncRNA) HOXA transcript at the distal tip (HOTTIP) have revealed bidirectional properties concerning the promotion and survival of cancer cells [93,94]. However, by studying knockout models, Jiang *et al.* argue that HOTTIP suppresses HNSCC progression. Flow cytometry tests studying the overexpression of HOTTIP revealed inhibition

of cancer cell proliferation. In contrast, trans well assays of HOTTIP overexpression revealed an increase in the invasion capacity with respect to their control. HOTTIP suppressed HNSCC by the competitive binding to miR-19a-3p and miR-19b-3p and the upregulation of the NF-κB pathway [95].

He *et al.* observed that the upregulation of FGD5-AS1, a lncRNA derived from pancreatic tumor exosomes, was found to promote the cancer's progression by activating M2 macrophage polarization via NF-κB signaling. The NF-κB/ STAT3 pathway was activated as a result of the lncRNA's interaction with p300. This increased the nuclear localization and therefore the transcription of the STAT3/ NF-κB and subsequently the malignancy of the tumors [96].

3.2. EGFR pathway

The epidermal growth factor receptor (EGFR) pathway is an essential pathway involved in the differentiation and proliferation of mammalian cells. The binding of EGFR, a type of kinase, to its ligands, such as epidermal growth factor, causes homodimerization and autophosphorylation. This reaction helps these tyrosine kinases behave like docking sites for a variety of molecules entangled in downstream signal transduction pathways like PI3K/AKT or RAS-RAF-MEK-MAPK pathways [97]. EGFR signaling also leads to the activation of transcription factors that cause the expression of genes that are important for cell cycle progression, such as cyclin D. This drives cells through the G1 phase and into the S phase [98].

Overexpression of EGFR and its ligands has been noted early on in several cancers, including HNC, and increases with tumor progression, leading to a poorer prognosis [99]. The EGFR-PI3K-AKT-mTOR signaling in HNSCC contributes heavily to the inactivation of PTEN and radioresistance due to accelerated tumor repopulation following irradiation, which supplements this claim [100].

Wang *et al.* hypothesize that the exosomal miRNA miR-148a-3p, highly present in gliomas, can induce increased proliferation and angiogenesis. The miRNA activates the EGFR/MAPK pathway by repressing the tumor-suppressing ERBB receptor feedback inhibitor 1 (ERRFI1) gene [101]. The overexpression of EGFR has been related to 80-90% of HNSCC, contributing to radioresistance and poor prognosis [102]. Erlotinib is a Tyrosine Kinase Inhibitor that inhibits canonical EGFR signaling. Zheng *et al.* suggest that exosomes secreted from the erlotinib-resistant cells spread the erlotinib resistance and pro-tumorigenic alterations like in the case of miR-7704, miR-21-5p and miR-3960 [103]. The expression of lncRNA metastatic lung cancer cell-derived exosome transmitted lncRNA 1 (MLETA1) through the miR-186-5p/EGFR and miR-497-5p/IGF1R axes positively correlated with EGFR, revealing a high correlation with tumor metastasis with a poor prognosis [104].

3.3. Cyclin-CDK pathway

Cyclin D1 is a crucial protein in cell cycle control and is necessary for the G1 to S transition [105]. Retinoblastoma protein (Rb) is a tumor suppressor that usually shuts down cell cycle progression and hyperphosphorylation of Rb prevents it from binding E2F, thus allowing the transcription of genes necessary for S phase entry. On inactivation of Rb by retinoblastoma protein (Rb) by the active cyclin D-CDK4/6 complex, E2F transcription factors are released. These E2F transcription factors help activate the expression of genes needed for S phase entry and DNA production, driving cells through the G1/S checkpoint. Cyclin D, the regulatory subunit, binds to and activates the catalytic subunits CDK4 and

CDK6 [106]. The stimulation of the growth factor induces the formation of the complex. Frequent overexpression or amplification of cyclin D in cancer leads to the constitutive activation of CDK4/6 and hyperphosphorylation of Rb, even without the need for stimulation via growth factors [107]. Overexpression of cyclin D1 is associated with poor outcomes in many cancers, including breast, lung, head and neck, and mantle cell lymphoma [108].

Zhu *et al.* highlighted that the overexpression of lncRNA CASC15 was characteristic of LSSC and positively correlated with cyclin D1, leading to cell proliferation. These circumstances were, however, counteracted by the overexpression of CASC15 suppressor miR-365 as shown in Figure 2 [109]. MiR-195 is associated with cancer regulation through Cyclin, BCL-2, YAP1 expression, apoptosis, and metastasis. The negative correlation of novel lncRNA MASCC1 counteracts Cyclin D1 and BCL-2 expression inhibition by miR-195 in HNSCC [110]. Classically, cyclin-CDK complexes drive the events of the cell cycle in the interphase. It has been proven that circRNAs act as sponges to the key modulators of the cell cycle, miRNAs that interact with cyclin-CDK complexes, in order to aid tumor progression. The upregulation of circRNA oxysterol binding protein-like 10 (circ_OSBPL10) in OSCC has effects on properties like colony formation, cell cycle progression, and proliferation. Circ_OSBPL10 silencing upregulates miR-299-3p, a direct regulator of cyclin-dependent kinase 6 (CDK6), affecting the functional properties of the cell [111]. Circular Yes-associated protein (circYap) inhibits OSCC cell proliferation by inhibiting the formation of Cyclin D1CDK4 complexes by interacting with CDK4, leading to the retention of Cyclin D1 in the cytoplasm and cell cycle arrest in OSCC cells [112].

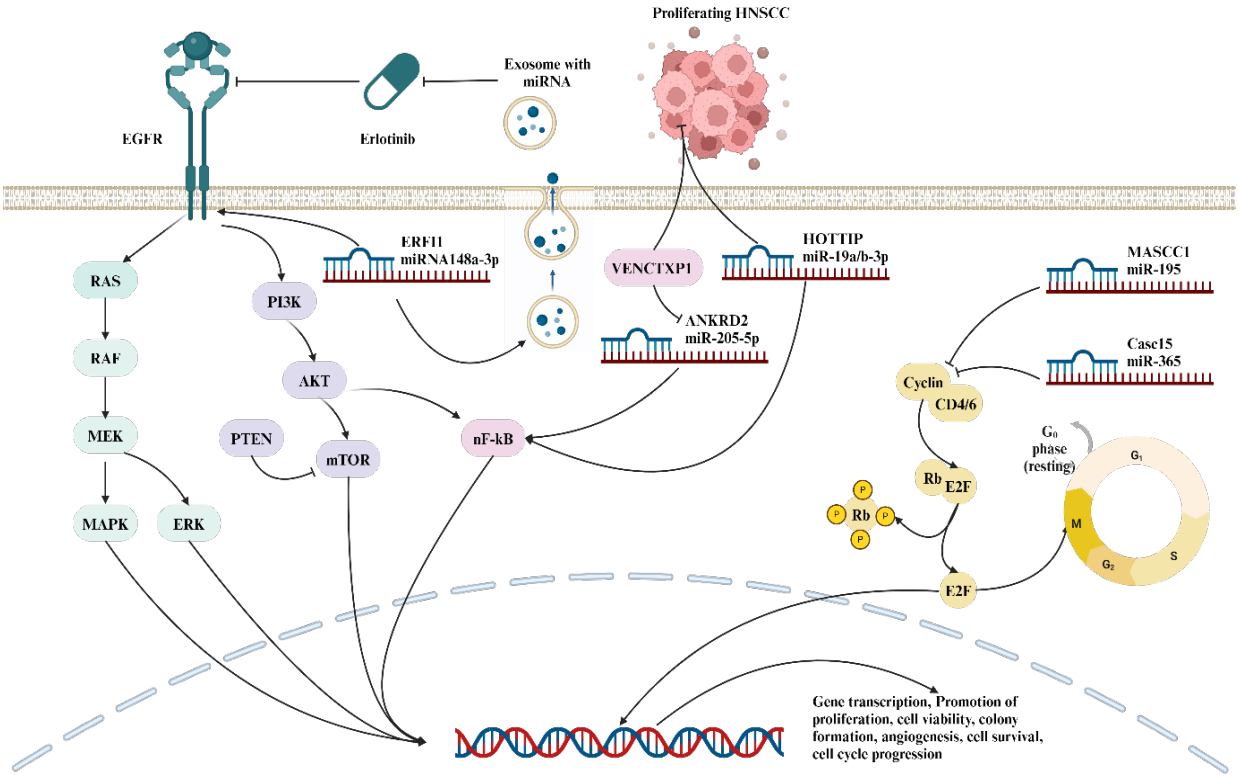

Figure 2. Diagram representing various signaling pathways implicated in the progression of HNCs as well as the interplay of exRNAs in the regulation of said pathways.

3.4. HIF-1α pathway

The HIF-1α (hypoxia-inducible factor-1α) pathway is a critical manager of the cell's reaction and solution to low oxygen conditions (hypoxia). The degradation of the HIF-1α is rapid in normal oxygen conditions [113]. However, in hypoxia, the hydroxylation and subsequent degradation are prevented, thus forming a dimer. This dimer binds to hypoxia response elements (HREs) in the promoters of target genes, leading to the transcriptional activation of hundreds of genes involved in the cellular adaptation to hypoxia, including VEGF for angiogenesis, erythropoiesis, GLUT 1 for glucose metabolism, and cell survival [114].

Hypoxia-induced long non-coding RNA (lncRNA) like the HIF1A Antisense RNA 2 (HIF1A-AS2) can evade immune response in hypoxic microenvironments through the degradation of major histocompatibility complex class I (MHC-I). HIF1A-AS2 was significantly linked to an advanced stage of clinical outcomes while also being a direct target of HIF-1alpha. In the tumor microenvironment (TME), tumor-derived exosomes (TEXs) containing miRNAs and lncRNAs are integral to the interactions of the tumor cells with the stroma surrounding them. Under hypoxia-like conditions, two lncRNAs (HIF1A-AS2 and H19) were significantly adjusted, in concordance with the upregulation of HIF1A-AS2, in HNSCC tissues and exosomal compartments in comparison to their normal counterparts [115]. In the same time, the upregulation of H19 was found to show poor prognosis for glioblastomas in hypoxic conditions, due to expression of stronger migration and invasion capabilities.

Silencing of HA synthases (HAS1, HAS2, AND HAS3), which have varying, often contradictory functions in different types of cells, decreases the synthesis of Hyaluronan (HA) [116]. The silencing of HAS2 tends to suppress malignancy in breast cancer cells while simultaneously promoting progression in OSCC cells [117]. Hypoxia induces the expression of the lncRNA Exogenous Hyaluronan synthase two antisense 1 (HAS2-AS1) in a HIF-1α dependent manner. In OSCC cells, the overexpression of HAS2-AS1 restored HAS2 expression in hypoxic conditions similar to HIF-1α, leading to cell invasiveness [118].

3.5 PI3K-AKT-mTOR pathway

Phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway is a commonly implicated pathway in cancer involved in facets like tumor progression and metastasis. Activating this pathway confers regulation over cell growth, angiogenesis, metabolism, and resistance to anticancer therapies [119].

Cytokines, hormones, and growth factors usually initiate the PI3K pathway. PI3K contains three different subunits, of which the p85 regulatory subunit is the most crucial. P85 binds to several proteins present in the intracellular and transmembrane space like tyrosine kinase-linked receptors, protein kinase C (PKC), Src, Src homology two domain-containing protein tyrosine phosphatase 1 (SHP1), Rac, and mutated Ras [120]. In addition, PI3KCA (the gene coding for PI3K) is regularly mutated or amplified in many common types of cancer [121]. Signaling proteins like AKT and PDK bind to the lipid products of PI3K, thus activating the growth and survival of cancer cells [122].

Exosomal miR-934 derived from colorectal cancer activates the PI3K/AKT pathway, by downregulation of PTEN expression, leading to increased liver metastasis due to the activation of CXCL13/CXCR5/NFκB/p65/miR-934 positive feedback loop. The knockdown of an RNA-binding protein called hnRNPA2B1, responsible for the transport of miR-934, could decrease this reaction [123].

Li *et al.* suggest that overexpression of lncRNAs like MAGI2-AS3 and CCDC144NL-AS1 may increase the activation and subsequent PI3K pathway cascade in order to promote migration, invasion, and proliferation of cells in OSCC [124]. Exosomes in some cancer cells contain Actin-binding protein (ANLN)-210 mRNA. These mRNAs polarize M2 macrophages, promoting HNSCC cancer cell survival via the canonical PI3K/AKT signaling pathway [125]. HOTAIR lncRNA, employs a similar mechanism where the PI3K/p-AKT/EMT pathway facilitates the migration and invasion in Laryngeal squamous cell carcinoma (LSCC) by inducing the polarization of M2 macrophages [126].

3.6. TP53 pathway

TP53, the tumor suppressor often called the "guardian of the genome," is crucial to maintaining genome stability. The TP53 gene encodes for the p53 transcription factor. When under cellular stress like hypoxia, DNA damage, or oncogene activation, p53 induces the transcription of target genes in DNA repair, apoptosis, and cell cycle arrest [127]. Tumorigenesis, poor response to chemotherapy, and chromosomal instability are responses to mutations in the TP53 gene. In addition, this gene is the most frequently mutated in HNSCC [128].

Sestrin 1 (SESN1), a gene that helps mitigate DNA damage by repairing damaged cells in the G1 cell cycle checkpoint, is activated by wild-type TP53. High expression of SESN1 repressor miR-377-3p was bound to a poor prognosis in patients with a TP53 mutation [129]. The repression of TP53 downregulates miRNA-107, miRNA-215, and miRNA-34a, while miRNA-125b gets upregulated due to its association with AKT1 [130]. The overexpression of miRNA-34a was found to influence TP53 and also enhance the transcriptional activity of p53

Patients with resection margins (RMs) with TP53 mutations have a higher chance of developing recurrence locally. The assessment of TP53-dependent microRNAs like miR-21-5p, miR-21-3p, miR-96-5p, and miR-429 revealed that good responders showed a significant decrease in expression and vice versa [131]. The TINCR (Terminal Differentiation-Induced Non-Coding RNA) miRNA, found prevalently in HNSCC, acts as a tumor suppressor as it is upregulated in aTP53-dependent fashion when keratinocytes suffer DNA damage due to UV irradiation [132].

LINC02434, AL139327.2, and AC126175.1 are three lncRNAs whose high-risk score model was built based on the expression rates and the coefficient of multivariable Cox regression. HNSCC shows a positive correlation with late-stage aggressive outcomes and TP53 mutations, classifying it as an indicator of poor prognoses in HNSCC [133].

4. Exploring therapeutic potentials and future prospects of exosomal RNA in cancer

Modulating the expression of exRNA presents an encouraging strategy for the treatment of HNCs. ExRNAs, comprising miRNA, mRNA, and lncRNA, play significant roles in oncogenic processes, including tumor progression, metastasis, and immune evasion. Manipulating the expression of specific exRNAs linked to cancer growth and resistance allows for the customization of therapies aimed at inhibiting tumor-promoting pathways. Silencing oncogenic miRNAs or restoring tumor-suppressive miRNAs via exosome-based delivery systems such as lipid nanoparticles and engineered exosomes may effectively decrease tumor burden and enhance therapeutic outcomes in HNCs. Targeting exRNA pathways may assist in overcoming resistance to conventional treatments, including chemotherapy and

radiation, thereby providing a novel and potentially more effective strategy for managing this aggressive cancer type.

Further research and preclinical studies may indicate the potential of exRNA-based therapies for head and neck cancers. Experimental models demonstrate that exRNAs can influence the tumor microenvironment, inhibit angiogenesis, and suppress metastasis, providing a comprehensive strategy for cancer treatment. Initial clinical trials are investigating the effectiveness of targeting exosomal pathways, including the inhibition of exosome release and modification of their cargo, to reduce tumor growth. Research indicates that exosomal miRNA signatures may function as dependable biomarkers for early detection, prognosis, and the monitoring of treatment responses. Certain miRNAs delivered through exosomes have demonstrated the potential to enhance the sensitivity of HNC tumors to radiotherapy, thereby decreasing recurrence rates. Clinical translation of these therapies remains in the early stages, necessitating further research to confirm their safety and efficacy in larger patient populations.

ExRNA-based therapies exhibit promising potential; however, numerous challenges persist. The variability of exosomes and their intricate biogenesis present challenges in the standardization of therapeutic strategies. The accurate targeting of exosomes to tumor cells while sparing normal tissues presents a considerable challenge. A significant challenge is the large-scale production of exosomes for clinical applications, ensuring their stability, functionality, and bioavailability are preserved. Future research must address these limitations by developing more efficient delivery systems, enhancing the understanding of exosome biology, and refining methods for isolating and characterizing exRNA. Advancements in nanotechnology, bioengineering, and personalized medicine are anticipated to significantly enhance the therapeutic potential of exRNA in head and neck cancers, facilitating the development of more effective and individualized cancer treatments.

5. Conclusion

This review emphasizes the increasing recognition of exRNAs as crucial players in the field of HNC. Exosomes are small extracellular vesicles secreted by the cells to carry a variety of molecules like RNAs and proteins. Recent developments have shown that these exRNAs are not just by-products of cellular processes but are actively involved in inter-cellular communication, modulating the tumor microenvironment (TME), promoting tumor growth, and contributing to metastasis. Techniques such as Next Generation sequencing (NGS) and advanced bioinformatics tools have allowed researchers to identify and characterize the exRNA profiles specific to HNC. These profiles serve as potential biomarkers in the early detection of HNC. The use of these liquid biopsies to detect exRNAs in bodily fluids such as saliva and blood has emerged as a non-invasive diagnostic tool that could revolutionize cancer diagnosis and monitoring. These molecules have been shown to modulate the key signaling pathways like PI3K/AKT and Wnt/β-catenin pathways. By targeting these, it has been understood that exRNAs influence cell proliferation, apoptosis, angiogenesis, and immune responses. This study highlights that certain exRNAs like lncRNA HOTAIR and circRNA CircPVT1, can be targeted to inhibit tumor growth and metastasis. While significant advancements have been made in understanding the role of exRNAs, the field is still in its early stages and more research is needed to overcome the challenges associated with therapeutic stability and delivery.

In the field of both diagnosis and prognosis, exRNAs serve as important markers, helping predict the evolution of the disease and the likely response to treatment. By monitoring exRNAs, clinicians could

make informed decisions regarding the necessity of more aggressive treatments or closer follow-up, ultimately leading to better patient management in the case of HNC. ExRNAs could also serve as novel therapeutic targets in the treatment of HNC, by specifically targeting exRNAs involved in oncogenic processes to try and prevent its metastasis. Additionally, the use of exRNA mimics or inhibitors can be used to restore the function of tumor-suppressive exRNAs. Continued development of liquid biopsy techniques could enhance the sensitivity and specificity of exRNA detection making it possible to detect it at an earlier stage. Furthermore, the potential for combination therapies, where exRNA-based treatments are used alongside conventional therapeutic languages such as chemotherapy and radiation therapy, represents an exciting way of enhancing overall treatment outcomes.

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Authors' contribution

Conceptualization, Vasanth Kanth Thasma Loganathan and Shriya Pattabiram, Kandasamy Nagarajan ArulJothi; Resources, Kruthika Prakash and Kirubakaran Rangasamy; Supervision, Vasanth Kanth Thasma Loganathan, Srisri Satishkartik, Shriya Pattabiram, Kandasamy Nagarajan Aruljothi and Kirubakaran Rangasamy; Writing—Original draft, Vasanth Kanth Thasma Loganathan, Srisri Satishkartik, Shriya Pattabiram, Aswini Suresh Kumar, Sayantani Chattopadhyay, Vanshikaa Karthikeyan and Kruthika Prakash; Visualization, Srisri Satishkartik, Aswini Suresh kumar, Sayantani Chattopadhyay and Vanshikaa Karthikeyan; Writing—Review and editing, Vasanth Kanth Thasma Loganathan, Srisri Satishkartik, Aswini Suresh Kumar, Sayantani Chattopadhyay, Vanshikaa Karthikeyan, Kruthika Prakash, Kirubakaran Rangasamy and Kandasamy Nagarajan ArulJothi; Project Administration, Vasanth Kanth Thasma Loganathan, Srisri Satishkartik and Kandasamy Nagarajan ArulJothi. All authors have read and agreed to the published version of the manuscript.

Conflicts of interests

The authors declare no conflict of interest.

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