Emerging roles of circular RNAs in non-small cell lung cancer (Review)

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Abstract. Circular RNAs (circRNAs) are a class of novel endogenous transcripts with limited protein-coding abilities. CircRNAs have been demonstrated to function as critical regulators of tumor development and distant metastasis through binding to microRNAs (miRNAs) and interacting with RNA-binding proteins, thereby regulating transcription and translation. Emerging evidence has illustrated that certain circRNAs can serve as biomarkers for diagnosis and prognosis of cancer, and/or serve as potential therapeutic targets. Expression of functional circRNAs is commonly dysregulated in cancer and this is correlated with advanced Tumor-Node-Metastasis stage, lymph node status, distant metastasis, poor differentiation and shorter overall survival of cancer patients. Recently, an increasing number of studies have shown that circRNAs are closely associated with NSCLC. Functional experiments have revealed that circRNAs are intricately associated with the pathological progression of NSCLC. The present review provides an overview of the regulatory effect of circRNAs in the development and progression of NSCLC, taking into consideration various physiological and pathological processes, such as proliferation, apoptosis, invasion and migration, and their potential value as biomarkers and therapeutic targets.

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Abbreviations: NSCLC, non-small cell lung cancer; circRNAs, circular RNAs; miRNA, microRNA; TNM, tumor node metastasis; EMT, epithelial-mesenchymal transition; SCLC, small cell lung cancer; ceRNA, competing endogenous RNA; ecircRNAs, exonic circRNAs; ciRNAs, intronic circRNAs; EIciRNAs, exon-intron circRNA; tricRNAs, tRNA intronic circRNAs; MRE, microRNA response element

Key words: circRNAs, non-small cell lung cancer, carcinogenesis

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1. Introduction

Lung cancer is the most common type of malignant tumor, and is the primary cause of cancer-related death worldwide (1). As the primary cause of mortality in men and women, the number of deaths from lung cancer was 135,720, which accounted for 22.5% of all cancer-related deaths in the United States in 2020 (2). Small cell lung cancer (SCLC) and non-SCLC (NSCLC), the two primary types of lung cancer, account for >85% of all lung cancer cases (3). Despite the progress in clinical management that has been made in the last few years, the 5-year overall survival (OS) rate of NSCLC is 15-21% (1,2). The primary cause of the low 5-year survival rate is that the majority of patients are diagnosed with advanced stage cancer with distant metastases at the first presentation (3). Additionally, cancer recurrence and drug resistance contribute to the high mortality rates of NSCLC (4). The poor prognosis of NSCLC can be attributed to the complicated and unclear molecular mechanisms underlying its development and progression (5,6). Therefore, it is vital to identify novel biomarkers or therapeutic targets to improve the prognosis of NSCLC.

Multiple factors may participate in the development and progression of NSCLC, including proliferation, autophagy,

apoptosis, invasion, metastasis and drug resistance (7,8). One of the most fundamental characteristics of cancer cells is its ability to sustain chronic proliferation. However, proliferation and division of malignant cells is uncontrolled (9). Autophagy serves an important and complicated role in tumor development. Upregulation of autophagy in cancer therapy can promote the survival or death of tumor cells (10). Abnormal regulation of cell death, whether too little or too much, may contribute to several diseases. Aberrant initiation of apoptosis may lead to malignant transformation of NSCLC cells (11,12). Metastasis of NSCLC is a significant obstacle reducing the OS of NSCLC patients, and is considered a core step in the malignant progress of NSCLC (13). Although chemotherapy prolongs the OS of patients with NSCLC, tumor cells may acquire resistance, resulting in poor therapeutic effects, tumor metastasis and recurrence (7). Therefore, it is necessary to identify and determine the relationship between novel biomarkers and malignant behavior in NSCLC.

CircRNAs are a novel group of non-coding RNAs that do not possess 3' and 5'ends, but instead form a closed-loop dissimilar to linear RNAs (14). CircRNAs were first detected in a virus by Sanger in 1976, and were initially deemed as irrelevant byproducts without any significant biological functions for a period of time (15). In the last decade, owing to the rapid advance of RNA-sequencing technologies, researchers have re-evaluated the crucial functions of circRNAs in the regulation of gene expression and in multiple diseases, such as carcinomas (16). Additionally, previous studies have indicated that circRNAs are conserved, stable and abundantly expressed in tissues and exosomes (17,18). According to reverse transcription quantitative (RT-qPCR) and reverse transcription-droplet digital (RT-ddPCR) qualification, 343 differentially-expressed circRNAs were identified between the plasma of patients with gastric cancer and healthy controls (19). CircRNAs are closely associated with tumorigenesis, development, proliferation, apoptosis, invasion and migration of various physiological and pathological processes in tumors (20). CircRNAs are extensively and stably expressed in the plasma and exosomes, indicating that they may serve as promising biomarkers in the prognosis and therapeutics of malignancies (21). The present review summarizes the relationship between circRNAs and the biological behaviors of NSCLC.

2. Classification and functions of circRNAs

Classification of circRNAs. CircRNAs can be divided into four groups, exonic circRNAs (ecircRNAs), intronic circRNAs (ciRNAs), exon-intron circRNA (EIciRNAs) (Fig. 1) and tRNA intronic circRNAs (tricRNAs). EcircRNAs are generated from single or several exons. The majority of circRNAs are ecircRNAs, accounting for >80% of currently identified circRNAs. CircRNAs are primarily localized in the cytoplasm and may act as miRNA sponges, indirectly participating in the regulation of gene expression. CiRNAs are intron-derived circRNAs. CiRNAs are abundantly present in the nucleus and may modulate the expression of their parental genes. EIciRNAs contain both introns and exons that can regulate their parental genes in a *cis* manner. TricRNAs are derived from tRNA introns and can form stable circRNAs via pre-tRNA splicing (15,22).

Functions of circRNAs. With the number of studies on circRNAs increasing, our understanding of the biological functions of circRNAs is ever growing. As shown in Fig. 2, an increasing number of studies have shown that circRNAs exhibit multiple functions, such as functioning as miRNA sponges, interacting with proteins, translation into proteins and regulation of transcription (23).

CircRNAs can sponge miRNAs. Recently, several studies have found that circRNA are primarily located in the cytoplasm. CircRNAs compete with miRNAs to regulate gene expression via miRNA response elements (MREs) (24). CircRNAs can increase the levels of the target genes of miRNAs, and circRNAs with this competitive function are termed competing endogenous (ce)RNAs. As a well-studied function of circRNAs, ceRNAs are widely involved in various circRNA-related diseases, particularly in cancer. For example, the expression of miR-7 is affected by ciRS-7 which possesses >70 selectively conserved binding sites for miR-7. When ciRS-7 efficiently binds to miR-7, the expression of miR-7 is attenuated and the activity of miR-7-target genes is increased. The ciRS-7/miR-7 axis participates in numerous diseases, such as breast cancer (25), cervical cancer (26), gastric carcinoma (27) and hepatocellular carcinoma (28,29). CircRNA zinc finger protein 609 (Circ-ZNF609) improves vascular endothelial dysfunction through upregulating the expression of myocyte enhancer factor 2A by serving as a ceRNA of miR-615-5p (30).

CircRNAs can bind with RNA-binding proteins (RBPs). CircRNAs can also bind to proteins directly. Thus, they can act as protein sponges, similar in principal to their function as miRNA sponges. CircRNAs contain a high density of binding sites for RBPs, and they may affect the activity of related proteins through binding with them directly (31). A circular transcript from forkhead box O3 (Circ-Foxo3) is related with cell cycle progression. Circ-Foxo3 affects cell cycle progression via regulation of a G1/S transition through binding with cyclin-dependent kinase 2 (CDK2) and p21 (32). Circ-Foxo3 facilitates Foxo3 expression via interacting with the MDM proto-oncogene (MDM2) and p53, which leads to MDM2-induced p53 ubiquitination and subsequent degradation (33).

CircRNAs can be translated into proteins. Although most circRNAs serve as miRNA sponges and indirectly regulate the expression of mRNAs, emerging evidence has shown that certain circRNAs are translatable (34). CircRNAs may contain an open-reading frame (ORF), N6-methyladenosine modifications and/or internal ribosome entry site (IRES) elements. Hence, circRNAs can be translated into proteins accordingly (35,36). Moreover, circRNAs can translate into proteins via a rolling circle amplification mechanism in eukaryotic cells (37). Circ-ZNF609 contains an ORF and it can be translated into a protein via a splicing event (38). CircRNA F-box and WD repeat domain containing 7, expression of which is high in the brain, encodes F-box and WD repeat domains containing 7-185aa, and inhibits proliferation and cell cycle progression in cancer cells (39). A circular form of SNF2 histone linker PHD RING helicase, which contains an ORF driven by the IRES, is translated into SNF2 histone linker

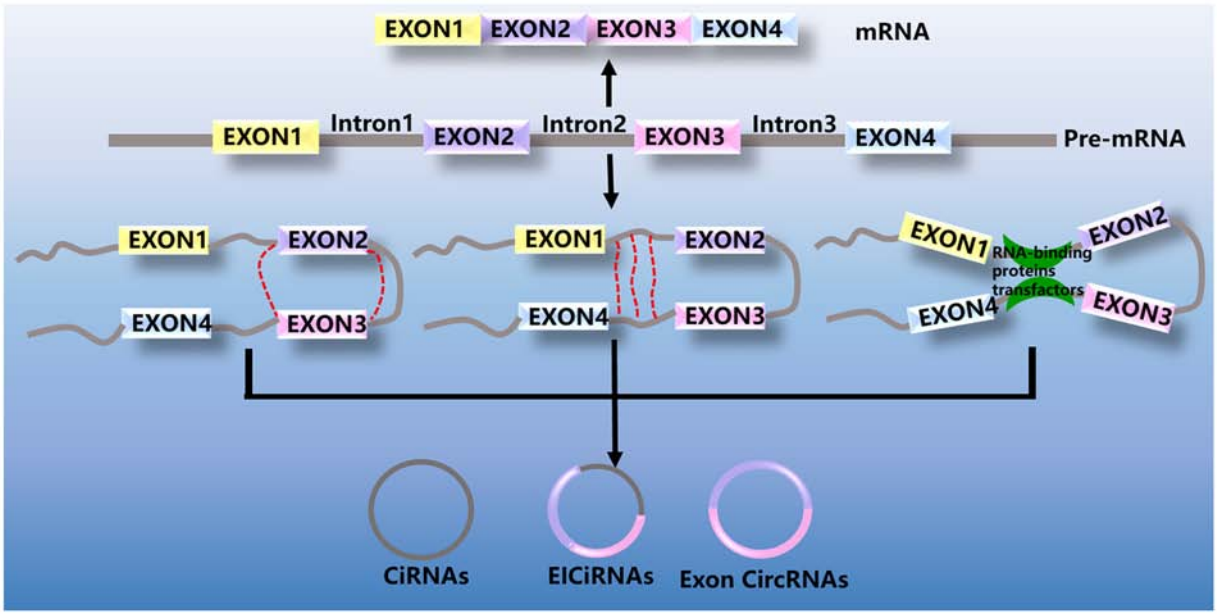

Figure 1. Biogenesis of circRNAs. CircRNA, circular RNA; ciRNAs, intronic circRNAs; EIciRNAs, exon-intron circRNA. CircRNA, circular RNA.

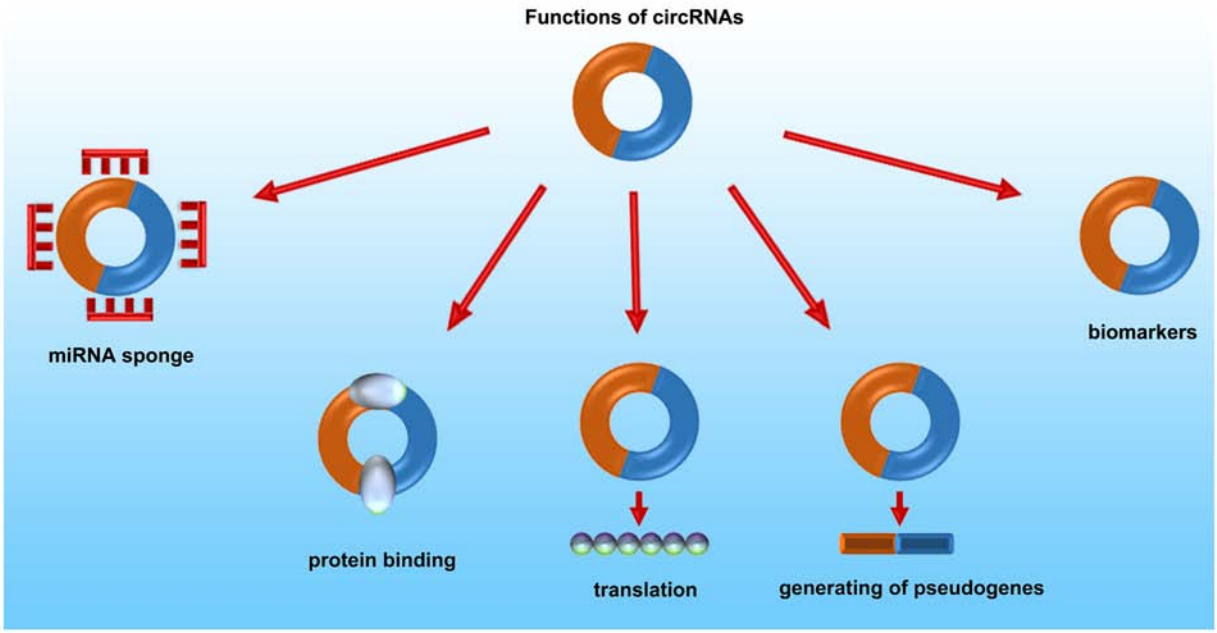

Figure 2. Biological functions of circRNAs. CircRNA, circular RNA; miRNA, microRNA.

PHD RING helicase-146aa, which is a cancer suppressor in human glioblastoma (40).

CircRNAs can regulate transcription. CiRNAs are primarily located in the nucleus, and possess very little MRE activity for sponging miRNAs (41). Knockdown of ciRNAs decreases the expression of their parental genes. CiRNAs can regulate PolII transcription in a cis manner via an RNA-RNA interaction, and can mediate the expression of their parental genes (42,43). EIciRNAs interact with UI small nuclear ribonucleoprotein (snRNP) and PolII to regulate gene expression via RNA-RNA interactions. UI snRNP is indispensable for EIciRNA-mediated regulation of expression of parental genes (44). Ci-ankrd52, which accumulates at transcription sites, is an intron-derived circRNA that is produced from ANKRD52, and Ci-ankrd52 can interact with PolII to regulate transcription of the parent genes (45).

CircRNAs enable derivation of pseudogenes. Pseudogenes are non-functional residues that were formed during the evolution of a gene family, and they serve as essential markers in the field of evolutionary and comparative genomics (46). Pseudogenes may participate in cellular differentiation and cancer progression (47). There is an exon-exon junction in a reversed order in circRNA-derived pseudogenes. In both mice and humans, numerous circRNA-derived pseudogenes have

been detected by a computational pipeline (CIRCpseudo) (48). In all mouse strains and in the rat reference genome, there are dozens of low-confidence circular SATB homeobox 1-derived pseudogenes. In the gorilla and chimp genomes, researchers identified the homologous sequences of human circular protein kinase, DNA-activated, catalytic submit-derived and circular calmodulin regulated spectrin associated protein 1-derived pseudogenes (49). Though reverse transcription may be involved in the non-colinear exon-exon junctions of pseudogenes, the mechanism of reverse transcription and translocation of circRNAs are still not distinct (23).

CircRNAs may serve as promising biomarkers. CircRNAs are stably expressed both intracellularly and in the plasma, due to their unique annular structure (14). RNA-seq analyses indicated that >1,000 circRNAs have been identified in human exosomes and may transfer biological activity to other cells (50). CircRNAs generated from cancer cells can enter into the blood circulation and can be detected easily, and may thus be used to distinguish between cancerous and healthy individuals (14). Exosomal circRNAs are thus potential biological markers of various types of cancer including NSCLC (17).

Roles of circRNAs in cancer. CircRNAs are extensively implicated in the pathological progression of multiple types of cancer, including gastric cancer, hepatocellular carcinoma, lung cancer, colorectal cancer and bladder cancer, amongst others (51). Moreover, these circRNAs exhibit dual roles; serving as oncogenes and tumor suppressors dependent on the type and potentially stage of cancer (52,53). In the following section, the roles of circRNAs in NSCLC are discussed in additional detail.

3. CircRNAs and NSCLC

Expression of circRNAs in NSCLC. With the development of next-generation sequencing technologies and advances in bioinformatics analysis, a large number of studies have shown that circRNAs are ectopically expressed in several types of tumors, including NSCLC. A total of 957 abnormally expressed circRNAs were identified by human circRNA microarray analysis in NSCLC tissues when compared with the adjacent normal tissue (54). In another study, 356 circRNAs were dysregulated in lung adenocarcinoma, including 204 upregulated circRNAs and 152 downregulated circRNAs (55). By utilizing circRNA chips, Mu *et al* (56) identified and annotated a total of 10,566 circRNAs in the peripheral whole blood of patients with lung adenocarcinoma. Amongst these, 78.14% of the circRNAs were exonic, and 3,009 circRNAs were upregulated, whereas 1,381 circRNAs were downregulated.

CircRNAs can be used as diagnostic biomarkers in NSCLC. CircRNAs produced by cancer cells can enter into the blood circulation and can be detected easily (57). Thus, they can be used to distinguish between patients with cancer from healthy individuals. Exosomal circRNAs are potential biological markers in a range of cancer types (14). With the development of circRNA research, a plethora of circRNAs may eventually be used as clinically diagnostic markers for the diagnosis of early-stage NSCLC (Table I).

The expression of hsa_circ_0014130 is associated with Tumor-Node-Metastasis (TNM) stage and lymphatic metastasis of NSCLC. Receiver operating characteristic (ROC) curves were used to determine the diagnostic potential of hsa_circ_0014130. The area under the ROC curve (AUC) was 0.878, the optimum critical value of hsa_circ_0014130 was 0.573, the sensitivity was 87% and specificity was 84.8%. Thus, hsa_circ_0014130 may serve as a biomarker for distinguishing NSCLC from normal tissues (58). CircRNA 100146 was shown to be augmented in 26 cases of NSCLC, and was associated with pathological stage and differentiation of lung cancer. ROC curve analysis indicated that the AUC was 0.643 (95% confidence interval: 0.521-0.764), the sensitivity was 72.5% and the specificity was 57.5%. Thus, circRNA 100146 may also be used as a diagnostic marker in NSCLC (59).

CircRNAs may serve as therapeutic targets in NSCLC. CircRNAs are stably expressed both intracellularly and in the plasma due to their annular structure. RNA-seq analyses indicated that >1,000 circRNAs are present in human exosomes and may transfer biological activity to other cells (50). Numerous circRNAs have been reported to be involved in the tumorigenesis and progression of NSCLC, and are being extensively assessed as potential therapeutic targets for the treatment of NSCLC (Table I).

Circular protein kinase C iota (CircPRKCI) is generated from exons 15 and 16 of the PRKCI gene (chr3:170013698-170015181) and is located at the 3q26.2 amplicon. CircPRKCI acts as a tumor promoting factor in lung adenocarcinoma (LAD), and circPRKCI is positively correlated with T stage and TNM stage in patients with LAD (60). Knockdown of circPRKCI led to a decrease in tumor size and tumor weight in nude mice. Patient-derived tumor xenografts (PDTXs) can be used as a translational model. Intratumoral injection of cholesterol-conjugated si-circPRKCI was used to clarify the therapeutic potential of circPRKCI. The findings showed that the growth of PDTX was decreased in the si-circPRKCI group. These results highlight the therapeutic potential of circPRKCI (60). At present, EGFR tyrosine kinase inhibitors (EGFR-TKIs) are widely used to treat NSCLC patients with EGFR-sensitive mutations. The combination of EGFR-TKIs (gefitinib) and knockdown of circPRKCI resulted in a more notable inhibitory effect than gefitinib or knockdown of circPRKCI alone. This suggests that a combination of EGFR-TKIs and attenuation of circPRKCI may exert a synergistic effect on reducing cancer progression (60).

Circular coiled-coil domain containing 66 (circCCDC66) is primarily located in the endoplasmic reticulum. CircCCDC66 is involved in several types of cancer and serves as a diagnostic and therapeutic biomarker (61). CircCCDC66 is highly expressed in LAD and in EGFR-resistant H1975 cells. EGFR is the primary target of TKIs for tyrosine kinase mutations, such as gefitinib and erlotinib in LAD chemotherapy. Knockdown of focal adhesion kinase (FAK) and hepatocyte growth factor reduces circRNA CCDC66 expression, separately. FAK was associated with metastasis and EMT. Meanwhile, administration of a FAK inhibitor, Y15, reduced metastasis. Conversely, nicotinic acetylcholine receptor α7 (nAchRα7) negatively regulates the expression of CCDC66β and circRNA CCDC66. The regulatory effect of nAchRα7 on circRNA CCDC66 is

Table I. CircRNAs act as prognostic or diagnostic biomarkers of non-small cell lung cancer.

First author, year	CircRNA name	circBase ID	Dysregulation	Correlation with clinical characteristics	Kaplan-Meier OS curves	ROC curve			(Refs.)
						AUC	Sensitivity	Specificity	
Zhang *et al*, 2018	Circ_0014130		Up	TNM stage, lymphatic metastasis	-	0.878	0.87	0.848	(58)
Chen *et al*, 2019	Circ_100146		Up	Pathological classification, differentiation grade	-	0.643	0.725	0.575	(59)
Qiu *et al*, 2018	CircPRKCI		Up	T stage, TNM stage	Poor prognosis/ independent factor (Multivariate analyses: HR=2.664, 95% CI: 1.327-5.347, P=0.006)	-	-	-	(60)
Chen *et al*, 2018	Circ_100395		Down	Cancer metastasis, TNM stage	Poor prognosis	-	-	-	(63)
Han *et al*, 2018	CircBANP		Up	Cancer metastasis, TNM stage	Poor prognosis	-	-	-	(64)
Zhang *et al*, 2018 and Su *et al*, 2018	Circular RNAciRS-7/CDR1as	hsa_circ_0001946	Up	TNM stage, lymphatic Metastasis, pathological classification	Shorter OS				(68,71)
Zhu *et al*, 2017	Circ_0013958	hsa_circ_0013958	Up	TNM stage, lymphatic metastasis	-	0.815	0.755	0.796	(72)
Wang *et al*, 2019	CircVANGL1		Up	T stage, TNM stage	Poor prognosis	-	-	-	(85)
Li *et al*, 2018	CircPVT1		Up	Distant metastasis	-	0.803	0.825	0.675	(86)
Wang *et al*, 2019	CircCRIM1		Down	TNM stage, lymphatic metastasis	Poor prognosis	-	-	-	(94)
SHI *et al*, 2020	CircLARP4		Down	-	Poor prognosis	-	-	-	(95)

CircRNA, circular RNA; TNM, Tumor-Node-Metastasis; ROC, receiver operating characteristic; AUC, area under the curve; OS, overall survival; HR, hazard ratio; CI, confidence interval.

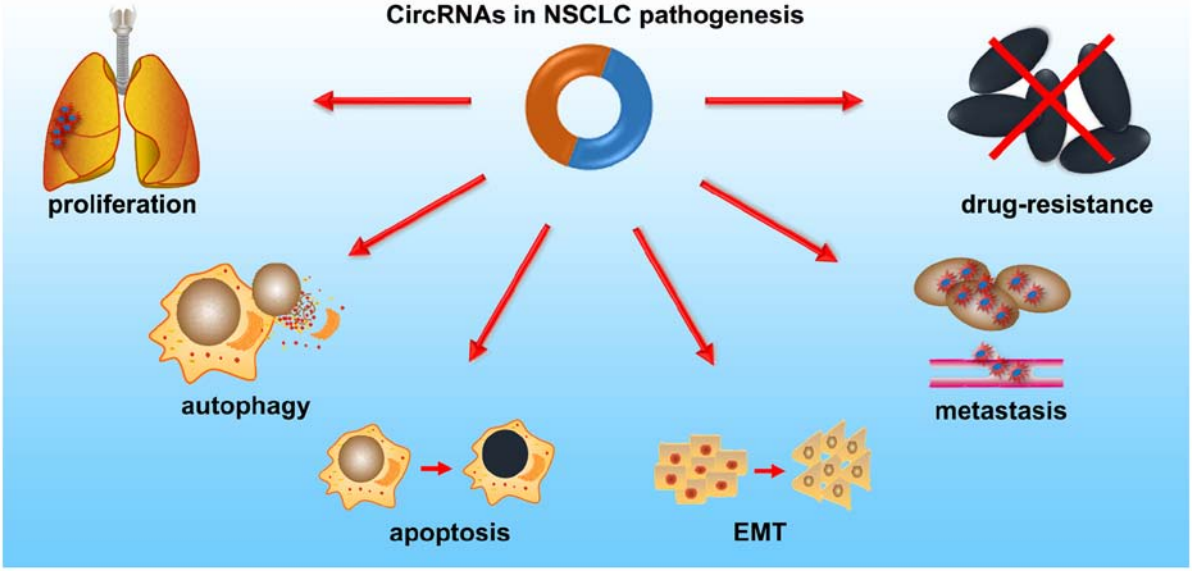

Figure 3. CircRNAs are extensively implicated in the pathogenesis of NSCLC. CircRNA, circular RNA; NSCLC, non-small cell lung cancer; EMT, epithelial-mesenchymal transition.

greater than that of FAK. Furthermore, knockdown of circRNA CCDC66 suppresses EMT and invasion, and augments cisplatin resistance in H23 cells. CircCCDC66 may thus serve as a novel therapeutic target for regulating EGFR-mediated tumorigenesis in NSCLC (62).

Prognostic potential of circRNAs in NSCLC. It is very important to evaluate the prognosis of patients with cancer. Aberrant expression of circRNAs has been reported to show extensive associations with clinical features of patients with NSCLC. Meanwhile, circRNAs are considered to possess valuable prognostic value as biomarkers in NSCLC.

According to a study with 69 cases of NSCLC, which used RT-qPCR qualification, hsa_circ_100395 expression was found to be lower in patients with advanced TNM stage. Additionally, Kaplan-Meier survival curve analysis showed that the survival rate of patients with lower expression of hsa_circ_100395 was lower (63). Circular BTG3 associated nuclear protein (Circ-BANP) was shown to be upregulated in lung cancer tissues and cell lines, and was higher in patients with stage III-IV cancer or in the metastatic tissue. Higher expression of circ-BANP was associated with reduced OS based on Kaplan-Meier curve analysis. Thus, circ-BANP may serve as an independent prognostic biomarker (64).

Based on the above studies, circRNAs may be used for the diagnosis, treatment and evaluation of prognosis of patients with NSCLC. In subsequent studies, increased attention should be paid to the molecular mechanisms by which circRNAs regulate cancer development/progression and in the clinical application of targeting circRNAs.

Functions of circRNAs in NSCLC. NSCLC pathogenesis is modulated by oncogenic or tumor suppressive circRNAs, via regulation of cell proliferation, autophagy, apoptosis, invasion, migration and EMT (Fig. 3). Additionally, circRNAs can act as independent prognostic biomarkers, and serve an important role in multidrug resistance (MDR) in NSCLC. The functions of circRNAs in NSCLC are discussed in the upcoming sections and are summarized in Table II.

CircRNAs in NSCLC proliferation and cell cycle progression. Cell cycle progression is an important factor in maintaining cell proliferation. The phenomenon of normal cells inhibiting division due to contact inhibition, is termed density-dependent inhibition of growth. When cells reach a finite density, they halt proliferation and the cell cycle arrests at the G0 phase of the cell cycle (65). Unrestricted cell proliferation and reduced apoptosis results in unlimited growth and distant metastasis of tumors. The proliferation of cancer cells represents a typical prognostic marker in the diagnosis of cancer (66). Abnormal expression of circRNAs leads to growth of NSCLC cells (67), suggesting that circRNAs serve a potential role in NSCLC treatments targeting unlimited proliferation.

CircRNAs modulate proliferation and cell cycle progression via sponging of miRNAs in NSCLC. CircRNAs serve their biological function in numerous ways. CircRNA-mediated sponging of miRNAs is the most-extensively studied circRNA mechanism.

CiRS-7, also known as cerebellar degeneration-related protein 1 antisense RNA (CDR1as), can absorb miRNAs, such as miR-7 and miR-671, and thus reduces the levels of CDR1 transcripts. CiRS-7 possesses over 70 binding sites with miR-7. Several reports have demonstrated that the CiRS-7/miR-7 axis contributes to several pathological processes, including NSCLC. CiRS-7 may increase cell viability and induce cell growth in NSCLC. CiRS-7 also significantly increases the expression of growth-related genes, including EGFR, cyclin E1 (CCNE1) and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit δ (PIK3CD) (68). Lower expression levels of EGFR maintains cell cycle arrest, facilitating mitosis and preventing cell apoptosis (69). CCNE1 induces rapid progression of cells through the G1/S phase via activation

Table II. Clinical value of circRNAs involved in the pathogenesis and progression of NSCLC.

First author, year	CircRNA name	circBase ID	Dysregulation	Function	Target gene/pathway	Effect in NSCLC	(Refs.)
Chen *et al*, 2019	Circ_100146	-	Up	miRNA sponge	miR-615-5p↓, miR-361-3p↓, SF3B3↑	Proliferation↑, invasion↑, apoptosis↓	(59)
Qiu *et al*, 2018	CircPRKCI	-	Up	miRNA sponge	miR-545↓, miR-589↓, E2F7↑	Proliferation↑, migration↑,	(60)
Joseph *et al*, 2018	CircCCDC66	-	Up	-	-	Invasion↑, EMT↑, drug resistance (cisplatin) ↑	(62)
Chen *et al*, 2018	Circ_100395	-	Down	miRNA sponge	miR-1228↓, TCF21↑	Proliferation↑, migration↑, invasion↑	(63)
Han *et al*, 2018	CircBANP	-	Up	miRNA sponge	miR-503↓, LARP1↑	Proliferation↑, migration↑, invasion↑	(64)
Zhang *et al*, 2018 and Su *et al*, 2018	Circular RNAciRS-7/ CDR1as	hsa_circ_0001946	Up	miRNA sponge	miR-7↓, EGFR↑, CCNE1↑, PIK3CD↑, NF-kB↑	Proliferation↑, migration↑. invasion↑, apoptosis↓	(68,71)
Zhu *et al*, 2017	Circ_0013958	hsa_circ_0013958	Up	miRNA sponge	miR-134↓, CCND1↑	Proliferation↑, invasion↑, apoptosis↓	(72)
Chi *et al*, 2019	CircPIP5K1A	hsa_circ_0014130	Up	miRNA sponge	miR-600↓, HIF-1α↑	Apoptosis↓, proliferation↑	(74)
Nan *et al*, 2018	CircNOL10	hsa_circ_0000977	Down	Protein binding, regulation of transcriptional	SCML1↑, HN polypeptide family	Apoptosis↑, proliferation↓, invasion↓	(75)
Chen *et al*, 2020	CircHIPK3	hsa_circ_0000284	Up	miRNA sponge	miR-124-3p↓, STAT3/PRKAA/AMPKa↑	Proliferation↑, invasion↑, migration↑, autophagy↑	(77)
Wang *et al*, 2019	CircVANGL1	-	Up	miRNA sponge	miR-195↓, Bax↑, Bcl-2↑	Migration↑, invasion↑, apoptosis↓	(85)
Li *et al*, 2018	CircPVT1	-	Up	miRNA sponge	miR-125b↓, E2F2↑	Proliferation↑, migration↑, invasion↑, apoptosis↓	(86)
Gao and Ye, 2020	CircSOX4	-	Up	miRNA sponge	miR-1270↓, PLAGL2↑	Proliferation↑, invasion↑, migration↑	(91)
Wang *et al*, 2018	CircPTK2	-	Down	miRNA sponge	miR-429↓, miR-200b-3p↓, TIF1γ↑	Invasion↓, TGF-β-induced EMT↓	(92)
Wang *et al*, 2019	CircCRIM1	hsa_circ_0002346	Down	miRNA sponge	miR-93↓, miR-182↓, LIFR↑, MMP13↑, PI3K/AKT/ JAK1↑	Invasion↓, metastasis↓	(94)
Shi *et al*, 2020	CircLARP4	-	Down	Protein binding	SMAD7↑	Invasion↓, migration↓	(95)
Wan *et al*, 2016	CircITCH	-	Down	miRNA sponge, regulation of parental genes	miR-7↓, miR-214↓, Wnt/β-catenin↓, ITCH↑	Proliferation↓	(96)

Table II. Continued.

First author, year	CircRNA name	circBase ID	Dysregulation	Function	Target gene/pathway	Effect in NSCLC	(Refs.)
Huang *et al*, 2019	Circ_0001946	-	Down	miRNA sponge	miR-7-5p↓, miR-671-5p↓, miR-3156-5p↓, miR-1270↓, NER↑	Proliferation↓, invasion↓, migration↓, drug resistance (cisplatin) ↓, apoptosis↑	(100)
Li *et al*, 2019	Circ_0002483	-	Down	miRNA sponge	miR-182-5p↓, miR-520q-3p↓, miR-582-3p↓, miR-587↓, GRB2↑, FOXO1↑, FOXO3↑	Proliferation↓, invasion↓, drug resistance (Taxol)↓	(101)
Xiao *et al*, 2020	Circ_103762	-	Up	Protein binding	CHOP↓	Proliferation↑, migration↑, invasion↑, drug resistance (MDR) ↑	(102)

CircRNA, circular RNA; miR/miRNA, microRNA; MDR, multi-drug resistance; NSCLC, non-small cell lung cancer; EMT, epithelial-mesenchymal transition.

of CDK2 (70). The effect of ciRS-7 on monitoring cell cycle progression is reversed by overexpression of miR-7. CiRS-7 also regulates cell proliferation, invasion, migration and apoptosis via targeting miR-7 to modulate nuclear factor-κB (NF-kB) (71).

Cyclin D1 (CCND1) is an important target of abnormally expressed circRNAs in NSCLC, such as circ_0013958 (72). Abnormal expression of circRNAs can regulate the cell cycle process and proliferation in NSCLC. The primary function of CCND1 is to promote cell proliferation. CCND1 can bind to and activate cyclin-dependent kinase CDK4, which is unique to the G1 phase (73). Circ_0013958 was identified as a sponge of miR-134, and circ_0013958 promotes the development of NSCLC via upregulation of oncogenic CCND1 (72).

A high-throughput microarray assay revealed that circular phosphatidylinositol-4-phophate 5-kinase type 1 alpha (circPIP5K1A) was significantly upregulated in NSCLC (58). CircPIP5K1A regulates the progression of NSCLC via activation of several signaling pathways. For example, circPIP5K1A promotes proliferation via a miR-600/hypoxia inducible factor-1α axis in NSCLC (74).

CircRNAs modulate proliferation and cell cycle progression via binding with RBPs and regulating transcription in NSCLC. Circular nucleolar protein 10 (circNOL10) is primarily expressed in the nucleus, and is generated from exons 6-12 of pre-NOL10 mRNA. The expression of circNOL10 is cooperatively regulated by pre-NOL10 methylation and by epithelial splicing regulatory protein 1, a splicing factor. CircNOL10 expression is low in lung cancer. CircNOL10 directly promotes the expression of sex comb on midleg-like 1 (SCML1) by suppression of ubiquitination, and also promotes the transcriptional regulatory effect of SCML1 on the humanin polypeptide family, ultimately inhibiting the progression of lung cancer (75).

CircRNAs participate in RNA splicing in NSCLC. The notable associations between the expression of circ-UBR5 and differentiation degree of NSCLC has been established. The differentiation of NSCLC is decreased following knockdown of circ-UBR5. Circ-UBR5 may thus be used to evaluate tumor differentiation, and as an indicator for the pathological grading of NSCLC. Circ-UBR5 binds to splicing regulatory factors, including KH domain containing RNA binding (QKI), NOVA alternative splicing regulator 1 and U1 snRNA. Circ-UBR5 additionally participates in differentiation via modulation of RNA splicing (76).

CircRNAs and NSCLC autophagy. Autophagy is a process of transporting damaged, denatured or aging proteins and organelles to lysosomes for digestion and degradation in cells. Autophagy serves an important and complicated role in tumor development. Upregulation of autophagy in cancer therapy can promote the survival or death of tumor cells (10). Abnormal activity of the mTOR signaling pathway, pathophysiological p53 expression and endoplasmic reticulum stress serve key roles in autophagy of NSCLC (12). Further studies have shown that autophagy is one of the most important pathogenic events in NSCLC development, leading to drug resistance, metastasis and poor prognosis (10).

CircRNAs modulate autophagy via sponging miRNAs in NSCLC. Circular homeodomain-interacting protein kinase 3 (circHIPK3) is derived from exon 2 of the HIPK3 gene, and circHIPK3 is primarily localized in the cytoplasm. Through an RFP-GFP-LC3B assay, Chen *et al* (77) reported that knockdown of circHIPK3 elevated autophagic flux in autophagy-induced cell lines (A549 and H838). CircHIPK3 may sponge miR-124-3p, a well-known tumor suppressor and autophagy regulator, and therefore indirectly regulate IL-6 receptor and STAT3. As a downstream factor of IL6R, STAT3 suppresses autophagy (78). Downregulation of circHIPK3 induces autophagy by modulation of miR-124-3p/STAT3/PRKAA/AMPKα signaling in NSCLC (77).

CircRNAs and NSCLC apoptosis. The unique morphology of cell death was first termed apoptosis by Kerr in 1972. Apoptosis is the process of programmed cell death which serves a crucial role in cell biology and life. The regulation of apoptosis must be strictly controlled (79). The imbalance in the expression ratio of pro-apoptotic proteins and anti-apoptotic proteins, such as the Bcl-2 protein family, p53 or inhibitor of apoptosis proteins are crucial for regulating cell death (80). The Caspases are generally divided into two groups. Caspase-1, 4, 5, 13 and 14, which primarily participate in the inflammatory process, and Caspase-2, 3, 6, 7, 8, 9 and 10, that either participate in initiation or execution of cell death (81). An increasing number of studies have illustrated that circRNAs regulate apoptosis in NSCLC, such as circ_0003645 (82) and circ_0074027 (83).

CircRNAs modulate apoptosis by sponging miRNAs in NSCLC. CircRNAs can affect the expression of apoptosis-related proteins by sponging miRNAs. The activation, expression and regulation of a series of proteins including the Caspase family of proteins, Bax and Bcl-2 family of proteins are involved in apoptosis. Circular VANGL planar cell polarity protein 1 (CircVANGL1) is generated from exons 3-4 of the VANGL1 gene. CircVANGL1 was reported as an oncogene in bladder cancer (84). Additionally, circVANGL1 was shown to reduce cell apoptotic rates in NSCLC. Silencing of circVANGL1 increased Bax expression and decreased Bcl-2 expression, and this effect was achieved by sponging of miR-195 in NSCLC (85).

Circular PVT1 (circPVT1) is generated from exon 3 of its host gene PVT1, and is flanked by two long introns (35,269 and 41,466 bp) on each side. In a total of 68 cases of NSCLC, the expression of circPVT1 was >2x higher than that in normal or paired paratumoral tissues (41/68 cases). Additionally, circPVT1 expression was significantly increased in 7 NSCLC cell lines compared with a human bronchial epithelial cell line (HBE cells). A luciferase assay showed that luciferase activity was promoted by c-Fos interacting with the circPVT1 promoter region. This results in upregulation of circPVT1 in NSCLC. C-Fos-induced circPVT1 modulates cell proliferation, invasion and migration, and induces cell apoptosis in NSCLC. CircPVT1 regulates carcinogenesis by downregulating miR-125b and upregulating E2F transcription factor 2 (E2F2) (86).

CircRNAs and NSCLC EMT, invasion and metastasis. Tumor metastasis refers to the process in which malignant tumor cells infiltrate into the surrounding tissues from their origin. The progression of tumor cell metastasis is divided into three stages: Adhesion, degradation and migration. Malignant tumor cells break through the basement membrane, move from its primary site (primary tumor) into lymphatic vessels, blood vessels or body cavities to 'target' tissues or organs, and form a distant secondary tumor with the same/similar histological type to that of the primary tumor (87). The EMT program is considered a key step and is closely involved in pathological states of tumor progression (88). EMT is considered as the driving factor of invasion and metastasis (89). Metastasis of NSCLC cells is a significant obstacle reducing the OS of NSCLC patients, and is considered a core step in the malignant progression of NSCLC (87). Thus, it is crucial that we improve our understanding of the mechanisms underlying metastasis. Moreover, it is widely accepted that circRNAs are related to the invasion and metastasis of NSCLC.

CircRNAs modulate EMT, invasion and metastasis through sponging miRNAs in NSCLC. Circular SRY-box transcription factor 4 (circ-SOX4) possesses a covalently closed cyclic structure, and has been shown to be upregulated in NSCLC. Reduced expression of Circ-SOX4 decreases the number of invasive and metastatic cells, and decreases the expression of the EMT related proteins, including N-cadherin, Vimentin, ZEB1, Slug, Twist, Snail, matrix metalloproteinase (MMP)2, MMP7 and MMP9. Overexpression of circ-SOX4 increases β-catenin expression in the cell nucleus and reduces its expression in the cell cytoplasm (increases translocation). Thus, circ-SOX4 results in activation of the Wnt pathway. c-MYC is upregulated upon Wnt pathway stimulation. Furthermore, c-MYC harbors two binding sites with circ-SOX4, and can increase its expression, highlighting the presence of a positive feedback loop between circ-SOX4 and c-MYC. Invasion, metastasis and EMT of NSCLC is promoted by circ-SOX4 via increased activity of the Wnt/β-catenin pathway, through increasing the expression of c-MYC (90). Gao and Ye (91) showed that circ-SOX4 was upregulated in LUAD Western blotting showed that expression of Wnt pathway related proteins and EMT representative proteins was increased when circ-SOX4 was overexpressed. These effects were altered via upregulated expression of miR-1270 and decreased expression of PLAG1 like zinc finger 2.

Three circRNAs, hsa_circ_0005273, hsa_circ_0008305, and hsa_circ_0003221, which are spliced from different exons of the pre-mRNA of PTK2, are all termed circular protein tyrosine kinase 2 (CircPTK2). In NSCLC, hsa_circ_0008305 expression is low in patients with distant metastasis. Hsa_circ_0008305 interacts with miR-429 and miR-200b-3p in NSCLC. The expression of tripartite motif containing 33 (TIF1γ) is reduced following overexpression of miR-429 and miR-200b-3p, as they can bind to the 3'-untralsted region (UTR) of TIF1γ. TIF1γ may mediate EMT and the TGF-β/Smad pathway by ubiquitinating Smad4. CircPTK2 participates in TGF-β-induced EMT and invasion. Mechanically, circPTK2 represses miR-429/miR-200b-3p expression and increases TIF1γ expression (92).

The process of tumor cell metastasis requires destruction of any physical barriers, such as the basement membrane and the extracellular matrix (ECM). MMPs are important proteases that degrade the ECM, and they serve an important role

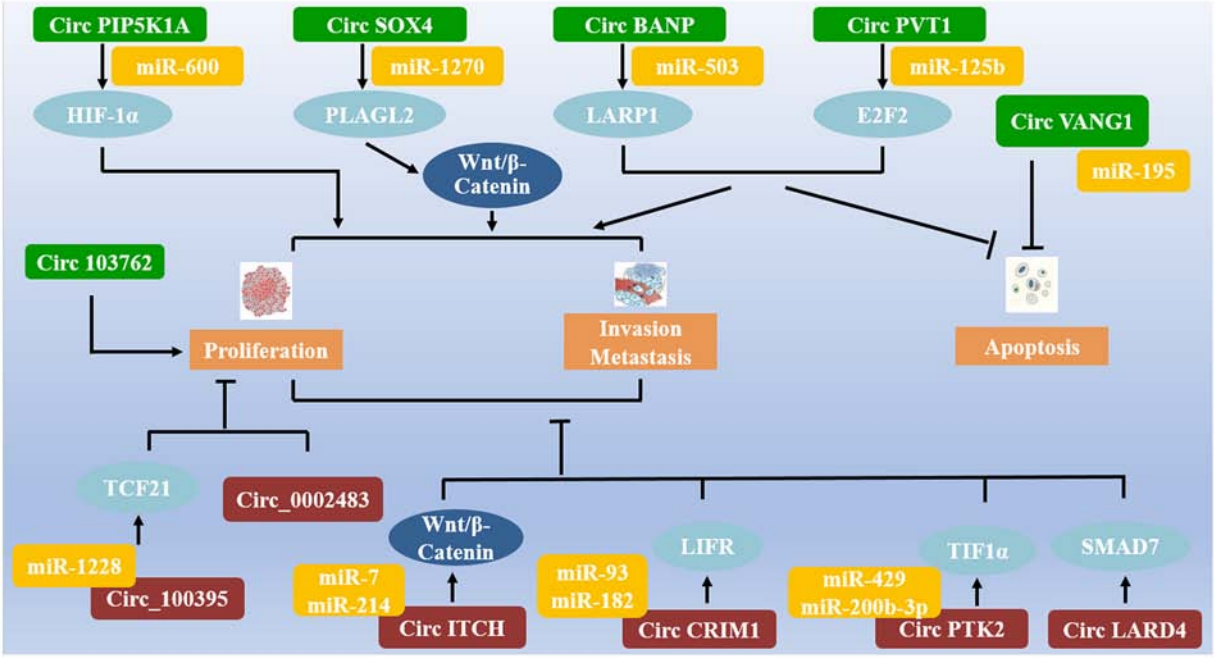

Figure 4. Several circRNAs and their downstream targets in the regulation of the pathological progression of NSCLC. CircRNA, circular RNA; NSCLC, non-small cell lung cancer; miR, microRNA.

in tumor invasion and metastasis (93). Circular cysteine rich transmembrane BMP regulator 1 (circCRIM1), also known as hsa_circ_0002346, is an exon-related circRNA. LUAC patients with TNM stage II and III and lymph node metastasis exhibited lower circCRIM1 expression levels than patients with TNM stage I LUAD. Functional experiments revealed that circCRIM1 represses invasion and metastasis in LADC. CircCRIM1 serves as a miR-182/93 sponge. Resulting in upregulation of leukemia inhibitory factor receptor and increases MMP13 expression via activation of the PI3K/AKT/JAK1 signaling pathway (94).

CircRNAs modulate EMT, invasion and metastasis through binding with RBPs in NSCLC. Circular La ribonucleoprotein 4 (circLARP4), derived from the LARP4 gene, acts as a La-related RBP. In NSCLC, downregulated expression of circLARP4 is associated with a worse prognosis. Overexpression of circLARP4 suppresses the metastatic ability of SPCA1 cells. Additionally, the protein levels of the SMAD family member 7 (SMAD7) is upregulated following circLARP4 overexpression. Thus, circLARP4 negatively regulates invasion and metastasis of NSCLC by upregulating of SMAD7 (95).

CircRNAs modulate EMT, invasion and metastasis through sponging miRNAs and regulating expression of their parental genes. Dysregulation of the Wnt/β-catenin signaling pathway modulates EMT progression in a range of cancer types. CircRNAs modulate the Wnt/β-catenin pathway through different mechanisms. Circ-ITCH is located on chromosome 20q11.22. Circ-ITCH expression is significantly decreased in lung cancer tissues, and its expression is positively correlated with its parental gene, ITCH. Circ-ITCH inhibits the activity of the Wnt/β-catenin pathway. Western blot analysis showed that overexpression of circ-ITCH suppressed the protein expression levels of β-catenin. Subsequently, mRNA expression of c-Myc and CCND1, the two downstream binding partners of β-catenin, was reduced following circ-ITCH upregulation/overexpression. Thus, circ-ITCH interacts with miR-7 and miR-214, and participates in the progression of lung cancer (96).

CircRNAs and NSCLC drug resistance. Chemotherapy is a common method for treating cancer, including NSCLC. It has been found that cells that are resistant to certain chemotherapeutic drugs may also possess resistance to other structurally unrelated drugs via different mechanisms. This phenomenon of broad drug resistance is termed MDR (97). Whilst certain factors in tumor cells underlying the development of resistance to chemoradiation and targeted therapy have been characterized, the process and the underlying molecular mechanisms are still not completely understood. Recent studies have described the roles of circRNAs in drug resistant NSCLC (98,99).

CircRNAs modulate drug resistance through sponging miRNAs in NSCLC. One circRNA can interact with multiple miRNAs to moderate the nucleotide excision repair (NER) signaling pathway. Hsa_circ_0001946 is an exon-derived circRNA that is produced from CDR1 with a length of 1,485 nt. Hsa_circ_0001946 is located in chrX: 139865339-139866824. FISH analysis indicated that hsa_circ_0001946 is primarily present in the cytoplasm. Hsa_circ_0001946 functions as a tumor suppressor in NSCLC. Upregulation of hsa_circ_0001946 enhances the cisplatin sensitivity of A549 cells. Moreover, silencing of hsa_circ_0001946 activates the NER signaling pathway, which decreased cisplatin sensitivity of lung cancer. Hsa_circ_0001946 is implicated in regulation of the sensitivity of NSCLC cells to cisplatin via modulation of the NER signaling pathway. Hsa_circ_0001946 sponges four

miRNAs (hsa-miR-7-5p, hsa-miR-671-5p hsa-miR-1270 and hsa-miR-3156-5p) to moderate NER signaling (100).

Circ_0002483 is located at chr8:141862969-141921766. Circ_0002483 is significantly downregulated in NSCLC tissue samples and in Taxol-resistant NSCLC cell lines. Lower levels of circ_0002483 is correlated with a poorer prognosis in patients with NSCLC. A Cell Counting Kit-8 assay showed that overexpression of circ_0002483 notably increased the sensitivity of NSCLC cells to Taxol. Using dual-luciferase reporter assays and an RNA immunoprecipitation assay, circ_0002483 was confirmed to competitively bind to miR-182-5p. Knockdown of miR-182-5p increases sensitivity to Taxol in A549 and H1299 cells. A luciferase assay indicated that miR-182-5p could bind to the 3'UTR of growth factor receptor bound protein 2 (GRB2), forkhead box O1 (FOXO1) and forkhead box O3 (FOXO3). Co-transfection of miR-182-5p and circ_0002483 restored GRB2, FOXO1 and FOXO3 expression and induced resistance to Taxol in NSCLC cells. These findings suggest that circ_0002483, serves as a miR-182-5p sponge, promotes GRB2, FOXO and FOXO3 expression and enhances the sensitivity of A549 and H1299 cells to the chemotherapeutic drug Taxol (101).

CircRNAs modulate drug resistance through binding with RBPs in NSCLC. CircRNA_103762 is significantly highly expressed in NSCLC tissues and cell lines, and its upregulated expression is closely correlated with shorter survival rates in patients with NSCLC. CircRNA_103762 is also upregulated in cisplatin-resistant H358/CDDP lung cancer cells. CircRNA_103762 represses the expression of DNA damage inducible transcript 3 and facilitates MDR in NSCLC (102).

CircRNAs modulate drug resistance through regulation of translation of their parental genes. Hsa_circ_0004350 and hsa_circ_0092857 are transcribed from eukaryotic translation initiation factor 3 subunit A (EIF3a). Hsa_circ_0004350 is located on chromosome 10:120.832.401-120.833.449 and hsa_circ_0092857 on chromosome 10:120.809.312-120.810.833, including three exons and two introns, and are differentially expressed in A549 and A549/DDP cells. Hsa_circ_0004350 and hsa_circ_0092857 are prominently associated with translation regulation based on analysis of data obtained from Metascape. Gene Ontology analysis showed that the overlapping RBPs of the two circEIF3as were regulators of translation, and they may exhibit functional synergy with their parental gene, EIF3a. Abnormal expression of hsa_circ_0004350 and hsa_circ_0092857 may impact the cisplatin resistance of lung cancer cells (103).

4. Conclusion

This review summarizes the findings of recent studies on circRNAs that may function as carcinogenic or tumor suppressor genes in NSCLC (Fig. 4). Several circRNAs participate in regulating the pathological progression of NSCLC. Compared with coding RNAs, miRNAs and lncRNAs, circRNA research is in its initial stages, and several problems still need to be addressed. To date several functions of circRNAs and their participation in the regulation of the progression of cancer have been identified, although considerably more remain undetermined. For NSCLC, in order to improve the prognosis and OS of patients, novel targeted therapeutic approaches are required. Further development of targeted circRNAs may become potential pivotal elements to improve our understanding of NSCLC. These mentioned circRNAs may serve as biomarkers of diagnosis and prediction in NSCLC. They may also serve as a means of non-invasive treatments. In future studies, additional attention should be paid to the role of circRNAs in the clinical diagnosis and treatment of NSCLC.

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Authors' contributions

SL, YL, GQ, YL, XL, FM, NL and TX wrote the original manuscript. YW, BQ and SX reviewed and edited the manuscript. All authors read and approved the final manuscript.

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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