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Review Article

CircRNAs: potency of protein translation and feasibility of novel biomarkers and therapeutic targets for head and neck cancers

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Abstract: Circular RNAs (circRNAs), a new star noncoding RNA (ncRNA), show stability, conservation, abundance, and tissue and stage specificity. They act as key regulators of biological processes. They target the mRNAs of many other different genes or signaling pathways, and closely link associated genes into regulatory networks. Growing evidence has demonstrated that circRNAs may play an important role in the carcinogenesis, progression and chemoradiation resistance of many cancers including head and neck cancers (HNC). CircRNA, like other ncRNA, such as miRNA, lncRNA, usually is considered to be non-protein coding transcript. However, recent studies indicated that abnormal translation of circRNAs may be involved in human diseases. In this review, we collected the origin, classification, characteristics, function of circRNAs, exosmal circRNAs, and then synthesize current study results to highlight aberration of circRNAs in various types of HNC, and try to clarify the molecular mechanisms of circRNAs affecting the pathogenesis and progression of HNC, as well as pay particular attention to provide a new avenue to the diagnosis and treatment strategy for HNC.

Keywords: Circular RNA, head and neck cancer, protein translation, biomarker, therapeutic target

Introduction

Head and neck cancers (HNC) are one of the most common malignancies worldwide with diverse biological behaviors. Its pathogenesis may be associated with three major etiological factors, including environmental factors, genetic factors, and Epstein-Barr virus or human papillomavirus infection [1]. Determination of the molecular mechanisms of HNC carcinogenesis and progression may enable the discovery of early diagnostic biomarkers and effective therapeutic targets. Recent advances have reported that deregulation of circular RNA (circRNA) might play critical role in the development of HNC [2-4], and provided a new clue as to the understanding of tumor biology and therapeutic strategy of HNC.

CircRNA is a type of single-stranded RNA which, unlike the better-known linear RNA, forms a covalently closed continuous loop without 5' caps and 3' tails [5, 6]. This feature confers numerous properties to circRNAs, many of which have gradually been identified and having been paid more and more attention. CircRNA is a rising star in the field of RNA molecular biology in recent years. In fact, circRNA was first found in viroid as early as 1970s [7], and was first found in human cells in 1990s in the study of delete in colorectal carcinoma gene [8], but its research has been annihilated for more than 20 years. Most RNA is considered linear, so circRNA is considered to be a genetic accident or an experimental artificial product. Until 2012, Salzman et al. [9] discovered a large amount of circRNA expression in human cells; then, in 2013, two important studies on circRNA acting as a molecular sponge [10, 11], suggesting that these circRNA molecules may play an important role in organisms. With the rapid development of high-throughput sequencing technology and bioinformatics, a large number of circRNA molecules have been found in different organisms, and

Table 1. Related properties of circRNAs

Categories	Properties
Classification	exonic circRNA (ecircRNA) intronic RNA (ciRNA) exonic-intronic circRNA (EIciRNA) circRNA produced by circularization of viral RNA genome, tRNA, rRNA and snRNA antisense circRNA originated from antisense transcripts
Biogenesis	exon-skipping or lariat-driven circularization direct back-splicing or intron-pairing-driven circularization RNA-binding-protein-driven circularization direct circularization of lariat introns circularization driven by tRNA splicing circularization driven by rRNA splicing
Features	high abundance and incredible diversity high stability evolutionary conservation tissue-specific expression specificity related with developmental-stage or -age expression competing endogenous RNAs or miRNA mediated activities ecircRNA mainly exists in the cytoplasm or exosomes, while EIciRNA and ciRNA mainly exist in the nucleus
Biofunctions	miRNA sponges RBP sponges gene transcription and expression regulators protein/peptide translators others

the golden age of rapid research of circRNA is coming.

Emerging evidence has demonstrated that circRNAs show abnormal expression in human diseases, including central nervous system diseases, cardiovascular diseases and cancers [6], indicating their marked potential in the prediction and prognosis of diseases and clinical treatment. Certainly, some circRNAs have also been shown to be deregulated in distinct HNC [3]. The important roles of circRNAs as miRNA sponge [11, 12], gene transcription and expression regulators [12], and RNA-binding proteins (RBP) sponges [13] are gradually uncovered. Furthermore, numerous of studies have confirmed the function of circRNAs in tumor cell proliferation, migration and invasion, apoptosis, angiogenesis, deterioration and recurrence of cancer, which may potentially serve as a novel biomarker and therapeutic target for cancer prevention and treatment [12, 14, 15].

CircRNAs in animals are a large class of particularly stable RNAs produced by circularization of specific exons or intron. Most circRNAs were not associated with translating ribosomes, therefore, circRNAs were deemed to be noncoding. However, recent findings revealed that some circRNAs could generate proteins *in vivo* [16-18]. Additionally, some exosomal circRNAs have been found and gradually used in the diagnosis and treatment of diseases, especially cancers. These new findings expand the landscape of circRNA applications and enhance the recognition of its function.

In the present review, we summarize the biological properties of circRNAs and the known molecular mechanisms, as well as their functions, especially those related to human tumors including HNC.

Biogenesis of circRNAs

Most circRNAs in organisms are synthesized by linear RNA precursor, which is accomplished by a variety of noncanonical splicing models. The process named backsplicing, where downstream exons are spliced to upstream exons in reverse order [19]. Currently, 6 models have been proposed for the formation of circRNAs [5, 20-24] (**Table 1**): (1) direct circularization of lariat introns, the 3' downstream of the lariat intron is trimmed to form a circular intronic RNA (ciRNA); (2) lariat-driven circularization (exon skipping), the exon-skipping event during alternative splicing promotes the 3' splice site (SS) of the exon to covalently splice to the 5'SS; (3) intron-pairing driven circularization (direct ba-

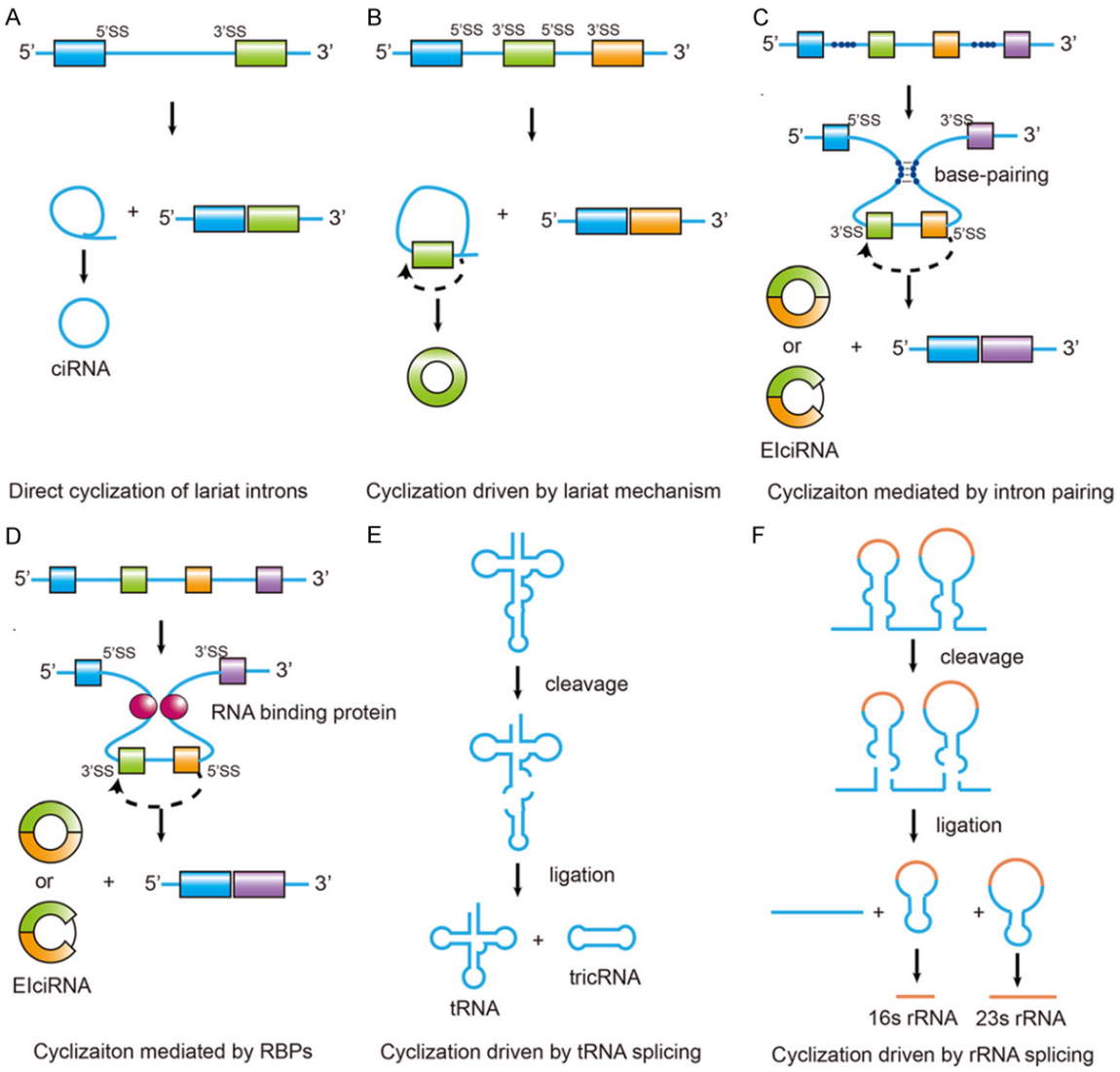

Figure 1. Zhang et al. Representative models for the production of circRNA [25]. A. Direct circularization of lariat introns. Canonical linear splicing generates a lariat structure. The 3' downstream of the lariat intron is trimmed to form a circular intronic RNA (ciRNA). B. Circularization driven by lariat mechanism. The exon-skipping event during alternative splicing promotes the 3' splice site (3'SS) of the exon to covalently splice to the 5'SS. C. Circularization mediated by intron pairing. Intron pairing brings the appropriate splice signals within proximity of each other, which promotes circularization. D. Circularization mediated by RNA-binding proteins (RBPs). RBPs bring the appropriate splice signals within proximity of each other, which promotes circularization. E. Circularization driven by tRNA splicing. F. Circularization driven by rRNA splicing.

cksplicing), intron pairing brings the appropriate splice signals within proximity of each other, which promotes circularization; (4) circularization mediated by RBPs and trans-acting factors, RBPs bring the appropriate splice signals within proximity of each other, which promotes circularization; (5) circularization driven by tRNA splicing; (6) circularization driven by rRNA splicing. Three of them are speculative models of backsplicing of exonic circRNA (ecircRNA) and exonic-intronic circRNA (EIciRNA), and other three are speculative patterns of ciRNAs processing maturation mechanism. The representative models of origin of circRNAs are diagrammed in **Figure 1**.

Classification of circRNAs

CircRNAs can be classified into five groups according to its biogenesis pattern [5, 24-27] (**Table 1**): (1) ecircRNA, composed of only exons; (2) retained-intron circRNA or EIciRNA,

composed of introns at the region between exons; (3) ciRNA, composed of only introns; (4) circRNA produced by circularization of viral RNA genome, tRNA, rRNA and snRNA; (5) antisense circRNA originated from antisense transcripts. Among circRNAs, ecircRNAs are the most, accounting for more than 80% of identified circRNAs. In addition, circRNA can be divided into intragenic circRNA and intergenic circRNA according to the parental gene location of circRNA.

Features of circRNAs

Increasing evidence revealed several highlighted characteristics of circRNAs [9, 12, 23, 27] (**Table 1**): (1) abundance and diversity: more than 20,000 different circRNAs have been identified in eukaryotes; (2) stability: circRNAs presented with more stable property than linear mRNAs due to their covalently closed loop structures which confer them resistant to RNase R; (3) conservation: circRNAs are highly conserved in different species, such as humans, mice, nematodes, zebrafish, drosophila, protists, and plants; (4) location: ecircRNA mainly exists in the cytoplasm or exosomes, while EIciRNA and ciRNA mainly exist in the nucleus; (5) specificity: circRNAs often exhibit tissue and developmental-stage specific expression; (6) some circRNAs contain miRNA binding sites and can competitively attenuate endogenous miRNA-mediated activities; (7) the sequence conservativeness of ecircRNA was higher than that of ciRNA and intergenic circRNA.

Biofunctions of circRNAs

At present circRNA has several putative functions as follows [16, 24, 28] (**Table 1** and **Figure 2**): (1) miRNA sponges, circRNAs can bind to miRNA as RNA sponge and increase downstream gene expression by regulating miRNA activities; (2) gene transcription and expression regulators, circRNAs that are considered as a type of alternative splicing isoforms may play a key role in regulating gene expression, leading to cancer related dysregulation. EIciRNAs and ciRNAs may regulate transcription and expression in the nucleus while ecircRNAs in the cytoplasm; (3) RBP sponges, circRNAs may bind to and sequester RBPs via their conserved seed matches, resulting in the formation of large RNA-protein complexes and affecting translation; (4) protein/peptide translators; (5) other undiscovered roles.

Here, we focus on interpreting the protein translation potential of circRNA. In general, circRNA is considered untranslatable. The first natural circRNA found able to encode protein is the genome of hepatitis virus back to 1980's [29]. Raising studies have demonstrated the potential of circRNAs in proteins translation [30]. Several circRNAs with protein coding ability were listed in **Table 2**. Chen et al. [31] showed that the eukaryotic ribosome can initiate translation mechanism on circRNA when the circRNA structure contains internal ribosome entry site. It has been confirmed that circZNF609 can be translated into protein functioning in myogenesis [32]. CircMbl3 is found to be translated in a splicing-dependent but cap-independent way in fly head extracts [33]. N^6-methyladenosine can promote the initiation of protein translation from circRNA in human cells, and a single N^6-methyladenosine residue in circRNA is sufficient to drive the translation, and suggests a role of circRNA-derived proteins in cellular responses to environmental stress [16]. Additionally, study on human glioma cell lines U251 and U373 has displayed a novel protein (FBXW7-185aa) encoded from circ-FBXW7, which contributes to inhibit glioma tumorigenesis [34]; and other two novel tumor suppressive proteins (SHPRH-146aa and PINT87aa) encoded respectively by circ-SHPRH and circPINTexon2 have been identified in glioblastoma [17, 35]. Moreover, computational analysis on sequencing of human transcriptomes has revealed the universal existence of circRNAs with coding potential, which provide a new direct for the functional studies of circRNAs. Meng et al. [36] have introduced an integrated tool to detect circRNAs with protein coding potential from high-throughput sequencing data, thus facilitating the investigation of circRNA translation as well as novel functions of circRNAs and circRNA-derived proteins. Until now, some circRNA-related databases were gradually constructed. For example, the circRNADb database (http://reprod.njmu.edu.cn/circrnadb/circRNADb.php) reveals in more detail whether endogenous circRNA can encode functional proteins in mammalian cells [37]; the circBank database (http://www.circbank.cn) exhibits 140783 circRNAs have protein coding potential. On the other hand, Bartsch et al. [38] had detailed a sucrose gradient-based method to evaluate the coding potential of candidate circRNAs (or any transcript of interest) and its association with the transla-

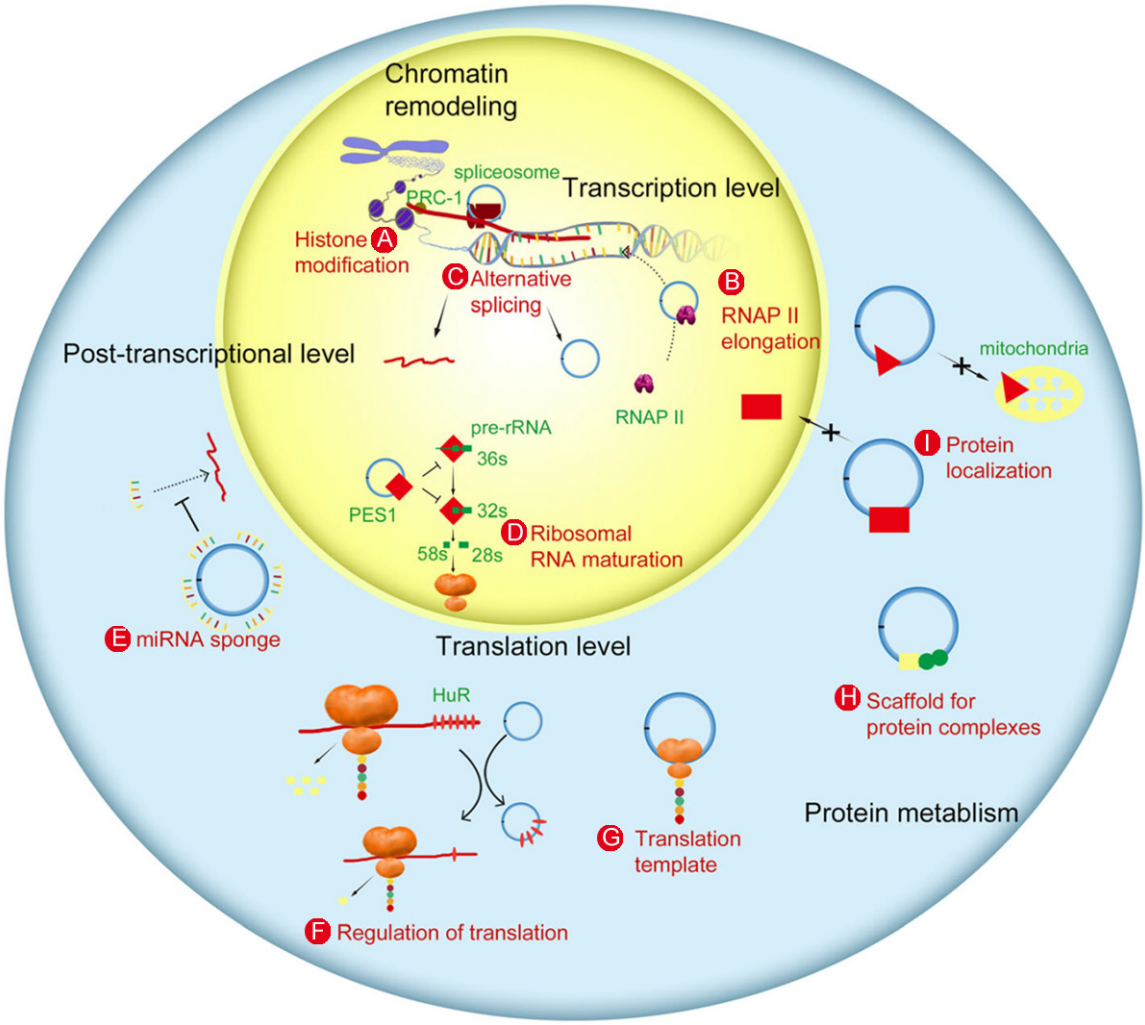

Figure 2. Zhang et al. The biological functions of circRNA [25]. CircRNAs can impact genetic output at almost every stage of a gene's life cycle-from epigenetic regulation to transcriptional and posttranscriptional control to translational control. A. Histone modification; B. RNAP II elongation; C. Alternative splicing; D. RNA maturation; E. miRNA sponge; F. Translation regulation; G. Translation; H. Scaffold for proteins; I. Protein localization.

Table 2. CircRNAs with protein translation function

CircRNA	Translated protein	Reference
circ-ZNF609	ZNF609	[32]
circMbl3	Mbl3	[33]
circ-FBXW7	FBXW7-185aa	[34]
circ-SHPRH	SHPRH-146aa	[17]
circPINTexon2	PINT87aa	[35]

tion machinery. Collectively, recent advances have proved the protein coding ability of circRNAs and abnormal translation of circRNAs may contribute to human diseases including tumors. The protein coding potency of circRNA will expand its territory for application and improve its clinical value.

Exosomal circRNAs

Exosomes are nanoscale extracellular vesicles of endocytic origin secreted by most types of cells and circulate in bodily fluids such as blood, urine, saliva, and breast milk [39]. Exosomes have emerged as critical mediators of intercellular communication in both physiological and pathological processes including cancer progression [40, 41]. Li et al. [42] first reported the enrichment and stability of abundant circRNAs in exosomes compared to the progenitor cells by using RNA-sequence analyses. Re-

Table 3. Exosomal circRNAs in cancers

CircRNA	Location	Cancer	Mechanism	Function	Reference
has_circ_007293	serum	PTC	-	-	[45]
has_circ_031752	serum	PTC	-	-	[45]
has_circ_020135	serum	PTC	-	-	[45]
circ-IARS	Plasma	PADC	Targets miRNA-122, ZO-1, RhoA, RhoA-GTP, and F-actin	Promotes tumor metastasis, increases endothelial monolayer permeability	[46]
circ-PDE8A	Plasma	PADC	Targets miRNA-338/MACC1/MET pathway	Promotes cell invasive growth	[47]
circPTGR1	Serum	HCC	Targets miRNA449a/MET pathway	Promotes tumor metastasis	[48]
ciRS-133	Plasma	GC	Targets miRNA-133/PRDM16 pathway	Promotes white adipose browning	[49]

Note: -, Not retrieved. PTC: Papillary thyroid carcinoma; PADC: Pancreatic ductal adenocarcinoma; HCC: Hepatocellular carcinoma; GC: Gastric cancer.

cently, circRNAs have also been reported to be enriched and stable in saliva, plasma, and even in some exosomes derived from serum, urine and tumor [42, 43], suggesting the potential of circRNAs as biomarkers. Moreover, based on the characteristics of exosomes, exosomes as therapeutic vectors began to be used in the treatment of tumors [44], suggesting the possibility of circRNA as a therapeutic target. Going forward, the application prospects of exosomal circRNAs are bright.

Using high-throughput sequencing and qRT-PCR, Yang et al. [45] identified three differentially expressed (DE) exosomal circRNAs including has_circ_007293, has_circ_031752 and has_circ_020135 in serum from patients with papillary thyroid carcinoma (PTC) compared with a benign thyroid goiter. Li et al. [46] found that exosomal circ-IARS expression in pancreatic ductal adenocarcinoma (PDAC) tissues and in plasma exosomes of patients with metastatic disease was positively correlated with liver metastasis, vascular invasion, and tumor-node-metastasis (TNM) stage and negatively correlated with postoperative survival time. Li et al. [47] also found that exosomal circ-PDE8A expression in plasma was associated with progression and prognosis in PDAC patients and circ-PDE8A promotes the invasive growth of PDAC cells via miRNA-338/MACC1/MET pathway. In addition, circPTGR1 was upregulated in serum exosomes from hepatocellular carcinoma (HCC) patients and was associated with the clinical stage and prognosis. Next experiments uncovered that exosomal circPTGR1 promotes HCC metastasis via miRNA449a/MET pathway [48]. Zhang et al. [49] found that exosomal circRNA ciRS-133 derived from gastric cancer could promote white adipose browning through targeting the miRNA-133/PRDM16 pathway. The above results imply that the presence of circRNAs in exosomes may be important indicator for early diagnosis and prognostic prediction in many cancers. Currently, accumulating evidence suggests that exosomal circRNAs can modulate cellular proliferation, invasion, migration, tumor metastasis and drug resistance through certain mechanisms such as targeting signaling pathways or sponging miRNAs [39, 46, 48-50] (**Table 3**). Up to now, the exoRBase database (http://www.exoRBase.org) was constructed and contains 58 330 circRNAs, 15 501 lncRNAs and 18 333 mRNAs in human blood exosomes [51]. Although only a few of circRNAs have established functional roles or clinical applications, exosomal circRNAs are a novel frontier in cancer research. More and more exosomal circRNAs have been identified and applied for cancer drug delivery system, cancer therapy and clinical treatment evaluation [52-54].

CircRNAs and head and neck cancers

Recent studies have indicated that circRNAs may have vital roles during the development and progression of multiform types of cancers including HNC [12, 55]. CircRNAs have become a novel area of interest in the early diagnosis and therapy of cancers due to their abundance, high stability and notable regulatory functions, and the enrichment and stability of exosomal circRNAs in body fluids, and the association with tumor chemoradiation. Herein, we review the relationship between circRNAs and HNC, and summarize the roles of circRNAs and their possible biological mechanisms in different HNC.

CircRNAs and nasopharyngeal carcinoma

Using the qRT-PCR, Shuai et al. [56] examined the expression level of circRNA_0000285 in 150 nasopharyngeal carcinoma (NPC) and 100

Table 4. Nasopharyngeal carcinoma and its associated circRNAs

CircRNA	Sample	Expression	Mechanism	Function	Reference
circRNA_000543	Tissue	Up	Sponges miRNA-9, targets PDGFRB	Radioresistant, poorer overall survival	[57]
circRNA_0000285	Tissue and serum	Up	-	Associated with tumor size, differentiation, metastasis, and TNM stage, independent prognostic factor, radiosensitivity	[56]
circHIPK3	Tissue and cell	Up	Sponges miRNA-4288, targets ELF3	Promotes cell proliferation and invasion	[58]

adjacent tissues, 150 serum samples from NPC patients and 100 serum samples from healthy controls. They found that circRNA_0000285 was significantly increased in NPC tissues and serum samples from patients with NPC, and significantly associated with tumor size, differentiation, cervical lymph node metastasis, distant metastasis and TNM stage. Additionally, univariate and multivariate analyses indicated that circRNA_0000285 may be a novel prognostic biomarker for NPC. Using the human circRNA microarray, Chen et al. [57] found that circRNA_000543 expression was upregulated in NPC tissues, and in tissues from patients with poorer overall survival. The following investigation demonstrated that circRNA_000543 knockdown sensitized NPC cells by targeting miRNA-9/platelet-derived growth factor receptor B axis. So circRNA_000543 may be a potential therapeutic target for NPC. Ke et al. [58] found that circHIPK3 was highly expressed in NPC tissues and cell lines and circHIPK3 depletion dramatically repressed tumor growth and metastasis *in vivo*. Next experiments revealed that circHIPK3 facilitated NPC progression through protecting ELF3 from miRNA-4288-mediated silencing. In brief, NPC and its associated circRNAs are shown in **Table 4**.

On the other hand, researchers have identified EBV-encoded circRNAs, such as EBV circBARTs and ebv_circ_RPMS1. They deemed that EBV circBARTs might contribute to viral oncogenesis and ebv_circ_RPMS1 may be a novel viral regulator of host and/or viral gene expression [59, 60]. The circRNAs originated from EBV and their functions and potential clinical applications in NPC need to be explored in the future.

CircRNAs and laryngeal cancer

Using microarray analysis, Xuan et al. [4] showed that 698 circRNAs were DE in laryngeal squamous cell carcinoma (LSCC) tissues, including 302 upregulated and 396 downregulated circRNA transcripts. Using qRT-PCR method, high expression of hsa_circ_100855 and low expression of hsa_circ_104912 are associated with T3-4 stage, lymph node metastasis, and advanced clinical stage of LSCC. Based on the microarray and bioinformatics analyses, Fan et al. [61] identified 506 DE circRNAs from LSCC and normal laryngeal mucosa tissues, and predicted that hsa_circ_0044520 and hsa_circ_0044529 play important regulatory roles by sponging hsa-miRNA-4726-5p and hsa-miRNA-4640-5p in the tumorigenesis of LSCC. Combined with qPCR methods, Gao et al. [62] confirmed that 382 circRNAs were DE in miRNA-145-5p overexpressed LSCC cells and revealed that miRNA-145-5p may be a core of the competing endogenous RNA (ceRNA) network to inhibit the LSCC progression. Lu et al. [63] detected 29 circRNAs were significantly upregulated and 19 circRNAs were significantly downregulated in the LSCC tissues by RNA-sequencing, then qRT-PCR validation result indicated that hsa_circ:chr20:31876585-31897648 may be a novel promising tumor suppresser in LSCC. Wu et al. [64] revealed that circRNA hg19_circ_0005033 promotes proliferation, migration and invasion of $CD133^{+}CD44^{+}$ laryngeal cancer (LC) stem cells, implicating circRNAs are associated with cancer immunity regulation. Zhang et al. [65] found that LSCC patients with high TNM stages, poorly differentiated tumors, lymph node metastases and poor prognosis had high ciRS-7 (CDR1as) expression level but low miRNA-7 level. *In vitro* and *in vivo* studies demonstrated that CDR1as is an oncogene, which promotes LSCC progression by regulating miRNA-7 signal, and suggested that CDR1as/miRNA-7 pathway was a key pathway in LSCC progression. Subsequent reports also shed light on the roles and mechanisms of several circRNAs in LC. Hsa_circ_0023028 functions as an mi-

Table 5. Laryngeal cancer and its associated circRNAs

CircRNA	Sample	Expression	Mechanism	Function	Reference
hsa_circRNA_100855	Tissue	Up	-	Associated with T3-4 stage, lymph node metastasis, and later clinical stage	[4]
hsa_circRNA_104912	Tissue	Down	-	Associated with T3-4 stage, lymph node metastasis, poor differentiation, and later clinical stage	[4]
hsa_circ_0044520	Tissue	Up	Sponges hsa-miRNA-4726-5p	Collagen synthesis	[61]
hsa_circ_0044529	Tissue	Up	Sponges hsa-miRNA-4640-5p	Collagen synthesis	[61]
hg19_circ_0005033	Cell	Up	-	Proliferation, migration, invasion; chemotherapy resistance	[64]
ciRS-7(CDR1as)	Tissue and cell	Up	Sponges miRNA-7, targets ki-67, CCNE1 and PIK3CD	Promotes tumor growth	[65]
hsa_circ_0023028	Tissue and cell	Up	Sponges miRNA-194-5p	Promotes cell proliferation, migration, and invasion	[66]
circFLNA	Tissue and cell	Up	Sponges miRNA-486-3p, targets cyclin D1	Promotes cell migration, poor survival	[67]
circMYLK	Tissue and cell	Up	Sponges miRNA-195	Promotes cell proliferation and cell cycle transition	[68]

RNA-194-5p sponge to promote the proliferation, migration and invasion of LC cells [66]; upregulation of circFLNA contributes to LSCC migration by targeting circFLNA-miRNA-486-3p-FLNA axis [67]; circMYLK serves as an oncogene to promote cancer progression via miRNA-195/cyclin D1 axis in LSCC [68]. Collectively, LC and its associated circRNAs are displayed in **Table 5**.

Taken together, aforesaid studies indicate that circRNAs may play important roles in the development and progression of LSCC and may be helpful for the diagnosis and prognosis of this disease. These results may provide a potential therapeutic target for the treatment of LSCC.

CircRNAs and oral cancer

Using high-throughput sequencing technology or circRNA microarray analysis for human oral squamous cell carcinoma (OSCC), many circRNAs were DE between OSCC tissues and adjacent tissues [69-71]. Subsequently, a series of works confirmed some circRNAs play key roles in tumor development and progression of oral cancer (**Table 6**). CircRNA_100290 serves as a ceRNA to counteract miRNA-378a-mediated GLUT1 suppression, thus promoting glycolysis and cell proliferation in OSCC [72]. CircRNA_0109291 regulates cell growth and migration in OSCC and is associated with prognosis of OSCC patients [73]. Moreover, circDOCK1 regulates BIRC3 expression through competitively binding to miRNA-196a-5p as a ceRNA, and participates in the process of apoptosis of OSCC cells [74]. Li et al. [69] explored the regulatory role of the hsa_circ_0008309-miRNA-136-5p/hsa-miRNA-382-5P-ATXN1 network in OSCC and identified that hsa_circ_0008309 may inhibit miRNA-136-5p and miRNA-382-5p expression and increase ATXN1 expression in the OSCC cell lines. Su et al. [71] determined that upregulation of hsa_circ_0007059 suppresses cell growth, migration and invasion, and facilitates apoptosis of OSCC cells. Meanwhile, hsa_circ_0007059 was determined to affect malignant behavior via AKT/mTOR signaling pathway. Sun et al. [75] showed that hsa_circ_001242 was significantly downregulated in OSCC and may act as a potential novel biomarker for the diagnosis and treatment of OSCC. Su et al. [70] found that hsa_circ_0005379 expression is significantly lower in OSCC tissue compared to paired non-cancerous tissue and is associated with tumor size and differentiation. Next experiments manifested that hsa_circ_0005379 may be regulate OSCC malignancy through the EGFR pathway and may be a new therapeutic target for OSCC.

Additionally, Zhao et al. [76] obtained 32 dysregulated cricRNAs in the saliva from the OSCC patients by microarray. Among these DE circRNAs, the expression levels of salivary hsa_circ_0001874 and hsa_circ_0001971 were correlated with the TNM stage, severity of oral mucosal lesions and outcome of surgical treatment. This result indicated the potential of

Table 6. Oral cancer and its associated circRNAs

CircRNA	Sample	Expression	Mechanism	Function	Reference
has_circRNA_100290	Tissue	Up	Sponges miRNA-378a, targets GLUT1	Promotes glycolysis and cell proliferation	[72]
circRNA_0109291	Tissue and cell	Up	-	Promotes cell proliferation, migration and apoptosis, poorer prognosis	[73]
circDOCK1	Cell and tissue	Up	Sponges miRNA-196a-5p, targets BIRC3	Suppresses cell apoptosis	[74]
hsa_circ_0008309	Tissue and cell	Down	Sponges miRNA-136-5P and miRNA-382-5P, targets ATXN1	Correlated with pathological differentiation	[69]
hsa_circ_0001874	Saliva	Up	-	Correlated with TNM stage and tumor grade	[76]
hsa_circ_0001971	Saliva	Up	-	Correlated with TNM stage	[76]
hsa_circ_0007059	Tissue and cell	Down	Targets AKT/mTOR pathway	Promotes cell growth, migration, invasion, and suppresses cell apoptosis	[71]
hsa_circ_001242	Tissue and cell	Down	-	Negatively correlated with tumor size and T stage	[75]
hsa_circ_0005379	Tissue and cell	Down	Targets EGFR pathway	Associated with tumor size and differentiation; drug sensitivity	[70]

Table 7. Hypopharyngeal cancer and its associated circRNAs

circRNA	Sample	Expression	Mechanism	Function	Reference
hsa_circ_0008287	Tissue	Down	Sponges hsa-miRNA-548c-3p, targets ErbB and Hippo pathways	-	[77]
hsa_circ_0005027	Tissue	Down	Sponges hsa-miRNA-548c-3p, targets ErbB and Hippo pathways	-	[77]

salivary hsa_circ_0001874 and hsa_circ_0001971 as new biomarkers for the diagnosis of OSCC.

CircRNAs and tongue cancer

By high-throughput sequencing, Qiu et al. [77] revealed 322 DE circRNAs in tongue squamous cell carcinoma (TSCC) tissue. Then RT-PCR results showed that circRNA expression in TSCC tissue was higher than that in adjacent tissue. Bioinformatics analyses indicated that the DE circRNAs might promote the development and progression of TSCC.

CircRNAs and hypopharyngeal cancer

Cao et al. [78] discovered that 2392 circRNAs are DE between hypopharyngeal squamous cell carcinoma (HSCC) and adjacent normal tissues by microarray. Of the circRNAs, 1304 are upregulated, including hsa_circ_0024108, hsa_circ_0058106 and hsa_circ_0058107, while 1088 are downregulated, including hsa_circ_0001189, hsa_circ_0002260 and hsa_circ_0036722. The functions of these circRNAs in HSCC have not been well characterized. Feng et al. [79] demonstrated that 173 circRNAs were DE between HSCC and adjacent normal tissues by circRNA sequencing, including 71 upregulated and 102 downregulated circRNAs. And they also demonstrated that a ceRNA subnetwork, consisting of two circRNAs (hsa_circ_0008287 and hsa_circ_0005027) and one miRNA (has-miRNA-548c-3p), which significantly affects both ErbB and Hippo signaling pathways (**Table 7**).

CircRNAs and thyroid cancer

Compared with normal thyroid tissues, 88 significantly upregulated circRNAs and 10 downregulated circRNAs were found in PTC tissues. Compared with benign thyroid lesions, 129 circRNAs and 226 circRNAs were significantly upregulated and downregulated in PTC tissues. Further overlap analysis suggested that hsa_circRNA_100395/miRNA-141-3p/miRNA-200a-3p axis may be involved in the pathogenesis of PTC [80]. Based on circRNA, miRNA and mRNA databases, Liu et al. [81] constructed a circRNA-miRNA-hubgene subnetwork of PTC including the 2 DE circRNAs, 3 DE miRNAs, and 4 DE mRNAs. These results indicated ceRNAs are the key regulator in the pathogenesis of PTC. However, this hypothesis needs further verification. Next, Ren et al. [82] have also

Table 8. Thyroid cancer and its associated circRNAs

CircRNA	Sample	Expression	Mechanism	Function	Reference
circ_0067934	Tissue and cell	Up	Targets EMT and PI3K/AKT pathways	Poor prognosis, promotes cell proliferation, migration, and invasion and inhibits apoptosis	[83]
circRNA_102171	Tissue and cell	Up	Targets CTNNBIP1-dependent Wnt/β-catenin pathway	Promotes cell proliferation, migration and invasion while inhibits apoptosis	[84]
hsa_circ_0137287	Tissue	Down	-	Correlated with aggressive clinicopathologic characteristics	[85]
circZFR	Tissue	Up	Sponges miRNA-1261, targets C8orf4	Promotes cell proliferation, migration and invasion	[86]
circ-ITCH	Tissue	Down	Sponges miRNA-22-3p, targets CBL/β-catenin pathway	Promotes cell proliferation and invasion and suppresses apoptosis	[87]
circBACH2	Tissue and cell	Up	Sponges miRNA-139-5p, targets LMO4	Promotes cell proliferation, migration, and invasion	[88]
circ_0039411	Tissue and cell	Up	Sponges miRNA-1179 and miRNA-1205	Promotes tumorigenesis and progression	[93]
circ_0058124	Tissue and cell	Up	Sponges miRNA-218-5p, targets NUMB	Promotes tumorigenesis and invasiveness	[94]
circ_0025033	Tissue and cell	Up	Sponges miRNA-1231 and miRNA-1304	Promotes cell proliferation and invasion	[92]
circ_0008274	Tissue and cell	Up	Targets AMPK/mTOR pathway	Promotes cell proliferation and invasion	[14]
circNUP214	Tissue and cell	Up	Sponges miRNA-145, targets ZEB2	Promotes cell proliferation, invasion, migration, and tumorigenesis	[91]
circ_0004458	Tissue and cell	Up	Sponges miRNA-885-5p, targets RAC1	Promotes cell proliferation and suppresses cell cycle arrest and apoptosis	[90]
circRNA_NEK6	Tissue and cell	Up	Sponges miRNA-370-3p, targets FZD8 and Wnt pathway	Promotes cell growth and invasion	[89]

found 206 up- and 177 downregulated circRNAs in PTC tissues by microarray. Their study suggested that PTC-related hsa_circRNA_047771 and hsa_circRNA_007148 may serve as potential diagnostic biomarkers and prognostic predictors for PTC patients. Furthermore, a series of studies have identified that a number of thyroid cancer-related circRNAs play a critical role in PTC pathogenesis and progression. Circ_0067934 could improve the development of PTC by promoting epithelial-mesenchymal-transition (EMT) and PI3K/AKT signaling pathways [83]. CircRNA_102171 overexpression promotes PTC progression through activating Wnt/β-catenin pathway in a CTNNBIP1-dependent way [84]. Decreased expression of hsa_circ_0137287 predicts aggressive clinicopathologic characteristics in PTC [85]. CircRNA circZFR exerted oncogenic roles via regulating miRNA-1261/C8orf4 axis in PTC, which suggested circZFR might be a potential therapeutic target [86]. CircRNA circ-ITCH suppresses PTC progression through miRNA-22-3p/CBL/β-catenin pathway [87]. CircBACH2 was highly expressed in PTC tissues and PTC cell lines, and the circBACH2/miRNA-139-5p/LMO4 axis could be targeted as a potential treatment strategy for PTC [88]. Moreover, growing evidence shows that certain circRNAs act as a crucial ceRNA contribute to the tumorigenesis and progression of PTC, such as circ_0039411, circ_0058124, circ_0025033, circ_0008274, circNUP214, circ_0004458 and circRNA_NEK6 [14, 89-94]. In summary, thyroid cancer and its associated circRNAs are exhibited in **Table 8**.

CircRNAs and esophageal cancer

Firstly, profiling and bioinformatics analyses revealed many DE circRNAs in esophageal cancer (EC) [95-97], these results indicated that dysregulated circRNAs were involved in the tumorigenesis and progression of EC. Secondly, the circRNA-mediated interacted networks of esophageal squamous cell carcinoma (ESCC) were built, and the construction of network can facilitate a better understanding of circRNA-related mechanisms in ESCC [2, 96, 98]. Finally, several dysregulated circRNAs were confirmed that they play important roles in ESCC. Li et al. [99] reported that cir-ITCH expression was usually low in ESCC compared to the peritumoral tissue and cir-ITCH acted as sponge of miRNA-7, miRNA-17 and miRNA-214. Their reports also indicate that cir-ITCH may have an inhibitory effect on ESCC by regulating the Wnt/β-catenin pathway. Study by Xia et al. has verified circ_0067934 is upregulated in ESCC tissue and promotes ESCC cell proliferation [100]. Dysregulation of circRNA_100876 expression leads to poor prognosis in ESCC by

Table 9. Esophageal cancer and its associated circRNAs

CircRNA	Sample	Expression	Mechanism	Function	Reference
cir-ITCH	Tissue	Down	Sponges miRNA-7, miRNA-17, miRNA-214, targets Wnt/β-catenin pathway	Inhibits tumor progression	[99]
has_circ_0067934	Tissue	Up	-	Poor differentiation and lower TNM stage, promotes proliferation and migration	[100]
circRNA_100876	Tissue and cell	Up	-	Poor prognosis, promotes cell proliferation and metastasis	[101]
ciRS-7	Tissue and cell	Up	Sponges miRNA-7, miRNA-876-5p, targets KLF4, HOXB13, MAGE-A, and NF-κB pathways	Promotes cell growth, migration, invasion, and metastasis	[102-104]
hsa_circ_0000337	Tissue and cell	Up	Sponges miRNA-670-5p	Promotes cell proliferation, migration and invasion	[105]
hsa_circ_0006168	Tissue and cell	Up	Sponges miRNA-100, targets rapamycin (mTOR)	Promotes cell proliferation, migration and invasion	[106]
circ0043898	Tissue	Down	Targets histone H3 and BMI1	Inhibits cell proliferation, migration and invasion and induces cell apoptosis and death	[107]
circVRK1	Tissue and cell	Down	Sponges miRNA-624-3p, targets PTEN/PI3K/AKT pathway	Tumor suppression, radioresistance	[110]
circ-TTC17	Tissue, cell and plasma	Up	Sponges miRNAs	Promotes cell proliferation and migration	[108]
circ-SMAD7	Plasma and tissue	Down	-	Promotes cell proliferation and migration	[109]

accelerating cell proliferation and metastasis [101]. CircRNA ciRS-7 was significantly upregulated in the ESCC tissues and cells, and can trigger the growth, migration, invasion and metastasis of ESCC cells via miRNA-7/KLF4 & NF-κB signals, or miRNA-7/HOXB13 & NF-κB signaling pathways or miRNA-876-5p/MAGE-A family axis [102-104]. Further functional experiments indicated targeted inhibition of ciRS-7 might be a potential approach for ESCC treatment. Studies have also identified that upregulated circ_0000337 and circ_0006168 promote cell proliferation, migration and invasion of ESCC [105, 106]. Wang et al. [107] determined that circ0043898 is presented as tumor inhibitor and could be a candidate biomarker in the therapeutic target and diagnosis of EC. Furthermore, recent works revealed that upregulated circ-TTC17 while downregulated circ-SMAD7 play a key role in affecting the proliferation and migration of ESCC cells [108, 109]. Additionally, He et al. [109] determined that circVRK1 was downregulated in ESCC tissues and cell lines, and identified that circVRK1 suppressed ESCC progression by regulating miRNA-624-3p/PTEN axis and PI3K/AKT signaling pathway. Altogether, above findings suggest that the deregulated expression of circRNAs is involved in the development and progression of EC through certain molecular mechanisms including miRNA sponges and regulation of pathways (**Table 9**).

CircRNAs in chemoradiation resistance of HNC

As we know, the emergence of chemoradiation resistance can lead to treatment failure and poor prognosis. At present, studies have found altered circRNA expression in radioresistant or chemoresistant tumors, such as EC, breast cancer, glioma, osteosarcoma, colorectal cancer, cervical cancer, prostate cancer, pancreatic cancer and non-small cell lung cancer, etc. [95, 111-120]. These results indicate possible involvement of these dysregulated circRNAs in the development of chemoradiation resistance. Through biological analysis, some circRNAs have been found to influence the chemoradiation resistance of cancer cells by regulating specific genes or signaling pathways [121-126].

Previous studies have also reported the deregulation of circRNAs were closely associated with chemoradiation resistance of HNC. Su et al. [95] first detected 57 upregulated circRNAs and 17 downregulated circRNAs in human radioresistant EC cell line, this suggested aberrant expression of circRNAs might be associated with the radioresistance of EC. Shuai et al. [56] found that circRNA_0000285 expression was significantly increased in patients with radioresistant NPC compared with patients with radiosensitive NPC. Chen et al. [57] found that circRNA_000543 expression was higher in

radioresistant NPC samples than in radiosensitive NPC samples. Further investigations demonstrated that circRNA_000543 can target miRNA-9/platelet-derived growth factor receptor B axis. These works indicated that circRNA_0000285 and circRNA_000543 may be involved in NPC radiosensitivity. Wu et al. [64] revealed DE circRNAs in $CD133^{+}CD44^{+}$ LC stem cells, and identified that circRNA hg19_circ_0005033 promotes cisplatin-chemotherapy resistance of LC stem cells. Su et al. [70] found that hsa_circ_0005379 expression level was significantly downregulated in OSCC tissue, while overexpression of hsa_circ_0005379 could significantly enhance the sensitivity of OSCC to the cetuximab drug *in vitro* and *in vivo*.

Although there is still very little study regarding circRNAs and chemoradiation resistance in HNC, it has a great potential that circRNAs can be used as novel biomarkers to predict the efficiency of chemoradiation and prognosis or recurrence in radiation or chemotherapy-resistant cancers. Moreover, some circRNAs will become the novel therapeutic targets for HNC patients with chemoradiation resistance.

Conclusions

Overall, the data elucidate the physiological and pathological roles of numerous circRNAs in human diseases, especially tumors. However, there remain a number of circRNAs to be investigated and specific molecular mechanisms to be explored. Since 2012, prostate cancer antigen 3 has be recognized as the first lncRNA biomarker by the FDA approval for clinical diagnosis of prostate cancer, efforts are being made to find more effective tumor markers. Compared with lncRNAs and miRNAs, circRNAs has the advantage of high stability, which can be regarded as promising clinical biomarkers for diagnosis, prognosis and therapy. In addition, since the first report of abundant presence of circRNAs in exosomes and the detection of exosomal circRNAs from cancer cells, the studies of circRNAs are coming into a new sight. Exosomal circRNAs may participate in the processes of cell growth, angiogenesis and EMT and be applied for targeted therapy of cancers. Exosomal circRNAs are likely to be regarded as a new class of exosome-based cancer biomarkers and to play potential biological function in cancer development and progression. Hence, the accurate detection of exosomal circRNAs and the effective development of treatment vector of exosomal circRNAs are two urgent programs for cancer patients.

To date, increasing studies have demonstrated that certain circRNAs were translated *in vivo*, and the coding potency of circRNAs may attribute to human diseases. However, we need overwhelming evidence to uncover the protein coding function of circRNAs, and to better understand the biological mechanism of circRNAs in related diseases, including tumors. This discovery gives circRNAs a new function and provides a new direction for research of circRNA in the future.

In conclusion, although the roles of circRNAs in HNC have just begun to be revealed, the potential application of circRNAs as diagnostic biomarkers and therapeutic targets will bring a board prospect for the biotherapy of HNC patients.

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Disclosure of conflict of interest

None.

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