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REVIEW – CANCER RESEARCH

Circular RNAs: pivotal molecular regulators and novel diagnostic and prognostic biomarkers in non-small cell lung cancer

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Abstract

Purpose Circular RNAs (circRNAs), a large class of non-coding RNAs with covalently closed-loop structures, are abundant, stable, conserved, and have tissue and developmental-stage specificities. The biological functions of circRNAs are varied. Moreover, circRNAs participate in various pathological processes, especially in multiple cancers. Lung cancer is the most frequent malignant tumor worldwide. Many studies have suggested that circRNAs are pivotal in non-small cell lung cancer. This article aims to provide a retrospective review of the latest research on the functions of circRNAs in non-small cell lung cancer. In particular, we focus our discussion on the role of circRNAs in cell-cycle regulation and the epithelial–mesenchymal transition, and also discuss the known regulatory molecular mechanisms of circRNAs in non-small cell lung cancer.

Methods We reviewed the literature on circRNAs and non-small cell lung cancer from PubMed databases. Specifically, we focused on the roles and mechanisms of circRNAs in regulating the cell cycle and the epithelial–mesenchymal transition.

Results Dysregulation of circRNAs is closely correlated with proliferation, migration, and invasion of non-small cell lung cancer, especially in terms of modulating cell-cycle regulation and the epithelial–mesenchymal transition.

Conclusion Taken together, circRNAs have potential as biomarkers for the diagnosis, prognosis, and treatment of non-small cell lung cancer.

Keywords Circular RNAs · Non-small cell lung cancer · Biomarker · Cell cycle · Epithelial–mesenchymal transition

Introduction

Lung cancer, the most frequent malignant tumor in both men and women, is the leading cause of cancer-related deaths worldwide (Torre et al. 2015; Chen et al. 2016; Siegel et al. 2018). According to its pathology, lung cancer can be divided into small cell lung cancer and non-small cell lung cancer (NSCLC), the latter of which accounts for 80% of all cases of lung cancer (Torre et al. 2015). At present, the standard therapy for NSCLC includes surgical resection, platinum-based dual chemotherapy, and target therapy. Despite improvement in diagnostic and therapeutic strategies over the past few decades, the diagnostic rate of stage-I lung cancer is only approximately 15% and the 5-year survival rate of advanced lung cancer is less than 20% (Wood et al. 2012; Collins et al. 2007; Wu et al. 2012). Therefore, it is urgent to identify more effective markers for early diagnosis and prognosis predication and to seek novel therapeutic targets.

Circular RNAs (circRNAs) were first discovered in RNA viruses by electron microscopy in 1976 (Sanger et al. 1976).

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Three years later, circRNAs were also confirmed to exist in the cytoplasm of eukaryotic cells, and were considered to be endogenous RNA-splicing products (Hsu and Coca-Prados 1979). This large class of long non-coding RNAs (lncRNAs) differs structurally from other lncRNAs in that circRNAs have a covalent junction linking the 3′ and 5 ends. However, for several decades of research following the discovery of circRNAs, the linkages of the ends to form circRNAs were mistaken for splicing errors (Cocquerelle et al. 1993). With the fast development of high-throughput sequencing and bioinformatic analysis in recent years, numerous circRNAs have been discovered in multiple human cell lines.

Recently, researchers have found that circRNAs can function as endogenous regulators in various diseases, such as in myocardial fibrosis and neuropsychiatric disorders. Surprising evidence has suggested that circRNAs may represent novel diagnostic, prognostic, and therapeutic markers of multiple cancers, such as gastric cancer, colorectal cancer, and hepatocellular carcinoma. However, the biological function of circRNAs in lung cancer remains less understood. Recently, a high-throughput microarray determined the expression profiles of circRNAs in lung adenocarcinoma (Zhao et al. 2017). There were 356 circRNAs that were significantly dysregulated; among them, 204 circRNAs were upregulated, while 152 circRNAs were down-regulated. This discovery indicates that circRNAs may play a pivotal role in the development and progression of NSCLC. The present study reviews the latest research on the roles of circRNAs in NSCLC. We review the biological functions of circRNAs in NSCLC and the potentiality of circRNAs as NSLC biomarkers. In addition, we focus on the roles of circRNAs in cell-cycle regulation and the epithelial–mesenchymal transition (EMT).

Classification and biogenesis of circRNAs

Based on their compositions, circRNAs are mainly divided into three categories, namely, exonic circRNAs (ecircRNAs), intronic circRNAs (ciRNAs), and exon–intron circRNAs (EIciRNAs). The majority of circRNAs are ecircRNAs, which are derived from exons and are predominantly localized in the cytoplasm (Zhang et al. 2014). In contrast, ciRNAs are derived from introns and are predominantly localized in the nucleus (Zhang et al. 2013). EIciRNAs, which are derived from exons and introns, are predominantly localized in the nucleus (Li et al. 2015b).

Zhang et al. found that flanking intronic complementary sequences are necessary for exon circularization (Zhang et al. 2014). A representative example is Alu sequences. In addition, Jeck et al. also confirmed that Alu sequences oriented in opposite directions can significantly promote exon circularization (Jeck et al. 2013). Furthermore, RNA-binding proteins (RBPs) can also affect the circularization of circRNAs. Simon et al. observed that quaking (QKI) can bind to upstream and downstream sequences of circRNA-forming exons, forming a bridge to link two introns (Conn et al. 2015). Moreover, both knockdown of QKI and insertion of QKI-binding sites into introns can effectively influence exon circularization. The splicing factor, muscleblind (MBL), also plays a similar role (Ashwal-Fluss et al. 2014). In addition, some other RBPs have been verified to be inhibitors of exon circularization (Ivanov et al. 2015).

Properties of circRNAs

The covalently closed continuous loop of circRNAs differentiates them from linear RNAs. The distinctive structures of circRNAs, which lack 5′ caps and 3′ tails, render them resistant to digestion by RNase R, the primary exoribonuclease of eukaryotic linear RNAs (Suzuki and Tsukahara 2014). Hence, circRNAs are more stable and have longer half-lives than canonical linear isoforms.

Reports on circRNAs have consistently confirmed that they are abundant and conserved. Different categories of circRNAs exhibit different characteristics in terms of their sequences and distributions. The ecircRNAs predominantly localize in the cytoplasm, while intronic and exon–intron circRNAs localize in the nucleus (Jeck et al. 2013; Zhang et al. 2013; Li et al. 2015b).

Recent studies have uncovered that the expression of circRNAs is tissue and developmental-stage specific (Salzman et al. 2013; Li et al. 2018c; Xu et al. 2017b). For instance, has_circRNA 2149 is expressed in $CD19^+$ leukocytes, but is not detected in $CD34^+$ leukocytes, neutrophils, or HEK293 cells (Qu et al. 2015). This specificity in expression indicates that circRNAs may play important roles in the regulation of multiple physiological and pathological processes.

Functions of circRNAs

microRNA (miRNA) sponges

In recent years, the post-transcriptional regulation of gene expression has been identified in which RNAs harbor the same microRNA response elements (MREs) and can sequester microRNAs from other targets (Karreth and Pandolfi 2013; Sumazin et al. 2011) (Fig. 1a). These MRE-harboring RNAs are termed as competing endogenous RNAs (ceRNAs) (Salmena et al. 2011). Many studies have demonstrated that miRNAs have multiple functions in physiological and pathological processes, such as cellular proliferation, differentiation, and apoptosis (Ebert et al. 2007). The novel crosstalk between miRNAs and ceRNAs can form a more

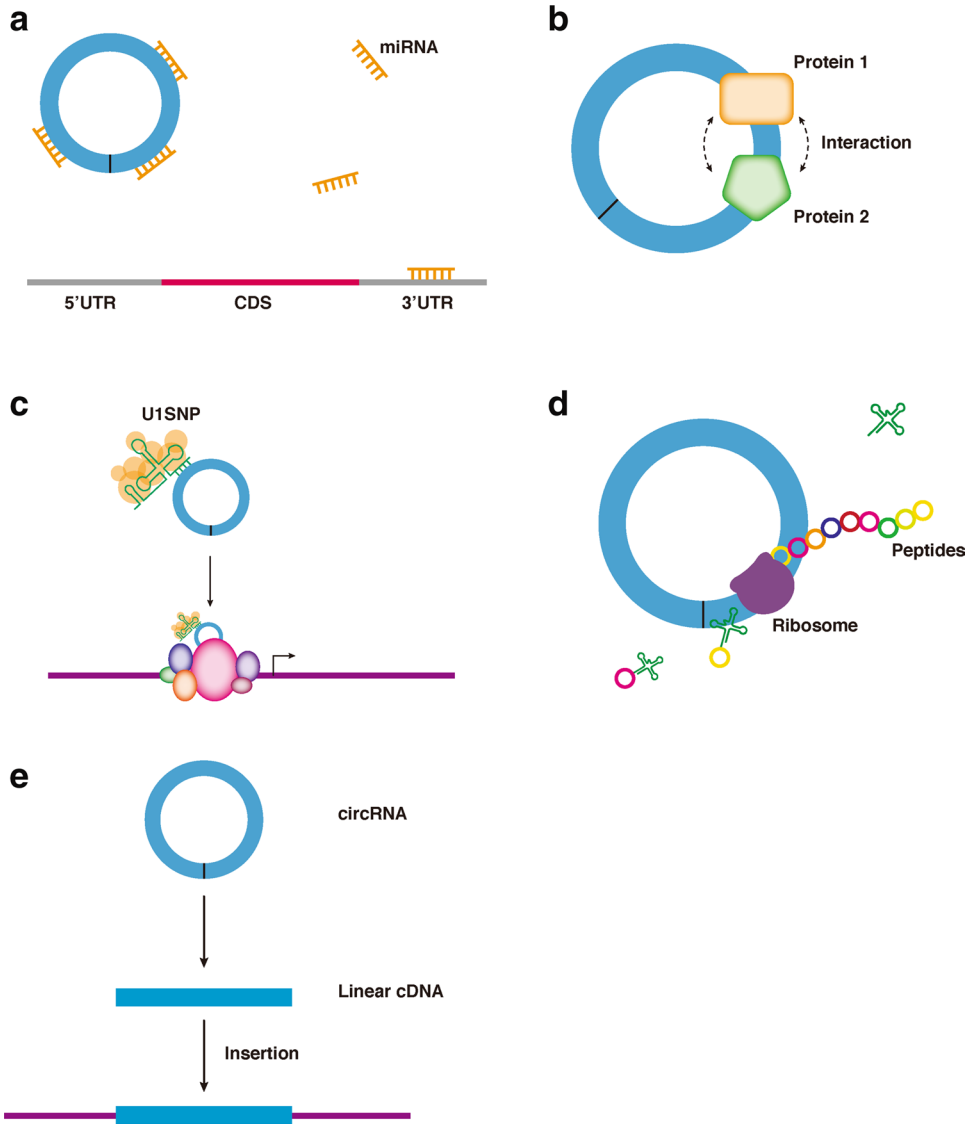

Fig. 1 Functions of circRNAs. **a** circRNAs harboring MREs can function as miRNA sponges. **b** circRNAs can simultaneously bind two different proteins to mediate interactions between proteins. **c** circRNAs can function as positive regulators of parental gene expression. **d** circRNAs can translate into proteins. **e** circRNAs can open their circular structure and insert into the genome to form pseudogenes

effective and systematic framework for post-transcriptional regulation. Recent studies have also shown that circRNAs participate in the regulation of gene expression by acting as ceRNAs (Hansen et al. 2013; Memczak et al. 2013). This function in protecting target genes from repression by miRNAs is referred to as circRNAs acting as miRNA sponges.

As a result of the diverse functions of miRNAs, circRNAs have been demonstrated to be involved in various diseases, especially in multiple types of cancer. For example, circCCDc66 can promote cellular proliferation, invasion, and metastasis of colorectal cancer by sponging miR-33b and miR-93 (Hsiao et al. 2017). In hepatocellular carcinoma, circ_0001445 (also named cSMARCA5, which is derived from the SMARCA5 gene) can function as miR-17-3p and miR-181b-5p sponges (Yu et al. 2018a). In addition, circABCB10 exerts its tumor-promoting effect by sponging miR-1271 (Liang et al. 2017). Another study found that circABCB10 also participates in the regulation of cancer cell invasion and metastasis by sponging miR-1252 rather than miR-1271 (Tian et al. 2019). Thus, one circRNA can sponge multiple miRNAs to form a complex and precise regulatory network.

Interaction with RNA-binding proteins

Several circRNAs possess conserved protein-binding sites and influence the function of corresponding proteins by interacting with them (Fig. 1b). The interaction between circRNAs and proteins may produce different results, including mediating the interaction of different proteins and altering subcellular localization of proteins.

A typical example is circFoxo3, which can interact with cyclin-dependent kinase 2 (Cdk2) and p21 (Du et al. 2016). The formation of a circFoxo3-Cdk2-p21 ternary complex

can inhibit the transition from the G1 phase to the S phase of the cell cycle, leading to G1 arrest. In addition, another study found that circFoxo3 can bind both mouse double minute 2 homolog (MDM2) and p53, facilitating MDM2-induced p53 ubiquitination and further degradation (Du et al. 2017a). In addition to mediating the interaction of proteins, circFoxo3 can also bind to and alter the subcellular localization of proteins (confining them to the cytoplasm), including ID1, E2F1, FAK, and HIF-1α (HIF1A) (Du et al. 2017b). Many other circRNAs, such as circ-Amotl1, can also interact with multiple proteins (Yang et al. 2017a).

Regulation of parental genes

Compared with ecircRNAs, which are predominantly located in the cytoplasm, ciRNAs and EIciRNAs are predominantly located in the nucleus and do not possess MREs; however, they can regulate the expressions of their parental genes at transcriptional and post-transcriptional levels (Fig. 1c). Li et al. discovered that EIciRNAs can promote the cis regulation of the expressions of their parental genes (Li et al. 2015b). EIciRNAs can interact with U1 small nuclear ribonucleoprotein particle (U1 snRNP) via specific RNA–RNA interactions to regulate RNA polymerase-II. A previous study showed that knocking down circEIF3J and circPAIP2 reduced the transcriptions of their parental genes, without affecting neighboring genes (Li et al. 2015b). Similar to EIciRNAs, ciRNAs can also promote the expressions of their parental genes by acting as positive regulators of RNA polymerase-II transcription (Zhang et al. 2013).

In addition, circRNAs can regulate parental gene expression at the post-transcriptional level. HuR, an RNA-binding protein, has been shown to be positively correlated with the expression of the PABPN1 gene. Recent evidence has demonstrated that circPABPH1, which is derived from the PABPN1 gene, interacts with HuR and results in the down-regulation of PABPN1 expression (Abdelmohsen et al. 2017).

Translation into proteins

Non-coding RNAs (ncRNAs) refer to RNAs without coding potential, including short ncRNAs (sncRNAs) and long ncRNAs (lncRNAs). An increasing number of studies have indicated that ncRNAs participate in the regulation of virtually every cellular process (Beermann et al. 2016). Although circRNAs have long been considered to be a subset of lncRNAs, recent evidence has demonstrated that circRNAs may have coding potential (Fig. 1d). The first study that discovered that circRNAs may have the ability to translate into proteins was published in 1986, and implied that the genome of the hepatitis δ virus can generate a 122-amino-acid protein from a circRNA (Kos et al. 1986). Since then, more studies have corroborated this phenomenon. The following features may be important for circRNAs to translate into proteins: (1) the open-reading frames (ORF) of circRNAs may need to be long enough, or greater than a minimum length (Abe et al. 2015; Bazzini et al. 2014); (2) the ORF may need to span splicing junctions (Wang and Wang 2015); and (3) upstream to the translation initiation site, some necessary regulatory elements may need to be present, such as N6-methyladenosine (m6A) modifications (Yang et al. 2017b) and the internal ribosome entry site (IRES) element (Filbin and Kieft 2009; Chen and Sarnow 1995). Circ-ZNF609 possesses a 753-nt ORF and has been shown to have protein-coding ability (Legnini et al. 2017). Skadener et al. found that ribo-circRNAs associate with translating ribosomes, bind to membrane-associated ribosomes, and take advantage of the start codon and termination codons of host mRNAs (Pamudurti et al. 2017). In addition, circ-SHPRH—containing an ORF driven by IRES—can code a functional 17-kDa SHPRH-146-aa protein (Zhang et al. 2018a). All of the above findings provide convincing evidence for the translational ability of circRNAs.

Other functions of circRNAs

In addition to the above functions, circRNAs have other potential functional mechanisms. Recently, researchers developed a computational pipeline (CIRCpseudo) according to the features of circRNAs and discovered circRFWD2-derived pseudogenes via this pipeline (Dong et al. 2016). This discovery suggests that circRNAs can change genomic DNA composition by forming pseudogenes (Fig. 1e).

Circular RNAs in non-small cell lung cancer

Increasing evidence suggests that circRNAs participate in pathophysiological processes in various diseases, especially in cancer. Research on circRNAs in lung cancer has been on the rise. Here, we provide a table of all of the circRNAs that have been shown to play roles in NSCLC (Table 1).

circ-Foxo3

The forkhead box O class (FOXO) is one subclass of the forkhead transcription factor superfamily and is evolutionarily conserved. Foxo3 has been identified to participate in the regulation of cellular proliferation, apoptosis, metabolism, and stress resistance (Van Der Vos and Coffer 2011). Increasing evidence suggests that Foxo3 is associated with tumorigenesis and tumor progression, such as in lung cancer (Myatt and Lam 2007; Cho et al. 2014). The Foxo3 gene can be transcribed into three isoforms, including linear Foxo3 (Foxo3 mRNA), circular Foxo3 (circ-Foxo3),

Table 1 Summary of the expression and functions of circRNAs in non-small cell lung cancer

circRNA	Expression pattern	Biomarker category	Key factors/pathway	Function	References
circ-ITCH	Down-regulation	Therapeutic target	miR-7/miR-214/Wnt/β-catenin pathway	Inhibits cell proliferation	Wan et al. (2016)
circ_001569	Upregulation	Therapeutic target	Wnt/β-catenin pathway	Promotes cell proliferation; its upregulation is associated with tumor differentiation, lymph node metastasis, advanced TNM stage, and shorter overall survival	Ding et al. (2018)
circ_008305 (circPTK2)	Down-regulation	Therapeutic target	miR-429/miR-200b-3p/TIF1γ/TGF-β-induced EMT	Inhibits cell invasion and metastasis	Wang et al. (2018b)
circ-0008717 (circABCB10)	Upregulation	Prognosis/therapeutic target	miR-1252/FOXR2 axis	Promotes cell proliferation, migration, and tumor growth	Tian et al. (2019)
cicc_0016760	Upregulation	Therapeutic target	miR-1287/GAGE1 axis	Promotes cell proliferation, migration invasion and inhibits cell apoptosis; its upregulation is associated with advanced TNM stages, lymph node metastasis, and shorter overall survival	Li et al. (2018d)
circ_Foxo3	Down-regulation	Diagnosis/therapeutic target	miR-155/Foxo3 gene; circ-Foxo3-p21-CDK2 ternary complex	Inhibits cell proliferation, migration, and invasion, and promotes apoptosis; arrests cell in G1/S phase	Zhang et al. (2018d), Du et al. (2016)
circ_0046264	Down-regulation	Therapeutic target	miR-1245/BRCA2	Inhibits cell proliferation, invasion, and tumor growth; promotes apoptosis	Yang et al. (2018)
circ_0001649	Down-regulation	Prognosis/therapeutic target	miR-331-3p/miR-338-5p	Inhibits cell proliferation, migration, invasion, and tumor growth; its down-regulation is associated with advanced TNM stage, positive lymph node metastasis and poor prognosis	Liu et al. (2018a)
circ_0000729	Upregulation	Diagnosis/prognosis	miR-375	Its overexpression is associated with advanced T stage and distant metastasis	Li et al. (2018b)
circ_0007385	Upregulation	Therapeutic target	miR-181	Promotes cell proliferation, migration, invasion, and tumor growth	Jiang et al. (2018)
circ_0020123	Upregulation	Prognosis/therapeutic target	miR-144-ZEB1/EZH2	Promotes cell proliferation, migration, invasion, and tumor growth, metastasis; its upregulation is associated with poor differentiation, lymph node metastasis, advanced TNM stage, and poor prognosis	Qu et al. (2018)
circ_BANP	Upregulation	Prognosis/therapeutic target	miR-503-LARP1	Promotes proliferation, migration, invasion, and inhibit apoptosis; its upregulation is associated with advanced TNM stage, lymph node metastasis, and poor prognosis	Han et al. (2018)
circ_0014130	Upregulation	Diagnosis		Its upregulation is associated with advanced TNM stage and lymph node metastasis	Zhang et al. (2018b)

Table 1 (continued)

circRNA	Expression pattern	Biomarker category	Key factors/pathway	Function	References
circ_0033155	Down-regulation	Diagnosis	PTEN	Inhibits cell proliferation, colony formation, and migration; its down-regulation is associated with lymph node metastasis	Gu et al. (2018)
circ_0067934	Upregulation	Prognosis/therapeutic target		Promotes cell proliferation; its upregulation is associated with lymph node metastasis, advanced TNM stage, and poor overall survival	Zou et al. (2018)
circ_0007534	Upregulation	Prognosis/therapeutic target	EMT pathway	Promotes cell proliferation, migration, invasion, tumor growth and inhibits cell apoptosis; its upregulation is associated with advanced TNM stage, lymph node metastasis, and poor overall survival	Qi et al. (2018)
circ_0003998	Upregulation	Prognosis/therapeutic target	miR-326/Notch1	Its upregulation is associated with larger tumor size, lymph node metastasis, and poor overall survival	Yu et al. (2018b)
circ_0000064	Upregulation	Diagnosis/prognosis		Promotes cell proliferation, migration, invasion, and inhibits apoptosis; regulates cell-cycle progression; its upregulation is associated with lymph node metastasis and TNM stage	Luo et al. (2017)
circ_0013958	Upregulation	Diagnosis	miR-134/cyclin D1	Promotes cell proliferation, migration, invasion, and inhibits cell apoptosis; its upregulation is associated with lymph node metastasis and TNM stage	Zhu et al. (2017)
circ_0043256	Upregulation	Therapeutic target	miR-1252/ITCH/Wnt/β-catenin pathway	Inhibits cell proliferation and promotes apoptosis; is involved in response to Cinnamaldehyde treatment	Tian et al. (2017)
circ_100833(FADS2)	Upregulation	Prognosis/therapeutic target	miR-498	Promotes cell proliferation, migration and invasion; its upregulation is associated with advanced TNM stage, lymph node metastasis, poor differentiation, and poor overall survival	Zhao et al. (2018)
circ_100395	Down-regulation	Diagnosis/prognosis	miR-1228/TCF21 pathway	Inhibits cell proliferation, migration, invasion, and tumor growth; arrests cells in G0/G1 phase; its down-regulation is associated with advanced TNM stage, lymph node metastases, and poor overall survival	Chen et al. (2018)
circ_0075930	Upregulation	Prognosis/therapeutic target	EMT	Promotes G0/G1-phase to S-phase transition; its upregulation is associated with tumors size and lymph node metastasis	Li et al. (2018a)

Table 1 (continued)

circRNA	Expression pattern	Biomarker category	Key factors/pathway	Function	References
circ_FARSA	Upregulation	Diagnosis	miR-330-5p/miR-326 FASN (fatty acid synthase)	Promotes cell migration and invasion	Hang et al. (2018)
circ_103595(MAN2B2)	Upregulation	Therapeutic target	miR-1275/FOXK1	Promotes cell proliferation and invasion	Ma et al. (2018)
circ_102231	Upregulation	Diagnosis/therapeutic target		Promotes cell proliferation and invasion; its upregulation is associated with advanced TNM stage, lymph node metastasis, and poor overall survival	Zong et al. (2018)
circ_103809	Upregulation	Prognosis	miR-4302/ZNF121/MYC	Promotes cell proliferation, invasion and tumor growth; its upregulation is associated with poor overall survival	Liu et al. (2018a, b)
ciRS-7/(CDR1as)	Upregulation	Prognosis	miR-7/EGFR/CCNE1/PIK3CD	Promotes cell proliferation and inhibits cell apoptosis; its upregulation is associated with lymph node metastasis, advanced TNM stage, and shorter overall survival	(Zhang et al. 2018c)
circ_0001346 (RNF13)	Down-regulation	Prognosis/therapeutic target	miR-93-5p	Inhibits cell migration and invasion; its down-regulation is associated with lymph node metastasis and TNM stage	Wang et al. (2018a)
circ_0067934	Upregulation	Prognosis/therapeutic target	EMT	Promotes cell proliferation, migration and invasion; its upregulation is associated with lymph node metastasis, distant metastasis, advanced TNM stage, and poor overall survival	Wang and Li (2018)
circ_100876 (CER)	Upregulation	Prognosis		Its upregulation is associated with lymph node metastasis, TNM stage, and shorter overall survival	Yao et al. (2017)
circ_0067934 (PRKCI)	Upregulation	Prognosis/therapeutic target	miR-545/miR-589-E2F7	Promotes cell proliferation, migration and tumor growth; its upregulation is associated with advanced TNM stage and shorter overall survival	Qiu et al. (2018)
Circ-UBR5	Down-regulation	Prognosis	QKI, NOVA1, U1 snRNA/spliceosome-mediated RNA-splicing regulation	Inhibits cell differentiation	Qin et al. (2018)
circ_0006916	Down-regulation	Therapeutic target	miR-522-3p/PHLPP1	Inhibits cell proliferation; inhibits G1-phase to S-phase transition	Dai et al. (2018)
circ_0012673	Upregulation	Therapeutic target	miR-22/ErbB3	Promotes cell proliferation and tumor growth; its upregulation is associated with tumor size	Wang et al. (2018c)
F-circEA-4a		Diagnosis/therapeutic target		Promotes cell migration and invasion	Tan et al. (2018a)
F-circEA-2a				Promotes cell migration and invasion	Tan et al. (2018b)

and pseudogene Foxo3 (Foxo3P), and all of them can act as tumor suppressers (Yang et al. 2016). In breast cancer, circ-Foxo3 is down-regulated, and overexpression of circ-Foxo3 increases the level of Foxo3 protein (Du et al. 2017a). However, the mechanism of this phenomenon is still unclear. Recently, it has been shown that the level of circ-Foxo3 expression is significantly down-regulated in NSCLC tissues and in several cell lines, and overexpression of circ-Foxo3 inhibits cancer cell proliferation, but induces apoptosis (Zhang et al. 2018d; Du et al. 2016). Zhang et al. also found enhancement of Foxo3 mRNA after overexpression of circ-Foxo3 (Zhang et al. 2018d). This study further revealed that circ-Foxo3 is mainly enriched in the cytoplasm and can function as a sponge of miR-155, which has a target gene of Foxo3. William et al. demonstrated a novel mechanism of the tumor-suppressive function of circ-Foxo3. Their study confirmed that circ-Foxo3 can interact with both cyclin-dependent kinase 2 (CDK2) and p21 to form a ternary complex, leading to the arrest of cell-cycle progression (Du et al. 2016). Hence, circ-Foxo3 plays an anti-oncogenic role by acting as an miRNA sponge and protein scaffold.

ciRS-7

ciRS-7 (also named CDR1as) is one of the earliest discovered and most well-known circRNAs and originates from the back-splicing of the CDR1 gene. Many studies demonstrate that ciRS-7 harbors more than 70 miRNA-binding sites and can specifically function as a sponge of miR-7 (Hansen et al. 2013; Memczak et al. 2013). As such, ciRS-7 participates in post-transcriptional regulation and plays a pivotal role in various diseases, including cancer (Pan et al. 2018; Weng et al. 2017; Xu et al. 2017a). To our surprise, a recent study discovered that ciRS-7 is associated with the development of NSCLC (Zhang et al. 2018c). The expression of ciRS-7 is significantly upregulated in NSCLC tissues and cell lines, and may promote cancer cell proliferation via ciRS-7/miR-7/EGFR/CCNE1/PIK3CD signaling (Zhang et al. 2018c). In addition, the higher expression level of ciRS-7 was further found to be associated with TNM stage and lymph node metastasis of NSCLC. Taken together, ciRS-7 has the prospect of being a prognostic marker of NSCLC.

circ-UBR5

It is well known that circRNAs exert their phenotype by sponging specific miRNAs in NSCLC and that they are mainly located in the cytoplasm (Wang et al. 2018c; Dai et al. 2018). Further in-depth research, however, has identified several circRNAs that play regulatory roles in the nucleus (Fang et al. 2019; Yang et al. 2017a). According to our recent work, there was only one functional circRNA (circ-UBR5) that was enriched in the nucleus and was previously considered as a circRNA without an obvious functional phenotype in NSCLC (Qin et al. 2018). Qin et al. found that the expression of circ-UBR5 is down-regulated in NSCLC and that this deregulation may be associated with tumor differentiation. In addition, a potential mechanism is that circ-UBR5 may participate in spliceosome-mediated RNA-splicing regulation via binding to splicing regulatory factor QKI and NOVA1, as well as U1 snRNA in the nucleus (Qin et al. 2018). All of these findings indicate that circ-UBR5 may serve as a prognostic marker by indicating the degree of NSCLC differentiation.

F-circEA

The genomes of tumor cells are generally unstable and incur various genomic alterations, such as point mutations, chromosomal amplifications, deletions, and translocations (Chin et al. 2011). It has previously been thought that fusion genes exert oncogenic phenotypes by encoding fusion proteins. Accumulated evidence has demonstrated that fusion genes not only encode fusion oncogenic proteins, but also generate specific circular RNAs (Guarnerio et al. 2016). A novel fusion gene has already been identified in NSCLC patients, which comprises portions of the echinoderm microtubule-associated protein-like 4 (EML4) gene and the anaplastic lymphoma kinase (ALK) gene, and has been named the EML4-ALK fusion gene (Soda et al. 2007). Its oncogenic phenotype has been identified, as have the differences between patients with the EML4-ALK fusion gene and patients with mutations of the epidermal growth factor receptor gene. Furthermore, the EML4-ALK fusion gene has been a therapeutic target in this subset of NSCLC patients (Katayama et al. 2011). New research has shown that the EML4-ALK fusion gene also encodes a specific circular RNA, named F-circEA, which is the product of the EML4-ALK variant 3b translocation (Tan et al. 2018a, b). More interestingly, this fusion gene can produce two different circRNAs, namely, F-circEA-2a and F-circEA-4a (Tan et al. 2018b). Both these circRNAs can promote cancer cell migration and invasion. F-circ-2a possesses an "AA" motif at the junction site, whereas F-circEA-4a possesses an "AAAA" motif at the junction site. It is noteworthy that F-circEA-4a is detected in both the cytoplasm and plasma membrane, whereas F-circ-2a is only detected in the cytoplasm in EML4-ALK-positive NSCLCs. These findings suggest that F-circEA-4a may be a novel liquid and non-invasive biopsy marker to monitor the EML4-ALK fusion gene and guide targeted therapy in NSCLC patients.

The mechanisms of circular RNAs in NSCLC

The expression levels of circRNAs are in equilibrium under normal physiological conditions, but are often dysregulated in NSLC. In addition, dysregulation of circRNAs may be

associated with the initiation and progression of tumors. Recent evidence has demonstrated that the majority of circRNAs associated with NSCLC act as endogenous miRNA sponges. In addition, miRNA–circRNA interactions consistently influence two common pathways—the cell cycle and the EMT. Next, we review circRNAs involved in these two pathways, some of which act as sponging miRNAs.

circRNAs participating in the regulation of cell cycle in lung cancer

The cell cycle is tightly controlled by a complex and elaborate regulatory network in which a series of biochemical switches trigger the processes of the cell cycle. The G1 phase is a period in which the cell integrates and interprets diverse signals, and makes further decisions about whether or not to enter the S phase. Evidently, the G1/S checkpoint is the most important one of all cell-cycle checkpoints, and many oncogenes and tumor repressor genes are associated with aberrations in the control of the G1/S (Massagué 2004; Kastan and Jiri 2004). Activation of G1 CDKs (cyclin-dependent kinases) is essential for the transition from the G1 phase to the S phase. Retinoblastoma protein (pRB), the product of the RB gene, is a well-known inhibitor of the G1/S transition, and its phosphorylation and dephosphorylation by CDKs represent its key regulatory mechanism. In early G1, activated cyclin-D-Cdk4/6 complexes regulate the phosphorylation of pRB, which releases E2F 1–3 (positive transcriptional factors). E2F 1–3 promote expression of cyclin E, which binds and activates Cdk2. When Cdk2 is activated, the pre-replicative complex (PRC) recruits multiple components to initiate DNA replication, leading to transition from the G1 phase to the S phase (Hochegger et al. 2008). All of the above findings represent the classical model for the transition from the G1 phase to the S phase. Rigorous regulation of Cdk2 activation and pRB phosphorylation ensures that cells with DNA damage and mutations cannot enter the S phase of cell cycle.

Many recent experiments have shown that circRNAs play important roles in regulation of the cell cycle. Aberrant expression of circRNAs allows cells to skip cell-cycle checkpoints and to enter the next phase of the cell cycle. Here, we briefly review a few circRNAs that have been shown to be involved in the regulation of G1/S checkpoint (Fig. 2).

The cyclin-dependent kinase inhibitor, p21 (a 165 amino-acid protein), is the central inhibitor of Cdk2 and can induce G1-phase cell-cycle arrest by binding to and inhibiting the catalytic activity of Cdk2 (Abbas and Dutta 2009). Recent research has found that circFoxo3 can directly bind with p21 and Cdk2 to form ternary complexes (Du et al. 2016). The ternary complexes inhibit the catalytic activity of Cdk2, leading to G1-phase arrest. Experimental data have confirmed that the expression level of circFoxo3 is significantly reduced in NSCLC (Zhang et al. 2018d; Du et al. 2016). The down-regulation of circFoxo3 disturbs the ternary complexes, which increases cell-cycle entry. Another circRNA that relates to the regulation of p21 is circPRKCI. The expression of circPRKCI is significantly increased during amplification of the PRKCI gene (the host gene of circPRKCI) (Qiu et al. 2018). Experimental results suggest that circRPKCI can function as miR-545 and miR-589 sponges, relieving its suppression to the E2F7 factor. In addition, E2F7 inhibits expression of p21 by binding to the promotor of CDKN1A (the host gene of p21) (Sun et al. 2016). Finally, the up-regulation of circPRKCI promotes proliferation of NSCLC cells.

In addition to the regulation of Cdk2 through p21, Cdk2 undergoes a different regulatory mechanism by restriction

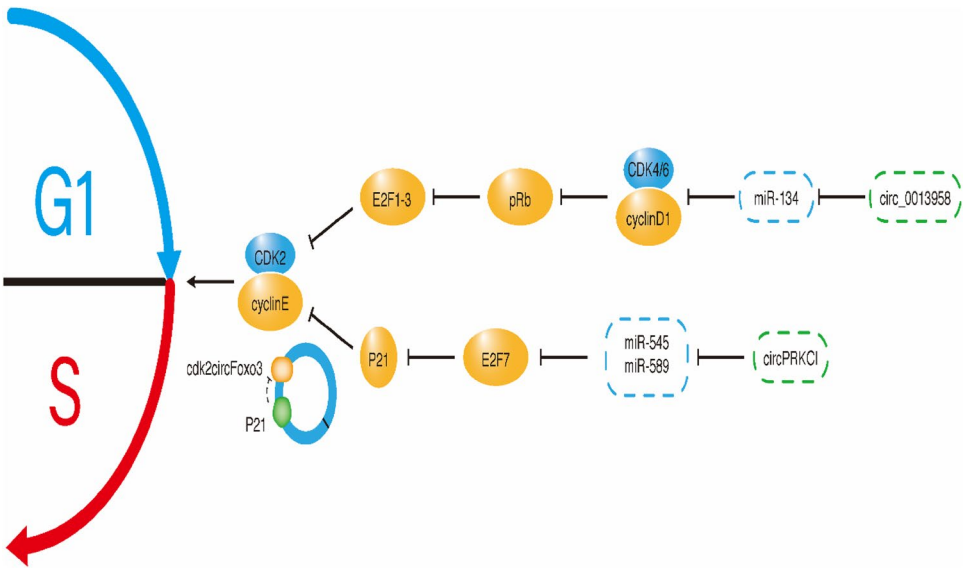

Fig. 2 Regulatory network of several circRNAs that are associated with the cell cycle in the pathogenesis of NSCLC

of the supply of cyclin E. A study found that the level of circ_0013958 is upregulated in NSCLC. Circ_0013958, localizing mainly in the cytoplasm, can increase the cyclin D1 level by sponging miR-134 (Zhu et al. 2017). The miR-134 can bind to the 3′-UTR of CCND1 (the host gene of cyclin D1). Cyclin D1 further binds to and activates Cdk4/6, leading to the release of E2F 1–3 factors and the promotion of cyclin E expression.

In addition to the above factors, there are several other circRNAs that are involved in the regulation of G1/S checkpoints. However, there are no clear regulatory mechanisms that have yet been elucidated. Specifically, circ-0003998 regulates cell proliferation by the circ-0003998/miR-326/Notch-1 pathway (Jiang et al. 2018). Surprisingly, we previously found that miR-326 can bind to the 3′-UTR of CCND1 mRNA, resulting in G1-phase arrest in NSCLC (Sun et al. 2016). A recent study indicated that the level of circ_100395 was down-regulated in NSCLC and regulated the miR-1228/TCF21 axis (Chen et al. 2018). In addition, the overexpression of circ_100395 can reduce the expression of Cyclin D1. Furthermore, miR-1228 can promote the development of hepatocellular carcinoma through a p53 feedback loop (Zhang et al. 2015) and p53 has been shown to be a positive regulator of p21. A study by Dai et al. found that overexpression of circ_0006916 can lead to accumulation of NSCLC cells in the G0/G1 phase and that circ_0006916 can function as an miR-522 sponge (Dai et al. 2018). We previously found that miR-522 can induce G1-phase arrest (Zhang et al. 2016; Tan et al. 2014). These results are in accordance with the results of Dai et al. In addition, researchers have discovered that knockdown of circ_0000064 and circ_0079530 inhibits the NSCLC cell transition from the G1 to S phases of the cell cycle; however, the underlying mechanism remains unknown (Luo et al. 2017; Li et al. 2018a).

circRNAs participating in the epithelial–mesenchymal transition

The EMT is an evolutionarily conserved biological process that polarizes epithelial cells and changes their phenotype to non-polarized mesenchymal cells, which confers migratory and invasive properties. The EMT is a fundamental physiological and pathological processes that is especially involved in the initiation of cancer cell migration, invasion, and metastasis (Yilmaz and Christofori 2009). Cells will undergo a series of changes in gene expression via a complex regulatory network. This network involves many complex signaling pathways, such as the transforming growth factor beta (TGF-β) signaling pathway (Massague 2008). TGF-β, a potent EMT inducer, can trigger the up-regulation of CDH1 transcriptional repressors (such as Snail1 Snail2, ZEB1, and ZEB2), leading to the down-regulation of E-cadherin (Peinado et al. 2007). The down-regulation of epithelial markers (such as E-cadherin) and up-regulation of mesenchymal markers (such as N-cadherin, vimentin, and fibronectin) are prominent features of cells after this transition (Mani et al. 2008). Thus, cellular phenotypes can be defined by detecting these classical markers.

circ_0007534, derived from the DDX42 gene, has been identified as a regulator of NSCLC cell migration and invasion (Qi et al. 2018). The protein levels of Snail, N-cadherin, and Vimentin were significantly increased, while E-cadherin was decreased when circ_0007534 was overexpressed, demonstrating that circ_0007534 can promote the EMT in NSCLC. The expression level of circ_0079530 is increased in NSCLC, and can promote cancer cell migration and invasion by regulating the EMT (Li et al. 2018a). Another oncogenic factor, circ_0067934, can also promote cancer metastasis by modulating the EMT (Wang and Li 2018). Epithelial markers were upregulated, while mesenchymal markers were down-regulated when circ_0067934 was silenced by si-RNA. All of the above oncogenic factors act as tumor promotors by regulating the EMT. In contrast, circ_0008305 is a tumor repressor by inhibiting the TGF-β-induced EMT (Wang et al. 2018b). A study by Wang et al. demonstrated this mechanism in detail. Specifically, circ_0008305 can upregulate the expression of TIF1γ by sponging miR-429 and miR-200b-3p, resulting in the inhibition of TGF-β-induced EMT. This study not only detected the markers of EMT, but also elucidated the corresponding regulatory mechanism (Fig. 3).

circRNAs as biomarkers of cancer

Recently, many studies have suggested that circRNAs may represent diagnostic, prognostic, and/or therapeutic markers of various types of cancer. circRNAs are abundant, conserved, and are stable because of their covalently closed structure. In addition, circRNAs can be detected in tissue, plasma, and saliva,which allow them to be widely detected as cancer biomarkers (Bahn et al. 2015; Memczak et al. 2015). In addition, circRNAs can be detected in exosomes, which are small membranous vesicles secreted by various cells (Li et al. 2015a). Interestingly, the expression profile of circRNAs in the sera of patients with colorectal cancer was found to be significantly different from that in healthy donors; in relation to the sera of healthy donors, 257 novel circRNAs were present and 67 circRNAs were missing in the sera of patients with colorectal cancer (Li et al. 2015a).

In addition, the expression of circRNAs is tissue and developmental-stage specific. Hence, dysregulation of distinctive circRNAs may be indicative of specific types of tumors (e.g., fusion circRNAs derived from fusion genes). In NSCLC, a specific circRNA, F-circEA, was discovered to be useful in the diagnosis of EML4-ALK-positive patients (Tan

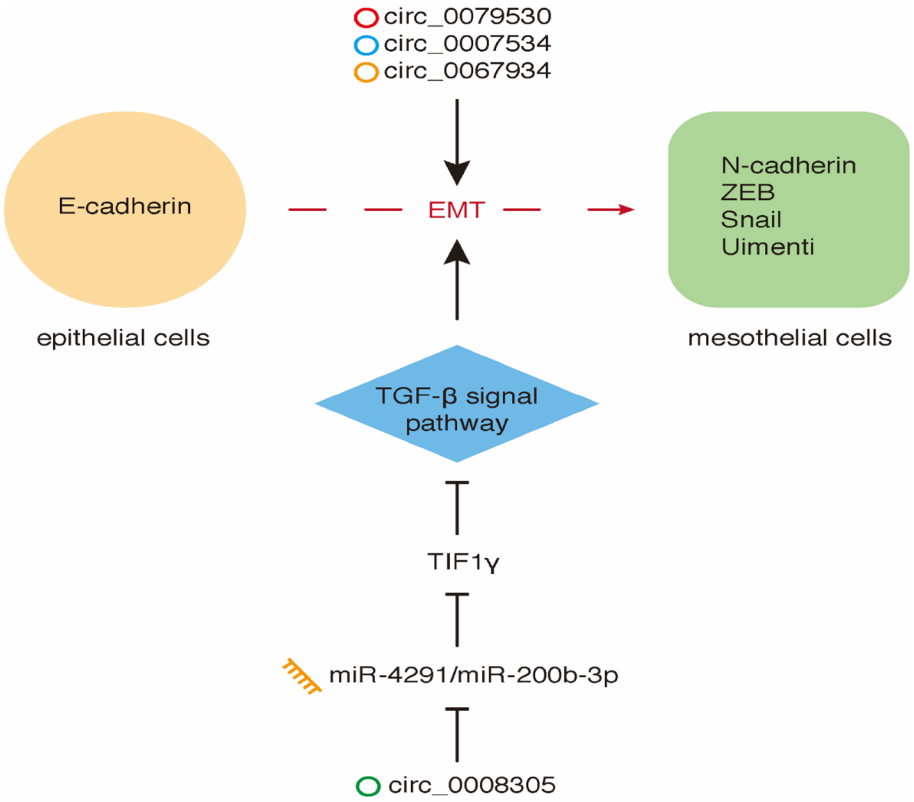

Fig. 3 Regulatory network of several circRNAs that are associated with the epithelial–mesenchymal transition in the pathogenesis of NSCLC

et al. 2018a). In leukemia, there is a distinctive F-circRNA, F-circM9, which is derived from MLL/AF9 translocation (Guarnerio et al. 2016).

Conclusion

Many studies have focused on ncRNAs as potential diagnostic biomarkers and therapeutic targets. Different aspects of ncRNA biology have been elucidated, including ncRNA biogenesis, modes of interactions, physiological functions, as well as their roles in disease contexts (especially in cancer) (Beermann et al. 2016; Guo et al. 2019). Among ncRNAs, the mechanisms of miRNAs are best understood. Along with the development of high-throughput sequencing technologies, the expression patterns of abundant circRNAs can be easily detected. Furthermore, different expression profiles of circRNAs between cancer patients and heathy individuals have been discovered. In gliomas, there are more than 476 circRNAs that are differentially expressed compared with those in control brain tissue (Song et al. 2016). In addition, a total of 527 circRNAs exhibited different expression profiles between hepatocellular-carcinoma and para-tumorous tissues (Fu et al. 2017) Compared with those in adjacent normal tissue, 356 circRNAs were differentially expressed in NSCLC (Zhao et al. 2017). All of these findings suggest that circRNAs play pivotal roles in the pathological progression of cancer and may be useful as cancer biomarkers.

NSCLC accounts for the majority of lung cancers and its early diagnosis is pivotal for increasing its survival rate. Thus, it is urgent to find novel biomarkers with high sensitivity and specificity for NSCLC. The dysregulation of circRNA expression is closely related to the development and progression of cancer. It is possible that circRNAs may represent novel and non-invasive biomarkers for cancer due to their closed-loop structures, resistance to RNAse R, and tissue and developmental-stage specificities. In particular, recent research has shown that circRNAs are enriched in exosomes, which can be detected in many types of bodily fluids (Zhou et al. 2018; Li et al. 2015a). Many experiments have demonstrated that circRNAs have potential as biomarkers for diagnosis, prognosis, and therapeutic interventions for NSCLC. The up-regulation and/or down-regulation of circRNAs are closely related to cellular proliferation, migration, invasion, and drug resistance. Although there are many advantages of circRNAs as biomarkers, the reliability of diagnosis by circRNAs still needs to be validated. In addition, there have been fewer studies on the roles of circRNAs in NSCLC compared with those for other cancers, and little is known about the mechanisms of circRNAs in NSCLC. Thus, more studies focused on the roles of circRNAs in NSCLC are needed.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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