

CircRNA: A novel potential strategy to treat thyroid cancer (Review)

GUOMAO ZHU^{1,2*}, XINGYU CHANG^{1,2*}, YUCHEN KANG³, XINZHU ZHAO^{1,2},
XULEI TANG^{1,2}, CHENGXU MA^{1,2} and SONGBO FU^{1,2}

¹Endocrinology Department, The First Hospital of Lanzhou University;

²Endocrine Disease Clinical Medical Research Center of Gansu Province, The First Hospital of Lanzhou University;

³The Second Clinical Medical College, Lanzhou University, Lanzhou, Gansu 730030, P.R. China

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Abstract. Thyroid cancer (TC) is the most common type of endocrine cancer. Over the last 50 years, the global incidence of TC has been increasing. The survival rate of TC is higher than that of most other types of cancer, but it depends on numerous factors, including the specific type of TC and stage of the disease. Circular RNAs (circRNAs) are a new class of long noncoding RNA with a closed loop structure that have a critical role in the complex gene regulatory network that controls the emergence of TC. The most important function of circRNAs is their ability to specifically bind to microRNAs. In addition, the biological functions of circRNAs also include interactions with proteins, regulation of the transcription of genes and acting as translation templates. Based on the characteristics of circRNAs, they have been identified as potential biomarkers for the diagnosis of tumors. In the present review,

the function and significance of circRNAs and their potential clinical implications for TC were summarized. Furthermore, possible treatment approaches involving the use of mesenchymal stem cells (MSCs) and exosomes derived from MSCs as carriers to load and transport circRNAs were discussed.

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1. Introduction

The pathogenesis of thyroid cancer (TC), the most frequent type of endocrine cancer, is related to a variety of gene mutations, such as those in v-raf murine sarcoma viral oncogene homolog B1 (BRAF), human telomerase reverse transcriptase promoter, TP53 and NRAS. However, almost no gene mutation has been proven to be able to guide treatment or influence clinical outcomes (1). In addition to age, sex, ethnicity and residential region of patients and subjects with a family history of TC, radiation exposure, obesity, smoking and even body height may be risk predictors of TC (2). In the last decade (2007-2016), the incidence of TC rose at an annual rate of ~3% among 20- to 39-year-olds and 4% among 15- to 19-year-olds. The 5-year survival rate of TC >99% (3) is related to numerous factors, including the unique type of TC (4), therapeutic treatment and overdiagnosis (2,5). According to the origin and histological characteristics of cancer cells, TC may be divided into the following types: Differentiated TC, originating from follicular cells, which accounts for >90% of all cases of TC (6) and includes two major subtypes, papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma; cancer originating from parafollicular cells is known as medullary thyroid carcinoma. However, anaplastic TC (ATC), the most heterogeneous tumor type among all TC subtypes, accounting for 1% of TC cases, is the cause of the majority of all TC-associated deaths

Correspondence to: Professor Songbo Fu, Endocrinology Department, The First Hospital of Lanzhou University, 1 Donggangxi Street, Lanzhou, Gansu 730030, P.R. China
E-mail: fusb@lzu.edu.cn

*Contributed equally

Abbreviations: TC, thyroid cancer; circRNA, circular RNA; miRNA, microRNA; MSC, mesenchymal stem cell; PTC, papillary thyroid carcinoma; ATC, anaplastic TC; UTR, untranslated region; CDRI, cerebellar degeneration-related protein 1; RBP, RNA binding proteins; CTNNBIP1, β -catenin interacting protein 1; TCF, T cell factor; RNA pol II, RNA polymerase II; DNMT1, DNA methyltransferase 1; IRES, internal ribosome entry site; m6A, N6-methyladenosine; TME, tumor microenvironment; EV, extracellular vesicles; KLF6, Krüppel-like zinc finger transcription factor 6; LMO4, LIM-only protein 4; AMPK, adenosine monophosphate-activated protein kinase; CDK, cyclin-dependent kinase; EMT, epithelial to mesenchymal transition; LDLR, low-density lipoprotein receptor; ATP, adenosine triphosphate; TGF, transforming growth factor; LNM, lymph node metastasis; AUC, area under receiver operating characteristic curve

Key words: thyroid cancer, circRNAs, mesenchymal stem cells, exosomes

and the chance of a cure is slim (7). In South Korea, the prevalence rate has increased 15-fold over the past decade, which may be related to overdiagnosis due to thyroid ultrasound examination, while the TC-related mortality rate has been stable (8). Although fine-needle aspiration biopsy is the gold standard for TC diagnosis, its invasiveness limits its application; therefore, there is an urgent requirement for a novel, less invasive method to balance the specificity and sensitivity to improve the accuracy of TC diagnosis.

Circular RNAs (circRNAs) are a new class of noncoding RNA (9) that are generated from linear precursor mRNAs in a nonclassical splicing square, which endows them with a unique closed continuous ring structure. According to their composition, circRNAs may be divided into three major types, the most common being exonic circular RNAs (ecircRNAs), and the others being circular intronic RNAs and exon-intron circular RNAs (EIciRNAs) (10). Based on recent studies, circRNAs have the following distinct features: i) Multiplicity: Different genes are able to encode circRNAs with different combinations of exons and introns due to alternative splicing (11); ii) Abundant expression: In fibroblasts, 14% of the transcribed genes are made up of circRNAs and the expression is much greater than that of their linear host RNAs (12); iii) Stability: CircRNAs are stable due to the lack of 5'-3' polarity and a polyadenylated tail and they accumulate in numerous cell types, such as neural tissues, (13,14); iv) Conservation: In mammals, circRNAs are likely to have a higher sequence conservation than other types of RNA (15). These features of circRNAs indicate their varied potential biological functions and clinical applications. CircRNAs are currently known to regulate gene expression directly or through interaction with micromolecules to process various biological functions accordingly (Fig. 1).

2. Functions of circRNAs

Due to their capacity to broadly regulate cell conditions, circRNAs may be associated with tumor progression. Abnormal expression of circRNAs has been detected in numerous cancer types, such as esophageal cancer (16), gastric cancer (17), colorectal cancer (18), hepatocarcinoma (19), glioma (20), bladder cancer (21) and TC (22). CircRNAs function as cell activity regulators, mainly through the following four aspects discussed below.

miRNA sponging. MicroRNAs (miRNAs) are single-stranded noncoding RNAs ~22 nucleotides in length and mediate post-transcriptional gene silencing in the cytoplasm by interacting with the 6 nt seed sequence on the 3'-untranslated region (3'UTR) of their target mRNAs (23,24). miRNAs have extensively vital roles in the post-transcriptional regulation of gene expression, including cell proliferation, migration, differentiation and apoptosis (25-31). Serving as the upstream molecules of miRNAs, circRNAs, which contain miRNA response elements, are able to bind various miRNAs and suppress their activity via a mechanism known as miRNA sponging (30,31). For instance, the first discovered miRNA sponge, antisense transcript of cerebellar degeneration-related protein 1 (CDR1), also termed CIRS-7, derived from the antisense transcript of the CDR1 gene, is able to negatively regulate miR-7 (30,31), which may be detected in gastric cancer and hepatocellular

carcinoma (32,33). This effect is also commonly observed in TC: CircFAT1 (e2) has a function similar to that of CIRS-7 and has a binding site for miR-873. CircFAT1 is highly expressed in PTC tissues and cells and serves as an miRNA sponge to vastly downregulate miR-873 expression, thus upregulating the activity of zinc finger E-box binding homeobox 1 and ultimately promoting the growth, migration and invasion of PTC cells (34).

Protein interaction. In addition to miRNA sponging, protein interaction is another important function of circRNAs. Among other proteins, RNA binding proteins (RBPs) are the most famous proteins involved in the regulation of RNA metabolism in different fields, such as formation, transportation, localization and translation (35). Errichelli *et al* (36) reported that fused in sarcoma was able to modify the formation of numerous circRNAs in mouse motor neurons derived *in vitro* by binding the introns flanking the back-splicing junctions. Sharing consensus sequences with other RBPs, the c-MYC protein is able to interact with circ-Amotl1, easing nuclear translocation of c-MYC, which appears to promote its stability and upregulate c-MYC targets (37). Similarly, nuclear translocation of signal transducer and activator of transcription 3 is also associated with circ-Amotl1 in a parallel manner (38). In addition, numerous circRNAs have been reported to interact with proteins in TC. CircRNA_102171 is highly expressed in PTC tissues, directly interacts with β -catenin interacting protein 1 (CTNNBIP1) and obstructs its association with the β -catenin/T cell factor (TCF)3/TCF4/lymphoid enhancer factor 1 complex to facilitate PTC progression (39). Existing evidence affirms that specific circRNAs are able to interact with different proteins (9,40), while certain proteins may also dynamically bind to different circRNAs (41,42). To date, research on circRNAs interacting with proteins in TC remains limited, probably due to it being more challenging to analyze the binding sequences of circRNAs in RBPs than those of mRNAs.

CircRNAs regulate gene expression. Through an intricate mechanism, circRNAs are able to modify gene transcription. With the assistance of U1 small nuclear ribonucleoproteins, exon-intron circular RNAs (EIciRNAs) influence the activity of RNA polymerase II (RNA pol II) via RNA-RNA interactions and then regulate the transcription of their parental gene, ultimately affecting protein translation (9,43). The nuclear circRNAs EIciPAIP2 and EIciEIF3J promote parental gene transcription in a similar manner (43). In addition to long non-coding RNAs, ciRNAs may also act as activators of RNA pol II and upregulate gene transcription. For instance, ci-ankrd52 enhances the expression of ankyrin repeat domain 52 protein (44). Certain circRNAs have the capacity to regulate gene expression at the translation level, in addition to the transcription level. Recently, Wu *et al* (45) reported that circYap impaired the interaction of PABP on the poly(A) tail with eIF4G on the 5'-cap of Yap mRNA. As a consequence, circYap suppressed gene translation at the initiation stage. It was also demonstrated that circRNAs may be associated with DNA modification. DNA methyltransferase 1 is a methyltransferase that controls DNA methylation and its promoter may be downregulated by circFECR1. Furthermore, circFECR1 is also

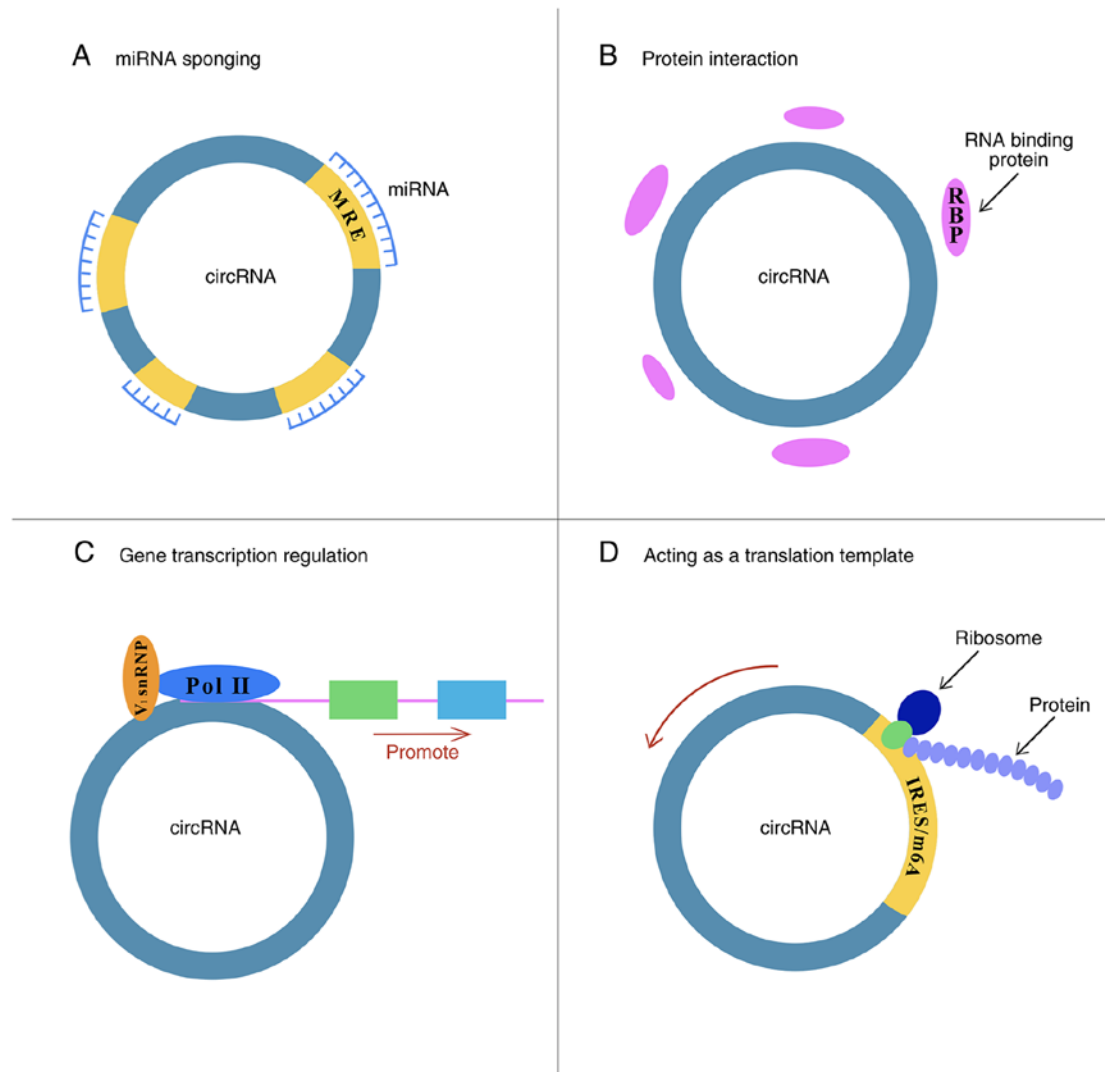


Figure 1. Functions of circRNAs. (A) miRNA sponging; (B) Protein interactions; (C) Gene transcription regulation; (D) Acting as a translation template. miRNA, microRNA; circRNA, circular RNA; POL II, RNA polymerase II; snRNP small nuclear ribonucleoprotein; RBP, RNA binding proteins.

able to recruit the demethylase TET1 to the Friend leukemia virus integration 1 promoter, resulting in the demethylation of DNA (46). As a consequence, circRNAs may regulate gene expression in different ways, while the effect of circRNAs on DNA replication remains unexplored.

Translation template. The two ends of a circRNA are connected by one covalent bond, which impairs its translation function. Furthermore, due to their loop structure lacking a 3' and 5'UTR, circRNAs have been classified as noncoding RNAs. However, most circRNAs are ecircRNAs with an open reading frame, which implies their translational potential (47-49). To date, their translational activity has been proven in diverse organisms (50). With regard to certain groups of circRNAs, specific elements are indispensable for cap-independent translation mechanisms for translation initiation, such as internal ribosome entry sites (IRESs) or N6-methyladenosine (m6A) (49). For instance, with the existence of an IRES and necessary splicing elements, circZNF609 may be translated into zinc finger protein 609 in a cap-independent way, but the translation activity is much lower than that of its linear counterpart (47). Another cap-independent translation mechanism

for circRNA is m6A in the circRNA 5'UTR, which interacts with eIF4G2 and eIF3A and recruits the 43S preinitiation complex to trigger translation. m6A modification may be found in more than one-tenth of circRNAs, the level of which is associated with circRNA translation efficiency (51,52). It has been inferred that rolling circle amplification may be a mechanism of enhancing the translation productivity of circRNAs, but this mechanism only produces long, repetitive peptides (53). Increasing evidence suggests the direct translation function of circRNAs (54,55). However, the proteins encoded by circRNAs remained to be analyzed. Perhaps these proteins are persistently produced at low levels due to the resistance to degradation and low translation activity characteristics of circRNAs.

In conclusion, circRNAs may regulate downstream gene and protein expression through various mechanisms and have numerous unique advantages over other noncoding RNAs. Although circRNAs have better stability and longer half-lives than their linear transcripts, the possibility of circRNA degradation in their *in vivo* transport when they are used as a therapeutic intervention remains to be investigated. The effects of circRNA degradation on disease and the biological effects

on surrounding cells and tissues require further study. Previous studies have revealed that circRNAs are more conserved among mammals than other RNAs (12), but of note, in-depth study and continuous discovery of novel circRNAs have indicated that numerous circRNAs expressed are species-specific and do not have any sequence homology (56).

3. Regulation of circRNAs in TC

Increasing evidence suggests that noncoding RNAs, including circRNAs, contribute to the formation of the tumor microenvironment (TME), communication between cancer cells and surrounding stromal cells (fibroblasts, immune cells, endothelial cells) and the impact of physicochemical parameters (57). Numerous studies have revealed that multiple functions of the TME may be influenced by circRNAs, not only in tumor invasiveness but also in tumor angiogenesis, epithelial to mesenchymal transition (EMT) and drug resistance (57,58). Thus, circRNAs may regulate the progression of TC invasion and evolution through different approaches (Fig. 2).

Regulation of invasiveness by circRNAs. Regulating cell invasiveness is of great importance in tumor progression. In recent years, certain oncogenic circRNAs have been indicated to regulate the invasiveness of TC. The upregulation of circ-ABCB10 in TC is negatively correlated with the expression of Krüppel-like zinc finger transcription factor 6 (KLF6) (59), which encodes a series of proteins that engage in the regulation of cancer development through alternative splicing, suggesting that circ-ABCB10 may promote the proliferation and invasion of TC by targeting KLF6 (60). As mentioned previously, circRNA_102171 is able to directly bind CTNNBIP1 and activate the Wnt/ β -catenin pathway. Ultimately, upregulated circRNA_102171 enhances the invasiveness of PTC (39). In addition to directly interacting with proteins, circRNAs also promote the progression of TC by sponging miRNAs. For instance, overexpressed circBACH2 in PTC sponges miR-139-5p, which is able to interact with the LIM-only protein 4 (LMO4) 3'UTR, resulting in the inhibition of LMO4. Therefore, the invasiveness of PTC is enhanced (61,62). Similarly, circRASSF2 is enriched in PTC. By downregulating miR-1178, circRASSF2 facilitates tumor growth and promotes invasiveness (63). To date, circ_0008274 (64), circ_0103552 (65), circ_FOXM1 (66), hsa_circ_0058124 (67), circ_FND3B (68) and circ_EIF3I (69) have been reported to participate in the regulation of different signaling pathways associated with the proliferation and apoptosis of TC cells.

Cell cycle. The cell cycle refers to the time interval, as determined by experiments, during which cells prepare and then equally replicate their genomes to form two daughter cells (70). Cancer is characterized by the abnormal activity of various cyclins that lead to uncontrolled proliferation of tumor cells (Fig. 3). The upregulation of circDOCK1 in TC is accompanied by increased expression of cyclin D1 and downregulation of p53, which leads to an imbalance of cyclin-dependent kinase (CDK) activity and rapid growth of tumor cells (71). Zhou *et al* (64) indicated that the expression of hsa_circ_0008274 in PTC was upregulated following activation of the adenosine monophosphate-activated protein kinase (AMPK)/mammalian target of rapamycin

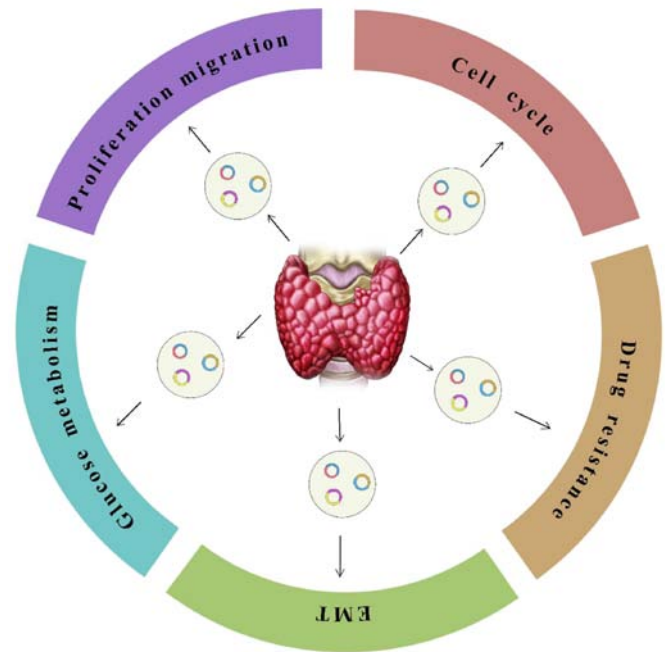


Figure 2. Functions of circRNAs in TC. CircRNAs are able to regulate TC progression through five aspects: Proliferation-migration (such as circRNA_102171, CircBACH2, circRASSF2, circ-ABCB10, hsa_circ_0008274, circ_0103552, circ_FOXM1, hsa_circ_0058124, circ_FND3B, circ_EIF3I), cell cycle (such as circDOCK1, hsa_circ_0008274, hsa_circ_0004458, hsa_circ_0011385), drug resistance (such as CircEIF6), EMT (such as circ_LDLR and circ_0067934) and glucose metabolism (such as hsa_circ_0011290). EMT, epithelial to mesenchymal transition; TC, thyroid cancer; circRNA, circular RNA.

signaling pathway. AMPK upregulates the p53-p21 axis and leads to cell cycle stagnation in G1/S phase (72). In PTC, circ_0004458 is upregulated. After circ_0004458 silencing, the cell cycle distribution was substantially decreased compared with that of the control group. Furthermore, hsa_circ_0004458 is able to regulate the expression of Rac1 through the specific sponging of miR-885-5p (73). Rac1 protein is able to activate extracellular regulated protein kinase 1/2 signaling induced by γ -irradiation and the subsequent G2/M checkpoint response (74). Circ_0001666 is highly expressed in PTC cells. After circ_0001666 downregulation, the cell cycle was blocked in G1 phase, decreasing the expression of cyclin D1 and cyclin E (75). When the overexpression of circ_PSD3 in PTC cells was abolished, the expression of cyclin D1 and CDK4 also decreased significantly, which inhibited the cell cycle progression of PTC cells (76). CircRNAs have a significant role in cell cycle regulation, bypassing cell cycle checkpoints in different ways to accelerate the progression of the cell cycle. Most current findings are from studies that performed knockout of cancer-promoting circRNAs and these types of experiments may reveal the effects of circRNAs on TC. The direct interactions between circRNA/miRNA or circRNA/protein and cell cycle proteins remain to be determined.

EMT. EMT is a process whereby epithelial cells lose their characteristics, such as cell-to-cell adhesion and cell polarity, and instead acquire interstitial characteristics during the process of cell culture; EMT is universally involved in physiological and pathological processes, particularly tumor metastasis (77-79). Therefore, analyzing the contribution of circRNAs to EMT and

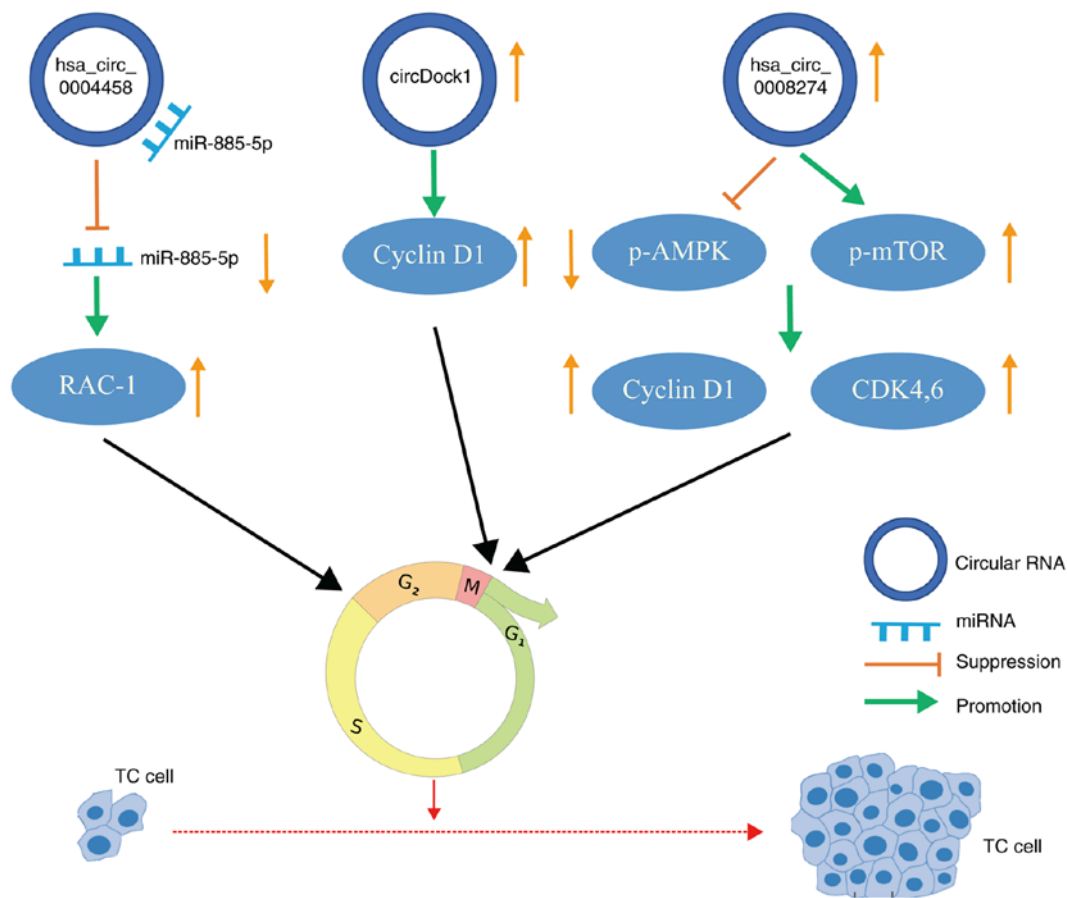


Figure 3. Interrelated regulation of circRNAs and the cell cycle. The biological role of circRNAs in the cell cycle and major interconnected regulatory networks in TC. Several fundamental effectors, including AMPK, cyclin D1, RAC1, CDK4 and CDK6, were identified in this network. The abnormal expression of certain circRNAs results in the bypass of key cellular checkpoints and ultimately neoplastic growth. TC, thyroid cancer; circRNA, circular RNA; p-AMPK, phosphorylated adenosine monophosphate kinase; mTOR, mammalian target of rapamycin; RAC1, RAC family small GTPase 1; CDK, cyclin-dependent kinase.

tumor metastasis may reveal them to be potential targets to inhibit the malignant progression of TC. CircRNA of low-density lipoprotein receptor (circ_LDLR) in PTC tissues was significantly upregulated compared with that in normal tissues and served as an miR-195-5p sponge to upregulate the expression of LDLR mRNA, which led to a decrease in the level of E-cadherin and an increase in the level of Twist1. Twist is able to downregulate epithelial markers such as E-cad and upregulate interstitial cell markers such as vimentin by altering the transcription of EMT-related genes (80,81), which promotes EMT transformation in PTC. In PTC cells with high expression of circ_102002, the expression of E-cadherin was downregulated and the expression of N-cadherin and mesenchymal phenotypic markers was upregulated, while PTC cells with low expression of circ_102002 produced the opposite result (82). CIRS-7 had a similar effect: E-cadherin was downregulated in PTC cells with high expression of CIRS-7, while vimentin levels were significantly increased (83). Similarly, another study suggested that circ_0067934 may promote TC by regulating EMT and PI3K/AKT signaling pathways (84). By sponging downstream targets, circRNAs may promote the invasion and metastasis of TC through their regulatory role in the EMT pathway, but the specific process and downstream pathway are largely elusive and require to be further explored.

Glucose metabolism. The glucose metabolism in tumor cells differs from that in normal cells. Only when oxygen is scarce do

normal cells rely on glycolysis rather than oxygen-consuming mitochondrial metabolism to create energy. However, tumor cells prefer to perform glycolysis in the cytosol regardless of whether oxygen is sufficient (85-87). This phenomenon was termed the Warburg effect and describes the ability of tumor cells to generate energy at a rapid rate, accompanied by low efficiency in adenosine triphosphate (ATP) production per molecule of glucose (88). Cancer cells undergo rapid growth and proliferation, and their energy demand may be satisfied via glycolysis. The Warburg effect has been suggested to be associated with the regulation of certain oncogenes and tumor suppressors, such as Akt, PI3K and Ras (89). However, the relationship between circRNAs and glucose metabolism in cancer remains to be fully proven. After circCCDC66 gene knockout, the glucose metabolism of PTC cells was significantly inhibited. Further analysis determined that circCCDC66 is able to act as a sponge for miR-211-5p to promote the expression of pyruvate dehydrogenase kinase 4, thereby increasing the level of glucose metabolism in PTC cells (90). However, circPUM1 was highly expressed in PTC. After downregulation of circPUM1, the expression of hexokinase 2 was downregulated and glycolysis in PTC was blocked (91). Similar research suggested that hsa_circ_0011290 was significantly upregulated in PTC (92). After specific silencing of hsa_circ_0011290 in cells, the glucose metabolism spectrum indicated that glucose uptake was inhibited, lactic acid production decreased, the

Table I. CircRNAs in TC.

A, Regulating invasiveness				
CircRNA	Expression level	Mechanism	Diagnostic and prognostic value	(Refs.)
circ_FAT1	Upregulated in PTC cell lines and tissues	Sponge miR-873 to regulate ZEB1	-	(34)
circ_102171	Upregulated in PTC tissues and cells	Interacts with CTNNBIP1 to block its interaction with the β -catenin/TCF3/TCF4/LEF1 complex	-	(39)
circ_BACH2	Upregulated in PTC tissues and cells	Sponges miR-139-5p and relieves suppression of the target LMO4	AUC was 0.8819	(61)
circ_RASSF2	Upregulated in PTC tissues and cells	Sponges miR-1178; downregulates TLR4 expression	-	(63)
circ_0008274	Upregulated in PTC tissues and cells	Modulates AMPK/mTOR signaling pathway	Associated with TNM stage and lymph node metastasis	(64)
circ_0103552	Upregulated in PTC tissues and cells	Sponges miR-127	-	(65)
circ_FOXM1	Upregulated in PTC tissues and cells	Sponges miR-1179; upregulates HMGB1 expression	Associated with tumor size, tumor stage and poor lymph node metastasis	(66)
hsa_circ_0058124	Upregulated in TC tissues and cells	Sponges miR-940; downregulates MAPK1 protein levels	-	(67,107)
circ_FNDC3B	Upregulated in PTC tissues and cells	Sponges miR-1178; downregulates TLR4 expression	-	(68)
circ EIF3I	Upregulated in PTC tissues	Sponges miR-149; upregulates KIF2A expression	-	(69)
circ_RAPGEF5	Upregulated in PTC tissues and cell	Sponges miR-198; upregulates FGFR1	AUC was 0.711	(107)
hsa_circ_0124055	Upregulated in TC tissues	-	AUC was 0.836	(114)
hsa_circ_0101622	Upregulated in TC tissues	-	AUC was 0.805	(114)
circ_ABCB10	Upregulated in TC tissues	Downregulates KLF6	-	(59)

B, Regulation of the cell cycle

CircRNA	Expression level	Mechanism	Diagnostic and prognostic value	(Refs.)
circDOCK1	Upregulated in TC tissues	Sponges miR-124; dampens signaling transduction of JAK/STAT/AMPK	-	(71)
has_circ_0008274	Upregulated in PTC tissues	Inhibits activation of the AMPK/mTOR signaling pathway	Associated with TNM stage and lymph node metastasis	(64)
hsa_circ_0004458	Upregulated in PTC tissues and cells	Sponges miR-885-5p; activation of RAC1	-	(73)
circ_0001666	Upregulated in PTC tissues and cells	Sponges miR-330-5p/ miR-193a-5p/ miR-326	Associated with lymph node metastasis	(75)
circ_PSD3	Upregulated in PTC tissues	Sponges miR-637 to regulate HEMGN and influence the PI3K/Akt signaling pathway	-	(76)

Table I. Continued.

C, Regulation of epithelial-mesenchymal transition

CircRNA	Expression level	Mechanism	Diagnostic and prognostic value	(Refs.)
circ_102002	Upregulated in PTC tissues and cells	Sponges miR-488-3p to regulate HAS2	-	(82)
circ_0067934	Upregulated in TC cells	Sponges miR-1304; upregulated CXCR1 expression	Correlated with AJCC grade, lymph node metastasis and survival rate	(84)
circ_LDLR	Upregulated in PTC tissues and cells	Sponges miR-195-5p to regulate LIPH expression	-	(80)
CIRS-7	Upregulated in PTC tissues and cells	Sponges miR-7 to regulate EGFR	-	(83)

D, Regulation of glucose metabolism

CircRNA	Expression level	Mechanism	Diagnostic and prognostic value	(Refs.)
circCCDC66	Upregulated in TC tissues and cells	Sponges miR-211-5p and upregulates PDK4	-	(100)
hsa_circ_0011290	Upregulated in PTC tissues	Sponges miR-1252; positively modulates FSTL1 expression	-	(92)
circPUM1	Upregulated in PTC tissues and cells	Sponges miR-21-5p; downregulates MAPK1	-	(91)

E, Enhancement of cisplatin resistance

CircRNA	Expression level	Mechanism	Diagnostic and prognostic value	(Refs.)
circEIF6	Upregulated in ATC tissues and cells	Sponges miR-144-3p; increases TGF- α expression	-	(99)

F, Other

CircRNA	Expression level	Mechanism	Diagnostic and prognostic value	(Refs.)
hsa_circ_0137287	Downregulated in PTC tissues	-	AUC was 0.8973 for predicting malignancy; The AUC was 0.6885 for predicting extrathyroidal extension; the AUC was 0.6691 for predicting lymph node metastasis	(105)
hsa_circRNA_007148	Upregulated in PTC tissues	-	AUC was 0.846; correlated with LNM	(106)
hsa_circRNA_047771	Downregulated in PTC tissues	-	Associated with BRAFV600 mutation, lymph node metastasis and advanced TNM stage. AUC was 0.876	(106)

TC, thyroid cancer; PTC, papillary thyroid carcinoma; ATC, anaplastic TC; circRNA, circular RNA; AUC, area under the receiver operating characteristic curve; AJCC, American Joint Committee on Cancer.

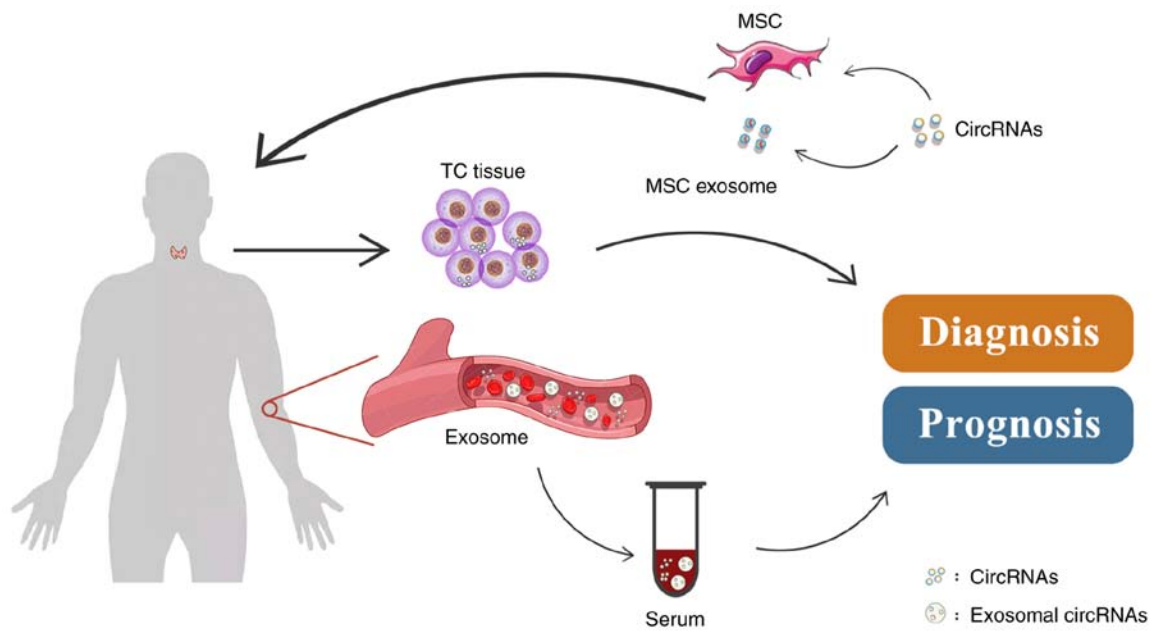


Figure 4. Clinical application of circRNAs. CircRNAs are differentially expressed in TC tissues, serum and plasma exosomes; therefore, they may be used as biomarkers for TC diagnosis and prognosis. MSCs and MSC exosomes may serve as transport vectors for circRNAs and as potential treatments for TC. MSC, mesenchymal stem cell; TC, thyroid cancer; circRNA, circular RNA.

ATP content increased, and cell proliferation and cell viability were significantly inhibited. CircRNAs have a certain effect on glycolysis in TC and may directly or indirectly regulate the activities of key enzymes in glucose metabolism, but their regulatory mode of action remains to be proven.

Drug resistance. Drug resistance refers to the tolerance of microorganisms, parasites and tumor cells to the effect of chemotherapy drugs. Drug resistance may develop prior to treatment or be acquired during treatment by tumors, which is a significant obstacle to overcome in tumor treatment (93). Platinum drugs such as cisplatin are extensively applied in treating human cancers and are considered successful standard therapies (94), but their effect is limited in ATC due to drug resistance (95–98). Liu *et al* (99) proved that circEIF6 acted as a regulator of drug resistance in ATC. CircEIF6 was upregulated in ATC tissues and cells and controlled transforming growth factor (TGF)- α by sponging miR-144-3p in cells treated with cisplatin. This signaling pathway (circEIF6/miR-144-3p/TGF- α axis) was confirmed to be associated with lowering the sensitivity of ATCs to cisplatin resistance. However, the study did not identify the TGF- α downstream signaling molecules, which requires further research.

In summary, numerous studies suggest that various circRNAs may promote or inhibit the occurrence of TC through different mechanisms (Table I). Most circRNAs function as miRNA sponges and by interacting with RBPs and a single circRNA may participate in different mechanisms of cancer development through sponging a variety of miRNAs. Thus, understanding the regulatory mechanism of circRNAs involved in the TC process may reveal novel therapeutic targets. However, there are limited studies on the subcellular localization and degradation mechanism of circRNAs in TC cells, which may become a novel starting point for research. The current research mainly focused on the regulatory network of circRNAs in PTC,

but studies on circRNAs involved in other types and subtypes of TC are scarce, suggesting that other types of TC may become the focus of future research. At the same time, the lack of diversity in experimental models to study the correlation between circRNAs and TC and other uncontrollable factors may also have a negative impact on the repeatability and reliability of the experimental results; thus, unifying the relevant experimental models may lead to more convincing results.

4. Clinical application prospect

CircRNAs as biomarkers in cancers. It is universally acknowledged that most cancer types may be cured if diagnosed at an early stage. Due to the high incidence of TC, the demand for early diagnosis is increasing. The features of circRNAs, such as their stability and diverse nature, enable them to accumulate in body fluids and tissues (12,100,101), making it possible for circRNAs to serve as cancer biomarkers. The biomarker value of circRNAs has been proven in different cancer types, such as breast cancer (102), lung cancer (103) and gastric cancer (104). Several circRNAs have been investigated as potential biomarkers through broad clinical sample testing, including tissues, serum and exosomes from patients with TC and healthy controls. These circRNAs have the potential to enable the early diagnosis of TC and predict recurrence and metastasis (Fig. 4).

Diagnostic biomarkers. Recently, through clinicopathologic factor association analysis, Lan *et al* (105) observed that the downregulation of hsa_circ_0137287 correlated with aggressive clinicopathologic characteristics of PTC, such as lymph node metastasis (LNM), advanced T stage, extrathyroidal extension and larger tumor size. The area under the receiver operating characteristic curve (AUC) was 0.8973, which demonstrated the potential of hsa_circ_0137287 to be a candidate diagnostic biomarker for PTC. Similarly, Ren *et al* (106)

identified hundreds of circRNAs upregulated or downregulated separately in PTC tissues. The expression of the most upregulated circRNA, hsa_circRNA_007148, was significantly associated with LNM. In addition, lower expression of hsa_circRNA_047771, which was the most downregulated circRNA, was associated with BRAF^{V600} mutation, LNM and advanced TNM stage. The AUC also supported the potential value of hsa_circRNA_047771 and hsa_circRNA_007148 as diagnostic biomarkers for PTC (106). However, due to the invasive and complex nature of detecting circRNAs in tissues, its value in TC diagnosis is limited. In addition, different subtypes of TC probably have different characteristic abnormal circRNAs, which may be the direction of further exploration.

Numerous studies have reported that circRNAs are able to stably accumulate in peripheral blood. Hence, serum circRNAs may be suitable and less invasive biomarkers. A study suggested that two upregulated circRNAs (circRAPGEF5 and hsa_circ_0058124) were stably enriched in the peripheral blood of patients with PTC (107). The results of a further expression analysis of circRAPGEF5 and hsa_circ_0058124 in serum samples during the treatment of patients with PTC indicated that the two circRNAs were markedly decreased along with systematic treatment, indicating that circRNAs may allow researchers to monitor the PTC process dynamically. The AUC for the ability to discriminate PTC from healthy controls was 0.711 for circRAPGEF5 and 0.790 for hsa_circ_0058124. Furthermore, the combination of the two circRNAs (circRAPGEF5 and hsa_circ_0058124) demonstrated a better diagnostic ability than a single circRNA in PTC identification, with an AUC value of 0.860. Therefore, the appropriate selection of a group of several circRNAs may be a novel diagnostic approach that may increase the sensitivity, specificity and accuracy of TC diagnosis and prognosis.

Exosomes are small vesicles with a single membrane, exhibiting the same topology as cells, and their diameter varies from 30 to 200 nm. Select proteins, lipids, nucleic acids and glycoconjugates may be packaged in exosomes (108). Several studies have proven that exosomes may act as carriers of circRNAs and transfer them between cancer cells (109), suggesting that circRNAs in exosomes have the potential to act as biomarkers. Wu *et al* (63) isolated serum exosomes from 60 serum samples collected from patients with PTC and healthy subjects and indicated that circRASSF2 was overexpressed in serum exosomes from patients with PTC. Logistic regression analysis demonstrated that upregulation of circRASSF2 was markedly associated with tumor stage and LNM. In addition, another study using high-throughput sequencing determined that circRNAs were differentially expressed in serum-derived exosomes collected from patients with PTC and patients with benign thyroid goiter. A total of three upregulated circRNAs and 19 downregulated circRNAs were detected in the former (110). However, no clinicopathologic factor association analysis was performed in that study.

Prognostic biomarkers. Prognosis is a prediction of the course and outcome of a disease. Prognostic evaluation in the early stage of a disease helps mitigate the effects of negative predictive factors. In addition, cancer patients heavily rely on prognostic evaluation to determine their life expectancy. The prognostic value of circRNAs has been proven in diverse cancer types, such as colon cancer (111), lung cancer (112) and

triple-negative breast cancer (113). Sun *et al* (114) indicated that hsa_circ_0124055 and hsa_circ_0101622 were overexpressed in the plasma of patients with TC, which was significantly associated with larger tumor size, poorer TNM stage or histological grade and LNM. Compared with the levels in plasma of patients prior to tumorectomy, hsa_circ_0124055 and hsa_circ_0101622 were markedly decreased after surgery. The patients with high hsa_circ_0124055 or hsa_circ_0101622 expression exhibited shorter overall survival. The AUCs were 0.836 for hsa_circ_0124055 and 0.805 for hsa_circ_0101622, which indicated their TC diagnostic significance. Furthermore, the AUC rose to 0.911 after combining hsa_circ_0124055 with hsa_circ_0101622. In addition, in patients with PTC, circFNDC3B was demonstrated to be upregulated in serum exosomes and tissues. The AUC for circFNDC3B was 0.891. Patients with PTC with low expression of circFNDC3B exhibited longer overall survival than those with high expression of circFNDC3B according to Kaplan-Meier survival curve analysis (68). Guo *et al* (22) discovered 8 circRNAs expressed abnormally in PTC tissues, 5 of which were associated with BRAFV600E, capsular invasion, advanced pT stage and LNM. These circRNAs, whose expression levels were associated with the overall survival of patients, may be potential prognostic biomarkers. However, to date, compared to the value reported for circRNA as diagnostic biomarkers, the value of prognostic circRNA biomarkers has not been widely demonstrated.

Potential intervention by mesenchymal stem cells. CircRNAs have attracted increasing attention in the field of tumor research and have potential for interventional, expression or regulatory therapy. There are certain problems associated with circRNAs due to their low targeting, potential off-target effects and biodegradation *in vivo*. Therefore, novel strategies to improve their regulatory effect and targeting of circRNAs require to be developed. The application of mesenchymal stem cell (MSC) therapy in the field of cancer has attracted increasing attention due to its unique chemotaxis, which brings hope to improve the accuracy of targeted therapy. In addition, exosomes secreted by MSCs are considered promising by researchers due to their special biological characteristics and superiority over MSCs. MSCs are multipotent stem cells with significant potential for regenerative medicine. The therapeutic potential of MSCs may be attributed to the key mechanism of homing, i.e. they are able to migrate to the injured site and differentiate into local components there (115-117).

There are numerous high-quality reviews (118,119) in the literature addressing the basic concept that MSCs have a good targeting transport effect and may thus be used as carriers to target the tumor growth site to exert effective clinical functions. The characteristics of MSCs suggest that they are able to transport therapeutic anticancer genes, making them a unique and promising choice for cancer treatment; part of their recently recognized functions have a wide variety of potential applications. The most important factors of tumorigenesis are epigenetic changes and genetic mutations in proto-oncogenes and tumor suppressor genes (117). Due to the unique characteristics of MSCs, TC may be treated by loading tumor suppressor genes or circRNAs into MSCs. The use of genetically engineered MSCs for gene-directed enzyme/prodrug therapy is a promising therapeutic approach. Kalimuthu *et al* (120) attempted to develop therapeutic MSCs containing an inducible suicide gene and confirmed its therapeutic

efficiency for ATC therapy. However, research on the use of MSCs as carriers loaded with circRNAs to treat TC is still in its infancy. In the future, more in-depth research in this area may be performed to provide novel approaches for tumor treatment.

Potential intervention by MSC-derived exosomes. Exosomes are small, lipid-membrane extracellular vesicles (EVs) that are formed by endocytosis, integration and efflux; they have a diameter of 30–150 nm, are stable in a variety of biological fluids, such as urine, plasma and serum (121), and may be used as drug or gene carriers. Therefore, to a certain extent, exosomes derived from MSCs may be a good and promising cargo-loading carrier for the treatment of tumors. Various studies have emphasized the function of miRNAs in TC. However, research concerning the influence of exosomal miRNAs on TC remains limited. Tang *et al* (122) aimed to uncover the regulatory effects of exosomal miR152 on TC and the underlying mechanisms. They isolated exosomal miR-152 from bone marrow mesenchymal stem cells (BMSCs) and cocultured it with TC cells to explore its potential for therapy. The results indicated that BMSC-derived exosomal miR-152 inhibited the proliferation, invasion and migration of TC cells and promoted cell apoptosis. The therapeutic potential of MSC-derived exosomes has been proven in non-small cell lung carcinoma (123), ischemic muscle injury (124) and liver fibrosis (125). However, the use of exosome-loaded circRNAs in TC has remained unexplored, to the best of our knowledge, suggesting that future studies may further explore related treatments for TC using similar strategies.

Taken together, the application of exosomes for the treatment of tumors is currently a major research hotspot and the utility of exosomes as carriers has also been widely and deeply discussed in the field of cancer. At present, diverse technologies may be used to isolate and analyze EVs, such as density gradient zone centrifugation (119), immunocapture by magnetic beads (126), exosome precipitation or chromatography (127). However, emptying native contents and loading the desired cargos may represent limitations in EV applications. Whether the biological efficacy of exosomes is only related to the cargo loaded into them or whether the effects of exosomes themselves on the delivery of goods have a role, as well as their molecular mechanisms, requires to be further studied. Exosomes are vesicles that mediate cellular communication via paracrine signaling (128); thus, whether the genes loaded into exosomes, such as circRNAs and miRNAs, are able to mediate intercellular communication and the underlying mechanisms of their mode of action require to be investigated.

5. Future directions and conclusion

CircRNAs are closed circular noncoding RNAs formed by reverse splicing of precursor RNAs. In recent years, circRNAs have received increasing attention in cancer research. The present study reviewed the role of circRNAs in TC, with an emphasis on their ability to promote and inhibit proliferation, invasion and metastasis of TC cells. CircRNAs affect tumor cells in different ways, such as through regulating the cell cycle, EMT and drug resistance. Of note, the mechanism by which circRNAs regulate glucose metabolism in TC has also been indicated to have an important role. At present, the clinical application of circRNAs is mainly focused on diagnostic and prognostic biomarkers.

CircRNAs have been widely studied as diagnostic and prognostic biomarkers. For the prognosis of patients with TC, regular detection of the expression level of specific circRNAs may bring new improvements to prognosis. In the present review, the possibility of using exosomes from MSCs and MSCs themselves as carriers to load circRNAs for treatment was described. At present, research on the biomarker function of circRNAs in TC is mainly focused on invasive TC tissue biopsy. Attention should be paid to noninvasive body fluids to improve their practicability as diagnostic and prognostic markers. In addition, the immunomodulatory effects of circRNAs in the TC tumor microenvironment, their effects on angiogenesis of TC cells and radiotherapy resistance have not been previously reported, which may become potential research directions in the future. In addition, the downstream signals of certain differentially expressed circRNAs in thyroid carcinoma have not been fully studied and the regulatory circRNA-miRNA-mRNA network mechanism and its effects on the tumor microenvironment, extracellular matrix and cellular communication require to be further studied. CircRNAs may become a novel research direction and may provide therapeutic targets. To date, studies have focused on the discovery signaling pathways of circRNAs acting as miRNA sponges in PTC. However, studies on other aspects, such as circRNAs affecting gene expression and directly affecting proteins, their role as a transcriptional template and the function of circRNAs in other types of TC are currently scarce. In general, circRNAs have great application prospects in the clinical treatment of tumors and the molecular mechanisms of their effect on tumor cells require further experimental research.

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Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Authors' contributions

GZ and XC contributed to the literature search and wrote the original draft of the manuscript. YK and XZ contributed to the revised version of the manuscript. XT and CM generated all the figures. SF contributed by performing the conceptualization.

All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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