The role of long non-coding RNAs in the pathogenesis of head and neck squamous cell carcinoma

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Head and neck cancers are a heterogeneous collection of malignancies of the upper aerodigestive tract, salivary glands, and thyroid. However, the molecular mechanisms underlying the carcinogenesis of head and neck squamous cell carcinomas (HNSCCs) remain poorly understood. Over the past decades, overwhelming evidence has demonstrated the regulatory roles of long non-coding RNAs (lncRNAs) in tumorigenesis, including HNSCC. Notably, these lncRNAs have vital roles in gene regulation and affect various aspects of cellular homeostasis, including proliferation, survival, and metastasis. They exert regulating functions by interacting with nucleic acids or proteins and affecting cancer cell signaling. lncRNAs represent a burgeoning field of cancer research, and we are only beginning to understand the importance and complicity of lncRNAs in HNSCC. In this review, we summarize the deregulation and function of lncRNAs in human HNSCC. We also review the working mechanism of lncRNAs in HNSCC pathogenesis and discuss the potential application of lncRNAs as diagnostic/prognostic tools and therapeutic targets in human HNSCC.

BACKGROUND
Head and neck squamous cell carcinoma (HNSCC) is a leading malignant disease and contributes to the global cancer burden.1 HNSCC develops in the upper airway’s epithelial cells and mucosal lining of food passages, such as the oral cavity, oropharynx, larynx, or hypopharynx. The majority of HNSCCs are associated with tobacco and alcohol use.2–3 Some subgroups, especially oropharynx cancers, are caused by human papillomavirus (HPV) infection.4 More than half of HNSCC patients are initially diagnosed at a locally advanced stage.5 Even with combined treatment involving surgery, radiotherapy, chemotherapy, and immunotherapy, the prognosis of HNSCC is still poor. For example, the 5-year survival rates range from approximately 60% in laryngeal carcinoma to roughly 25% in hypopharyngeal carcinoma.6 Besides the poor outcomes due to specific anatomical structures, patient survival is also threatened by cancer cells’ tendency to invade surrounding normal tissue and metastasize to cervical lymph nodes. Despite the therapeutic progress in the past two decades, novel strategies that provide earlier diagnosis, more effective treatment, and prognosis assessment are urgently needed. As the pathogenesis of HNSCC is a multistep process driven by the accumulation of various genetic and epigenetic alterations, a better understanding of the molecular mechanism of HNSCC is in need as a crucial factor for the future development of effective treatment.

Early studies on the molecular mechanism of cancer carcinogenesis have focused on protein-coding genes because proteins are traditionally accepted as the center of molecular biology. However, high-throughput transcriptome studies in the past few years have revealed many non-coding RNAs that outnumber the protein-coding genes in the human genome.7 These non-coding RNAs have been found to be implicated in diverse biological processes affecting development, differentiation, and physiology.8 Although some of these non-coding RNAs are small, most of them surpass 200 nucleotides in length and are cataloged as long non-coding RNAs (lncRNAs). It is now recognized that lncRNAs are exquisitely regulated and restricted to specific cell types greater than messenger RNA (mRNA).9 They frequently have an evolutionarily conserved function, a secondary structure, and microhomology regions, despite sharing minimal overall sequence similarity.10–12 LncRNAs can control chromatin modifications, chromosomal looping, DNA transcription, and edit and stabilize mRNAs that influence their translation or interact directly with proteins and other RNA species.11 Concerning cancer disease, lncRNAs are aberrantly expressed in different types of cancer and...
influence cell-cycle regulation, survival, immune response, or pluripotency, among functions altering cancer phenotypes. Several lncRNAs can also be transcriptionally regulated by key tumor suppressors or oncogenes. Increasing evidence has shown that lncRNAs are key players in human carcinogenesis, and we are only beginning to understand the importance and complicity of lncRNAs in HNSCC.

In this review, we describe the emerging roles of lncRNAs in HNSCC and outline the current knowledge on the functions and action mechanism of lncRNAs in HNSCC pathogenesis. We also discuss the potential applications of lncRNAs as diagnostic/prognostic tools and therapeutic targets and highlight the possible challenges in future studies.

**BIOLOGICAL ROLES OF lncRNAs IN HNSCC**

**LncRNA profile in HNSCC**

Multiple profiling studies based on microarray and whole-genome transcriptome sequencing platforms have pinpointed the aberrant expression pattern of lncRNAs in HNSCC. From the etiological aspect, by analyzing lncRNA expression across 426 HNSCC samples from The Cancer Genome Atlas (TCGA), researchers have shown significant associations between lncRNA-based clustering and DNA methylation, HPV infection, and TP53 mutation. A recent study has identified a DNA methylation-dysregulated four-lncRNA signature (DNAMeFourLncSig) from 596 DNA methylation-dysregulated lncRNAs in HNSCC, which could be an independent prognostic factor and may predict the chemotherapy response of HNSCC patients. Other studies have observed the hypomethylated lncRNA H19 as a potential prognostic biomarker in oral squamous cell carcinoma (OSCC) and the hypermethylated lncRNA HNF1A-AS1 as a tumor suppressor in laryngeal squamous cell carcinoma (LSCC), respectively. LncRNAs are also differentially expressed in HPV-positive and HPV-negative HNSCCs. By analyzing databases of the Atlas of Noncoding RNAs in Cancer (TANRIC) and TCGA, researchers have found 140 lncRNA transcripts significantly and differentially expressed between HPV-positive and HPV-negative tumors. Among them, lncRNA HOX transcript antisense RNA (HOTAIR), PROM1, CCAT1, and MUC19 are inversely correlated with the myeloid-derived suppressor cell collection of HPV-associated HNSCC. More recently, Song et al. have identified the Inc-IL17RA-11 transcription factor ER-alpha as the most likely HPV infection-associated factor promoting increased Inc-IL17RA-11 levels. As another critical etiology of HNSCC, over 70% of HNSCC patients carry TP53 oncogenic mutations. Researchers have also found that the expression of tumorigenic IncMIR205HG significantly increased in HNSCC with mutated TP53 compared with matched non-tumoral tissues. Moreover, Chaudhary et al. have found 133 lncRNAs to have differential abundance by 2-fold in the mutant versus wild-type TP53 samples, among which LINCO0460 is associated with cancer-related biological pathways, including epithelial-to-mesenchymal transition (EMT) and other inflammatory response pathways. The transcriptional regulation of tumor-suppressive lincRNA-p21 by the mutant p53/nuclear transcription factor Y sub-unit alpha (NF-YA) complex in HNSCC has also been confirmed, in which knockdown of NF-YA reversed the activation of lincRNA-p21 in mutant p53 cells, not wild-type p53 cells.

From the lifestyle aspect, it is widely accepted that alcohol consumption and tobacco abuse are implicated in the pathogenesis of HNSCC. By comparing the expression of lncRNAs in alcohol drinker and non-alcohol drinker HNSCC patients, researchers have identified a panel of lncRNAs dysregulated due to alcohol consumption. Among them, lnc-PSD4-1 and lnc-NETO-1 have been found differentially expressed due to alcohol consumption, and the low expression of the lnc-PSD4-1 isoform, lnc-PSD4-1:14, has been observed to exhibit a strong correlation with high survival rates of HNSCC patients. In terms of tobacco abuse, a research group from India has proved that out of 11 lncRNAs analyzed, 9 are expressed at a significantly high level in HNSCC patients who are tobacco chewers/smokers. In these 9 lncRNAs, Lnc-RoR, a regulator of reprogramming, has shown a strong association with tumor recurrence and poor therapeutic response. Taken together, these findings have shown us the close correlation between the lncRNA profile and etiology factors, as well as the living habits of HNSCC patients.

**Key IncRNAs in the pathogenesis of HNSCC**

Numerous lncRNAs have been found dysregulated in cancer cell lines and cancerous tissue of HNSCC. Based on their expression pattern in cancerous tissues and their functions in regulating cell behaviors, lncRNAs promote and suppress tumors. Among the most evaluated HNSCC-related lncRNAs, selected examples will be discussed as follows and are summarized in Figure 1.

**HOTAIR**

HOTAIR is coded by the homeobox C gene (HOXC) locus and exerts diverse functions in various malignancies. HOTAIR is aberrantly expressed in multiple human cancers, and it is a potential biomarker for assessing prognosis. HOTAIR functions as an oncogene by recruiting EZH2 to catalyze H3K27 triple-methylation (H3K27me3) to suppress downstream tumor suppressor genes. STAT3 can enhance HOTAIR transcription by interacting with pEZH2-serine21, thus promoting HNSCC cell growth via activation of PI3K/AKT. Targeting HOTAIR and EZH2 can also cause mitochondria-related apoptosis and inhibit the growth of HNSCC. Furthermore, high levels of HOTAIR have been reported to be correlated with poor prognosis in LSCC patients by inducing PTEN methylation. Besides, HOTAIR can upregulate stanniocalcin-2 (STC2) by sponging miR-206 and activating PI3K/AKT signaling pathway, thus promoting HNSCC cell proliferation, invasion, and migration.

**HOXA11-AS**

Homeobox A11 antisense (HOXA11-AS) is located on the HOXA gene cluster. It has been reported to be upregulated in various carcinomas. LSCC patients with T3–4 grade, neck nodal metastasis, or advanced clinical stage present a high HOXA11-AS level. Kaplan-Meier analysis has shown that high HOXA11-AS expression could predict a poor prognosis of LSCC patients. HOXA11-AS is also significantly
upregulated in hypopharyngeal squamous cell carcinoma (HSCC) tumors and is positively associated with lymph node metastasis. HOXA11-AS knockdown suppresses the proliferation and migration ability in HSCC FaDu cells by sponging miR-155. Furthermore, HOXA11-AS also targets miR-518-3p and enhances PDK1 level, thus promoting the malignancy of OSCC both in vitro and in vivo.

**MALAT1**
Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a nuclear-enriched lncRNA that is generally overexpressed in patients’ primary tumors and metastases. Chromatin immunoprecipitation (ChIP) and luciferase reporter assays have revealed that STAT3 could bind to the malat1 promoter region and transcriptionally activate MALAT1 expression. Then MALAT1 can interact reciprocally with miR-30a, thus inducing the EMT and accelerating HNSCC metastasis. MALAT1 targets p21 (RAC1)-activated kinase 1 (PAK1) through suppressing miR-140-5p, thus promoting the proliferation, migration, and invasion of tongue squamous cell carcinoma (TSCC) cells both in vitro and in vivo. MALAT1 also promotes the migration and invasion of LSCC Hep-2 and HSCC FaDu cells.

**ANRIL**
LncRNA antisense non-coding RNA in INK4 locus (ANRIL) functions as a competing endogenous RNA (ceRNA) for miR-125a-3p and upregulates fibroblast growth factor receptor-1, which can promote HNSCC growth. ANRIL promotes the proliferation, clonogenicity, and invasion of LSCC via the miR-181a/Snai2 axis. Moreover, it has been reported to activate the transforming growth factor β (TGF-β)/Smad pathway’s key molecules and promote the proliferation of OSCC cells.

**H19**
H19 is significantly overexpressed in a cohort of 65 primary tumor samples and 2 HNSCC cell lines, playing an essential role in tumor growth and progression. The expression of H19 is higher in metastasized tumors than in unmetastasized ones. Consistently, TSCC cells express a higher level of H19 than human squamous cells. H19 functions as a ceRNA to sponge miRNA let-7a, leading to an increase in a let-7a target HMG2, the key regulator of tumor metastasis. Upregulating in LSCC, H19 targets miR-148a-3p and increases the expression of DNA methyltransferase 1. Therefore, cellular DNA methylation levels are upregulated due to the high level of H19.

**MECHANISM OF lncRNAs IN HNSCC**
LncRNAs can form complex secondary and tertiary structures, thus providing multiple binding sites to RNAs and other biological molecules. LncRNAs can exert their functions in HNSCC through the following ways (Figure 2): (1) to regulate gene expression by interacting with chromatin-modifying enzymes or transcription factors; (2) to guide transcription factors and direct their localization to specific sites or block transcription factor binding sites; (3) to be involved in mRNA splicing; (4) to serve as a scaffold to facilitate the intermolecular interaction of target molecules; (5) to bind and sequester RNAs (for example, miRNAs); (6) to assemble with mRNAs to protect them from miRNA targeting and increase their stability; (7) to bind to proteins and regulate their stability (Table 1).

**LncRNAs interact with microRNAs**
Due to complementary base pairing, lncRNAs can bind miRNAs and keep them away from their target mRNAs. This function implies that dysregulated lncRNAs in HNSCC can act as ceRNAs, affecting gene expressions and tumor progression.

Increasing evidence has shown that lncRNAs work as miRNA sponges to promote HNSCC cells’ unrestrained proliferation. For example, LncMIR205HG is significantly increased in p53-mutated HNSCC and depletes miR-590-3p, leading to upregulated expression of cyclin B, CDK1, and YAP. LncRNA nuclear paraspeckle assembly transcript
Figure 2. Diverse molecular mechanism of lncRNAs in HNSCC

Some lncRNAs act in the nucleus of the cell (A) by inducing different epigenetic chromatin modifications and (B) by activating/inhibiting the transcription of nearby genes via interacting with transcription factors (TFs). (C) LncRNAs can also act post-transcriptionally, such as being involved in mRNA splicing. (D) LncRNAs have specific roles in the nucleus by their interaction with nuclear proteins. (E) Many lncRNAs leave the nucleus and inhibit miRNAs in the cytoplasm by sequestering them, or (F) interact with mRNA, inducing translation activation or repression, and (G) interact with cytoplasmic proteins, prolonging/shortening their half-life.

1 has been found to upregulate CDK6 by targeting miR-107 and inducing the proliferation of LSCC cells.63 AC026166.2-001 is downregulated in LSCC tissues, acting as a sponge of miR-24-3p, and regulates the expression of p27 and cyclin D1. The in vivo results have shown that AC026166.2-001 significantly suppresses LSCC xenografts’ growth and promotes apoptosis.64 LINC0052 sponges miR-608 that regulate epidermal growth factor receptor (EGFR) expression, promoting HNSCC cell proliferation in vitro and in vivo.65

LncRNAs also regulate the invasion of HNSCC cells by targeting microRNAs. For example, LINC00467 enhances HNSCC progression and EMT via the miR-299-5p/ubiquitin-specific protease-48 axis.66 HNSCC patients with a lower expression of ZFAS1 present a slightly longer disease-free survival and overall survival. ZFAS1 likely regulates the EMT process through miR-150-5p and its downstream target eukaryotic initiation factor 4E.67

LncRNAs can exert their functions through targeting several different miRNAs. For example, LncRNA KCNQ1 overlapping transcript 1 (KCNQ1OT1) has been shown to facilitate the proliferation of maxillary sinus squamous cell carcinoma cells through inhibiting miR-204 expression and restoring EphA7 expression.68 It can also sponge miR185-5p to promote the migration and proliferation of OSCC cells.69 Besides, KCNQ1OT1 contributes to the cisplatin resistance of TSCC through the miR-124-3p/TRIM14 axis.70 For other lncRNAs, XIST promotes the progression of LSCC via sponging both miR-144 and miR-125b-5p.71 FAM225A functions as a ceRNA, which sponges miR-590-3p and miR-1275, leading to the upregulation of their target integrin β3. FAK/P13K/Akt signaling is also activated to promote the proliferation and invasion of nasopharyngeal carcinoma (NPC) cells.72 In addition, LINC00460 has been found to bind miR-206 and increase the expression of STC2, AKT, and ERK, and the phosphorylation of AKT and ERK, which could inhibit the apoptosis and autophagy of HNSCC cells.73 LINC00460 also promotes HNSCC cell progression by sponging miR-612 to upregulate AKT2.74

On the other hand, key miRNAs or downstream targets in the tumor progression can also be regulated by the lncRNA-microRNA network. For instance, lncRNA maternally expressed 3 (MEG3) and CASC2 can reduce miR-21 expression and restrain the proliferation of HNSCC cells. These two lncRNAs are downregulated in HNSCC and thus promote tumor progression.52,53 LncRNA H19 and CCAT1 both target miR-let-7 and increase the expression of HMG2A, promoting EMT in HNSCC.44,54 The level of HMG2A is also enhanced by HOXC13-AS via sponging miR-383-3p, promoting cell proliferation, migration, and invasion.72 Small nuclear RNA host genes (SNHGs), as stable cytoplasmic lncRNAs, have been widely reported to be overexpressed in various tumors and promote disease progression. As an essential member of the SNHG family, SNHG1 promotes YAP1 expression and Hippo signaling activity by competitively sponging miR-375. Moreover, YAP1 can occupy the SNHG1 promoter to enhance its transcription, suggesting a positive feedback regulation loop between YAP1 and SNHG1.75 Similarly, SNHG12 promotes the proliferation and invasion of LSCC cells via sponging miR-129-5p and potentiating WW domain-containing E3 ubiquitin protein ligase 1 expression.46 A study also showed that the SNHG20/miR-197/LIN28 axis is vital in OSCC oncogenesis and stemness.47

LncRNAs bind and stabilize mRNAs

LncRNA can also directly interact with mRNAs and regulate the stability of miRNAs. Overexpression of lncRNA zinc finger E-box binding homeobox2 antisense RNA 1 has been found to promote EMT and metastasis of HNSCC. ZEB2-AS1 is a natural antisense transcript corresponding to the 5' UTR of ZEB2 and might increase their target sense mRNAs' stability by forming an RNA duplex. Researchers have demonstrated that the stability of ZEB2 mRNA is significantly impaired following ZEB2-AS1 inhibition.78 Another study has reported that aberrant upregulation of IncRNA WWTR1-AS1 is associated with malignant features and unfavorable prognosis of HNSCC by modulating WWTR1 mRNA stability.79 Leng et al. have found that increased HOXB-AS3 expression is associated with poor prognosis in OSCC. HOXB-AS3 and its encoded protein could promote OSCC cell proliferation and viability by directly binding with IGFBP2 to maintain c-Myc mRNA stability.80 Furthermore,
FOXD1-AS1 has been reported to express at a high level in OSCC and promote the malignant phenotypes via regulating the stability of FOXD1.58 Other studies have focused on DANCR, which is dramatically upregulated in human NPC and can bind to RNA-binding protein 3 and stabilize SOX2 mRNA, resulting in NPC cell proliferation.59 Besides, DANCER can also increase HIF-1α mRNA stability by interacting with NF90/NF45, leading to NPC metastasis and disease progression.49

LncRNAs interact with proteins
LncRNAs interact with proteins to modulate protein function, directing their localization or regulating protein-protein interaction. Overexpression of IncMX1-215 suppresses HNSCC proliferation and its metastatic capacity both in vitro and in vivo. The researchers have found that IncMX1-215 directly interacts with GCN5, a known H3K27 acetylase. Therefore, the binding of GCN5 to H3K27 interrupts acetylation and thus inhibits the expressions of PD-L1 and galectin-9.60 LINC00460 interacts with PRDX1 and facilitates PRDX1 entry into the nucleus. PRDX1 promoted the transcription of LINC00460 and EMT-related genes.

| LncRNAs (Refs) | Cancer type | Expression | Molecular mechanisms |
|---------------|-------------|------------|---------------------|
| A) Chromatin modification |
| HORAIR44      | OSCC        | up         | binds to EZH2 and H3K27me3, promoting tumor progression and metastasis |
| MXI-21546     | HNSCC       | down       | interacts with GCN5 (an H3K27 acetylase) to inhibit PD-L1 and galectin-9 expression |
| B) Transcription activator/inhibitor |
| LINC0046057   | HNSCC       | up         | interacts with PRDX1 and facilitates PRDX1 entry into the nucleus. PRDX1 promoted the transcription of LINC00460 and EMT-related genes |
| C) Splicing regulation |
| AC091729.756  | HNSCC       | up         | combines with SRSF2 and promotes HNSCC cell migration, proliferation and invasion, and tumor growth |
| D) Protein scaffold |
| FOXD2-AS149   | LSCC        | up         | binds to STAT3 and augmented STAT3 transcriptional activity by recruiting PRMT5 |
| (E) miRNA sponge |
| IncMIR205HG47 | HNSCC       | up         | targets miR-590-3p, upregulating the expression of cyclin B, CDK1, and YAP |
| LncRNA NEAT142 | LSCC        | up         | targets miR-107 and upregulates CDK6, inducing proliferation of LSCC cells |
| LINC0046747   | HNSCC       | up         | enhances HNSCC progression and EMT via the miR-299-5p/ubiquitin-specific protease-48 axis |
| LncRNA XIST557 | LSCC        | up         | sponges both miR-144 and miR-125b-5p |
| LncRNA SNHG142 | HNSCC       | up         | promotes YAP1 expression and Hippo signaling activity by competitively sponging miR-375 |
| LncRNA SNHG125 | LSCC        | up         | sponges miR-129-5p and potentiates WWP1 expression, promoting LSCC proliferation and invasion |
| (F) mRNA interaction |
| ZEB-AS155     | HNSCC       | Up         | increases the stability of their target sense mRNAs and promotes EMT and metastasis |
| (G) Protein interaction |
| MIR31HG56     | LSCC        | up         | targets HIF1A and p21 to regulate cell-cycle progression |
| DANCR47       | NPC         | up         | binds to NF90 and NF45 to increase HIF-1α mRNA stability and NPC cell invasion and metastasis |
| ST7-AS154     | LSCC        | up         | be required for the malignancy by interacting with CARM1 and protect CARM1 from ubiquitin-dependent degradation |
| CEBPA-AS150   | OSCC        | up         | interacts with CEBPA and promote tumorigenesis via CEBPA /Bcl2 |
| LincRNA-p2160 | HNSCC       | down       | binds to STAT3 to inhibit its phosphorylation, suppressing HNSCC tumor growth |

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and enhances Sox-2 self-association and transactivation activity. Thus, ST7-AS1 is required for LSCC cells’ malignancy through migration, tumorsphere formation assay, and in vivo implantation. In OSCC cells, using RNA pull-down assay, CEBPA-AS1 has been demonstrated to directly interact with CEBPA and promote tumorigenesis via CEBPA/Bcl2, and its high expression is correlated with poor prognosis.

**LncRNAs are involved in signaling pathways that synergistically drive tumor progression**

Aberrant expression of lncRNAs has been reported to be associated with dysregulation of several signaling pathways in cancerous tissues, such as the JAK/STAT3, TGF-β/Smad, and Wnt/β-catenin pathways (Figure 3).

**JAK/STAT3 signaling pathway**

STAT3 is frequently activated in cancer progression. STAT3 plays a vital role in the EMT and self-renewal of laryngeal cancer stem cells (CSCs), acting as a mediator that transduces intracellular and extracellular signals to the nucleus. FOXD2-AS1 acts as a scaffold for STAT3 and protein arginine N-methyltransferase 5 (PRMT5) and promotes STAT3 transcriptional activity, thus maintaining cancer stemness and promoting chemotherapy resistance. Conversely, lincRNA-p21 works as a tumor suppressor and its lower expression indicates a worse prognosis. High lincRNA-p21 level inhibits Janus kinase 2 (JAK2)/STAT3 signaling activation by directly binding to STAT3, inducing G1 arrest and apoptosis. Furthermore, activated STAT3 binds to the HOTAIR encoding gene’s promoter to increase HOTAIR transcription, thereby enhancing the EZH2-mediated epigenetic silencing of genes in HNSCC.

**TGF-β/Smad signaling pathway**

The role of TGF-β-induced tumor progression in advanced malignancy is well established. It has been proposed that TGF-β/Smad signaling transduction plays a leading role in inducing EMT of OSCC. The silence of lincRNA ANRIL increases the expression of TGF-β1 and p-Smad2/3 in OSCC cells. TGF-β causes the downregulation of lincRNA EPB41L4A-AS2. Overexpression of this lincRNA inhibits cell migration and invasion in the TGF-β-induced EMT model of HNSCC.

**Wnt/β-catenin signaling pathway**

The Wnt/β-catenin signaling pathway is one of the classical pathways in cell signaling transduction, promoting cell growth, proliferation, and invasion. Several lncRNAs have been reported to be involved in the Wnt/β-catenin signaling pathway. Higher ferritin heavy chain 1 pseudogene 3 expression in OSCC tissue is associated with T classification, N classification, and TNM staging. It promotes OSCC cell migration and invasion via enhancing the PI3K/Akt/GSK3β/Wnt/β-catenin pathway. In OSCC cell lines, low expression of lncRNA MEG3 leads to activation of the Wnt pathway and a higher expression of β-catenin. LncRNA urothelial cancer-associated 1 (UCA1) could regulate the Wnt/β-catenin pathway’s downstream targets, such as MMP9 and cyclin D1 (CCND1). The knockdown of TUG1 in OSCC cells inhibits the m6A and protein expression of β-catenin, CCND1, and c-myc. In LSCC cells, LncRNA NEF inhibits the Wnt/β-catenin pathway and promotes apoptosis. Moreover, lncRNA LINC00473 is upregulated in HNSCC cells and induces radioresistance through the Wnt/β-catenin pathway.

**LncRNAs and tumor immunity of HNSCC**

As the research hotspot in recent years, the role of lncRNAs in the tumor immunity of HNSCC has also been fully investigated. One study has highlighted the value of the 21 immune-related lncRNA pairs signature as a predictor of prognosis and immunotherapeutic response in HNSCC. Also, by pairing immune-related lncRNAs, Yin et al. have established a signature, concerning specific expression levels, to predict the immune landscape of HNSCC, thus guiding the clinical therapy of HNSCC patients.

In HNSCC patients with an elevated expression of the m6A-modified lncRNAs, the programmed cell death 1 ligand 1 (PD-L1) immune scores are significantly higher, with more infiltration of CD8+ T cells, Tregs, follicular helper T cells, and naive B cells. More specifically, Ma et al. have observed that ectopic expression of lncMX1-215 markedly inhibits the expression of TGF-β1 and p-Smad2/3.
of interferon-\(\alpha\)-induced, immunosuppression-related molecules of PD-L1 and galectin-9. Mechanistically, their study has suggested that lncMX1-215 negatively regulates immunosuppression by interrupting GCN5/H3K27ac binding in HNSCC, thus providing novel insights into immune checkpoint blockade treatment.\(^6\) Another group of researchers has found that lncRNA DCST1-AS1 can activate the NF-kB pathway to promote M2 macrophage polarization, thus advancing OSCC cancer progression.\(^9\) Li et al. have observed that silencing of lncRNA LINC02195 can decrease the MHC I protein expression. High LINC02195 expression is positively correlated with an increased number of CD8\(^+\) and CD4\(^+\) T cells in the HNSCC microenvironment.\(^9\)

In terms of the tumor microenvironment (TME), researchers have identified an immune-related seven-lncRNA prognostic signature (IRLPS), grouping HNSCC patients into high- and low-IRLPS subgroups. Then they found that low-IRLPS samples have more immune cell infiltration and are enriched in immune-related pathways, while high-IRLPS samples are enriched in metabolic pathways.\(^9\) In another study, based on bioinformatics analyses and functional assays, Zhong et al. have proved that certain lncRNAs (e.g., AL365361.1 and PCED1B-AS1) likely contribute to the modulation of TME in the high-immune-score HNSCC patients, achieved by regulating transcription of abundant immune-related genes, including CCR7 and TLR8.\(^9\) More recently, another group of researchers has demonstrated that lncRNA LURAP1L-AS1 plays a vital role in platelet-derived growth factor BB-induced activation of cancer-associated fibroblasts (CAFs) in TME through the positive regulation on NF-\(\kappa\)B signaling, which may become a potential target for the treatment of OSCC.\(^9\)

In recent years, studies on the role of lncRNAs and tumor metabolism have also emerged. Wang et al. have reported that lncRNA-p23154 binds to the 3’ UTR of the miR-378a-3p promoter and inhibits miR-378a-3p transcription, thus accelerating OSCC metastasis by regulating the glucose transporter 1 (GLUT-1)-mediated glycolysis.\(^9\) In another study also focusing on OSCC, researchers have shown that, by regulating miR-159-5p and GLUT-1, the lncRNA PVT1 promotes tumor cell proliferation, invasion, and migration, and inhibits apoptosis of cancer cells.\(^9\) Furthermore, Yang et al. have suggested that lncRNA H19 could promote glycolysis pathway in CAFs, thus accelerating the growth of OSCC through the H19/miR-675-5p/6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 axis.\(^9\) Studies on the role of lncRNAs in the metabolism of other nutrients in HNSCC, including protein and lipid, are relatively low, of which more research is needed in the future.

**Potential Clinical Implications of lncRNAs in HNSCC**

**LncRNAs as Biomarkers for HNSCC**

The heterogeneity of HNSCC is high due to the heterogeneity of the causes of the disease, including alcohol abuse, HPV infection, and diet. This makes the prognosis difficult to predict and the therapy challenging in HNSCC patients. The prognosis of HNSCC patients mainly depends on the TNM staging system and histologic grade. However, the therapeutic effect based on this is unfavorable. LncRNAs are involved in the tumorigenesis of HNSCC via different mechanisms. They are often tissue-specifically expressed, stable in human body fluids, and can be obtained with non-invasive methods. Therefore, LncRNAs are promising diagnostic and prognostic tumor biomarkers for HNSCC (Table 2).

Researchers have focused on lncRNAs with enhancer-like functions, which is a subclass of lncRNAs derived from the enhancer region of genes and could contribute to the activation of critical regulators of development and differentiation.\(^9\)\(^\)\(^9\) The TANRIC database and cBioPortal have been used to explore the RNA levels and clinical results of HNSCC patients.

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**Table 2. Selected examples of lncRNAs with a potential prognostic role for patients with HNSCC**

| Cancer type | Patients | Methods of analysis | Diagnostic/prognostic | Up/down | lncRNAs-mRNAs-miRNAs (Refs) |
|-------------|----------|---------------------|-----------------------|---------|-----------------------------|
| TSCC        | 12       | Integrated data analysis | P                     | up      | lncRNA AP001056.1\(^9\) |
| HNSCC       | 546      | Integrated data analysis | P                     | up      | lncRNA HOTTIP\(^9\)          |
| HSCC        | 53       | Microarray analysis   | P                     | up      | lncRNA UCA1\(^9\)           |
| HNSCC       | 192      | Orthogonal partial least-squares discrimination analysis | P            | –       | 3-lncRNA panel\(^9\)       |
| HNSCC       | 269      | Gene set enrichment analysis | P            | –       | 8-lncRNA signature\(^9\)    |
| HNSCC       | 425      | Cox regression analysis | P                     | –       | 3-lncRNA signature\(^9\)    |
| HNSCC       | 498      | Multivariate Cox regression and stratified analyses | P            | –       | 15-lncRNA signature\(^9\)   |
| HNSCC       | 28       | Single-factor survival analysis | P            | –       | 5-lncRNA signature\(^9\)    |
| HNSCC       | 499      | Univariate Cox regression survival analysis, robust likelihood-based survival model, and random sampling iterations | P            | –       | 4-lncRNA signature\(^9\)    |
| HNSCC       | 501      | Univariate Cox proportional hazards regression analysis | P            | –       | 4 lncRNAs-3 miRNAs-6 mRNAs\(^9\) |
| HNSCC       | 502      | Univariate Cox proportional hazards regression | P            | –       | 71 lncRNAs-8 miRNAs-16 mRNAs\(^9\) |
| HNSCC       | 755      | Univariate Cox regression survival analysis | P            | –       | 7 lncRNAs-mRNA\(^9\)        |

**Head and neck squamous cell carcinoma (HNSCC) is a leading malignant disease. Here, we summarize the deregulation and dysfunction of lncRNAs (200-nt to 100-kb transcripts lacking protein-coding potentials) in HNSCC, review their working mechanism in HNSCC pathogenesis, and discuss their potential application as diagnostic/prognostic tools and therapeutic targets.**
LncRNA and therapy resistance in HNSCC

HNSCC patients are frequently treated with surgery, together with radiotherapy or cisplatin-based chemotherapy. Patients with aggressive disease may also be treated with anti-EGFR antibodies or tyrosine kinase inhibitors (TKIs). However, therapy resistance has become the main obstacle to the effective treatment of HNSCC.

Emerging studies have focused on the role of lncRNAs in the chemoresistance of various cancers, including HNSCC. UCA1 is regarded as an oncogene that facilitates proliferation, enhances cisplatin chemoresistance, and suppresses apoptosis in OSCC cells, suggesting a potential therapeutic strategy targeting UCA1 in OSCC patients. Overexpressed MALAT1 can also promote the chemo-resistance of LSCC cell lines TU686 and LSC-1. FOXD2-AS1 acts as a scaffold for STAT3 and PRMT5 and promotes chemo-resistance. Interfering with FOXD2-AS1 using a short hairpin RNA can rescue LSCC’s chemotherapeutic sensitivity. The knockdown of LINC00958 expression enhances HNSCC cells’ sensitivity to cisplatin treatment as well as ionizing radiation. Furthermore, cisplatin treatment up-regulates inflammation-related Inc-IL7R expression in OSCC cells, resulting in decreased chemotherapeutic sensitivity of patients. TLR3 agonist polyinosine-polycytidylic acid treatment can negatively manipulate the expression of Inc-IL7R and strengthen the low-dose cisplatin-based chemotherapy with reduced side effects.

LncRNAs are also involved in the resistance to radiotherapy. LINC00473 is recognized as an oncogene to promote cell proliferation and inhibit apoptosis. Downregulation of LINC00473 can inhibit the Wnt/β-catenin signaling pathway and enhance the sensitivity of HNSCC cells to radiotherapy. LncRNA BLACAT1 promotes cell viability and inhibits cell apoptosis by modulating the presenilin-1 (PSEN1) gene. The knockdown of BLACAT1 improves the radiosensitivity of HNSCC cells. In HPV-positive HNSCC, HPV infection-induced expression of transcription factor ER-alpha can upregulate Inc-IL17RA-11 and its co-expressed genes that enhance HNSCC cells’ sensitivity to radiotherapy.

Therapeutics targeting the EGFR pathway have shown potential clinical activity in HNSCC. Despite the solid evidence that some HNSCCs are dependent on the EGFR signaling pathway, only moderate success has been achieved via treatment with anti-EGFR monoclonal antibodies or TKIs. Researchers have identified a synonymous mutation in EGFR, c.2361G > A (encoding p.Gln787Gln) in two HNSCC patients. This A/A genotype has shown greater sensitivity to TKIs than the G/A and G/G genotypes. Mechanically, G > A mutation decreases the stability of the IncRNA EGFR-AS1 and increases the sensitivity to TKIs. Overexpression of this lncRNA is sufficient to induce resistance to TKIs, while EGFR-AS1 knockdown could cause sensitivity to TKIs both in vitro and in vivo.

LncRNAs can act as ceRNAs and sponge miRNAs and mRNAs, therefore playing an essential role in tumor initiation and progression. Several studies have analyzed the lncRNA-mediated ceRNA crosstalk to construct a ceRNA network of HNSCC and identify key prognostic markers. For example, an analysis has found that four lncRNAs (RP11-366H4.1, HOTTIP, RP11-86516.2, and RP11-2751N1.1), three microRNAs (miR-99a, miR-337, and miR-137), and six mRNAs (NOSTRIN, TIMP4, GRB14, HOXB9, CELSR3, and ADGRD2) can be used as prognostic genes of HNSCC. Another study has constructed a lncRNA-miRNA-mRNA ceRNA network of HNSCC, including 8 miRNAs, 71 lncRNAs, and 16 mRNAs. There is also a study integrating the expression of mRNAs and lncRNAs to predict the survival in HNSCC. A seven-lncRNA-mRNA-based risk model has been developed, and the model has successfully predicted the survival of 755 HNSCC patient samples.

The markers above have been primarily studied in isolation, but more work has revealed that combining them might improve the predictive power. For example, Cao et al. have analyzed the RNA-seq data derived from the TANRIC database to identify a lncRNA prognostic signature model using the orthogonal partial least-squares discrimination analysis and 1.5-fold expression change criterion methods. A three-lncRNA panel (KTN1-AS1, LINC00460, and RP5-894A10.6) has been achieved to predict the overall survival of HNSCC patients. Using RNA-seq and clinical survival information of 269 patients from the GEO dataset, 8 prognosis-related IncRNAs, including AC010624.1, AC130456.4, LINC00608, LINC01300, MIR99AHC, AC008655.1, AC055785.2, and AC118553.1, have been obtained by univariate analysis, Cox LASSO regression, and multivariate analysis. Combined with clinical information, a nomogram containing an eight-lncRNA signature has been established. Furthermore, gene set enrichment analysis of the signature score has indicated that samples with high scores are mainly enriched in IL6/JAK/SATA3 signaling, complement pathways, and allograft rejection-related genes. This finding implicates that HNSCC patients with a poor prognosis might have dysfunctional immune systems. Another study has obtained a three-lncRNA expression signature with similar strategies, predicting HNSCC patient survival from data of 425 patients. Zhang et al. have analyzed the RNA-seq data from TCGA project. The most significant survival-associated enhancer lncRNA is AP001056.1, with a ligand for the T cell-specific cell surface receptor ICOS encoding an immune checkpoint protein as its regulated target. Researchers have obtained a total of 546 RNA-seq profiles of HNSCC patients with clinical outcome data from the TCGA database. HOTTIP has shown the most significant prognostic value and is significantly correlated with the clinical stage and histological grade of HNSCC patients. Moreover, the upregulation of UCA1 in HSCC is noticed in a cohort of 53 paired tumor and non-tumor samples. The high UCA1 level is significantly associated with advanced T category, later clinical stages, more lymphatic invasion, and worse prognosis.

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CONCLUSIONS AND FUTURE PERSPECTIVES

Over the past decades, lncRNA has emerged pivotal to understanding tumorigenesis. The advancement of high-throughput sequencing technology has greatly facilitated the expression profiling of lncRNAs in normal and cancer cells. Accumulating evidence has suggested that the deregulation of lncRNAs is closely associated with HNSCC carcinogenesis and metastasis. Functional analyses integrated with clinical data have shown that lncRNAs interact with RNAs/proteins and exert regulatory roles via controlling intracellular pathways, communications between cancer cells, and other cell types in the microenvironment. Further investigations may advance our knowledge of more functions of lncRNAs and unravel the sophisticated crosstalk in different cancer-related processes, such as EMT and CSC formation. In addition, how deregulation of lncRNA contributes to cancer heterogeneity remains to be clarified. Studies in these areas will better explain the pathway regulation and produce more therapeutic targets for this lethal and aggressive disease.

LncRNAs hold a great promise with regard to therapeutic applications for HNSCC, either by inhibiting or restoring lncRNAs that fine-tune cancer cells’ regulatory networks in a cancer-type-specific manner. The challenge now is that lncRNAs can simultaneously regulate multiple targets and be involved in complicated feedback mechanisms. To avoid adverse effects due to off-targeting, a thorough understanding of lncRNA functions and mechanisms in HNSCC-specific content is required to identify proper therapeutic lncRNA targets or mimics. Moreover, further investigations are needed in chemical technology, which may improve the stability of the therapeutic oligonucleotides and prolong their half-life in vivo, and nanotechnology, which can improve the efficiency of in vivo delivery.118

The application of lncRNAs as non-invasive biomarkers for early detection and prognostication for HNSCC has been proposed in the past few years. The challenges still exist and remain to be explored. The most important limitation is the varying results among studies, which may arise due to the lack of a consensus regarding the analytic methods used in different studies. Therefore, it is essential to validate the results in multiple laboratories on diverse populations to reach reproducibility. Further effort is required to validate these biomarkers’ specificity and sensitivity in prospective studies with larger patient cohorts and standardized methodology. In particular, it would be interesting to explore the potential values of employing lncRNA markers to predict treatment response and guide therapeutic decisions for personalized medicine.

In summary, lncRNA is emerging as an important regulator involved in HNSCC initiation and progression. A better understanding of the regulation and deregulation of lncRNA will shed light on the molecular mechanisms of HNSCC carcinogenesis. The opportunities for utilizing lncRNAs as novel diagnostic and therapeutic modalities for patients with HNSCC are fascinating and have been exemplified by the studies summarized in this review. Indeed, further investigations are required to translate the research findings into clinical applications.

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AUTHOR CONTRIBUTIONS

M.J. searched and reviewed the literature and wrote the original draft. F.L. validated the results, revised and edited the draft. A.-G.Y. conceptualized the idea and supervised the whole work. W.W. conceptualized the idea and supervised the whole work. R.Z. conceptualized the idea, supervised the whole work, and administrated the project.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Molecular Therapy: Oncolytics Vol. 24 March 2022 135
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