Molecular Therapy
Nucleic Acids
Review

Pathogenic mechanisms and the potential clinical value of circFoxo3 in cancers

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Circular RNAs (circRNAs) are covalently closed circular structures that can function in various physiological and pathological processes by acting as microRNA (miRNA) sponges, RNA-binding protein (RBP) sponges, mRNA transcriptional regulators, and protein translational templates. circFoxo3 is one of the most studied circRNAs and is generated from the tumor suppressor gene *Foxo3*. Increasing studies have demonstrated the multiple functions of circFoxo3 in the pathogenesis of different cancer types. circFoxo3 plays important roles in cancer development mainly by binding to various miRNAs. The diagnostic potential of circFoxo3 has been revealed in several cancers. Some research results have been found to contradict the results of other studies, and this may be due to insufficient sample sizes and inconsistencies in the experimental and nomenclature methods. In this review, we systematically summarize current knowledge about the biogenesis and functions of circRNAs, elucidate the roles of circFoxo3 in different cancers, and explore the clinical applications of circFoxo3.

Noncoding RNAs, such as microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), have been widely studied for their various roles in important disease progression processes and have been shown to be promising diagnostic and prognostic biomarkers for many diseases.[1–3] In recent years, circular RNA (circRNA), a special type of noncoding RNA with covalently closed loop structures, has gained much attention for its wide participation in various diseases, including cancer and cardiovascular disease (CVD).[4–7]

circRNAs were first documented in plant viruses[8] and were initially thought to have no function or very limited function.[9–11] Subsequently, circRNAs were found in many eukaryotes, such as yeasts, mice, and humans.[12–14] With the rapid development of prediction, detection, and screening technologies, different types of circRNAs have been discovered.[15–20] Growing mechanistic studies have proven that circRNAs have important biological functions in the physiological and pathological development of many organisms.[15,21–24] circRNAs can act as important regulatory factors at both the transcriptional and posttranscriptional levels by sponging miRNAs, binding to RNA-binding proteins (RBPs), regulating parental gene expression, or serving as translation templates.[25–29] ciRS-7/CDR1$_{as}$ and sex-determining region Y (Sry, a testis-specific circRNA) can bind to miRNAs to regulate the progression of various diseases.[15,16,30–32] circANRIL has been determined to sponge the PES1 protein (an essential 60S-preribosomal assembly factor) and inhibit ribosome biogenesis, thereby providing an atheroprotective role.[33] ciRNA-ankrd52 can bind to RNA polymerase II (RNA Pol II) of the precursor (pre-)mRNA of the *ANKRD52* gene to promote transcription.[25] circFBXW7 can be a template for translation to generate a functional protein that represses glioma tumorigenesis.[6] circFoxo3 is one of the most studied circRNAs. circFoxo3 is generated from the *Foxo3* gene, which is considered a tumor suppressor gene.[34,35] circFoxo3 has been shown to have multiple functions in various cancers.[28,36,37] In this review, we summarize the current knowledge on the biogenesis and functions of circRNAs, elucidate the roles of circFoxo3 in cancers, and explore the clinical application of circFoxo3.

The biogenesis and characteristics of circRNAs

circRNAs originate from pre-mRNAs by back-splicing, a unique mechanism that differs from canonical splicing, which generates mRNA.[38] There are three types of circRNAs: exonic circRNAs (ecircRNAs or ecRNAs),[20] circular intronic RNAs (ciRNAs),[25] and exon-intron circRNAs (EIciRNAs),[39] which can be generated from different mechanisms. Jeck et al.[38] reported two models of circRNA formation: lariat-driven circularization and intron pairing-driven circularization. In the lariat-driven circularization model, a 5′ splice donor (GU) and a 3′ splice acceptor (AG) in the introns can form a lariat that will then be internally spliced to generate an exonic circle (ecRNAs)[38,40] (Figure 1A). In the intron pairing-driven circularization model, the pairing of RNA base motifs (e.g., Alu repeats, the most highly repeated interspersed repeat element in humans that contributes to the occurrence of a wide range of diseases[41]) in the introns of pre-mRNA with reverse complementary sequences can facilitate the generation of ecRNAs (introns removed) or EIciRNAs (introns retained)[38] (Figure 1B). A growing number of studies have illustrated that the intron pairing-driven circularization occurs more frequently than lariat-driven circularization.[38,39] Moreover, some recent studies have suggested that RBPs, such as muscleblind

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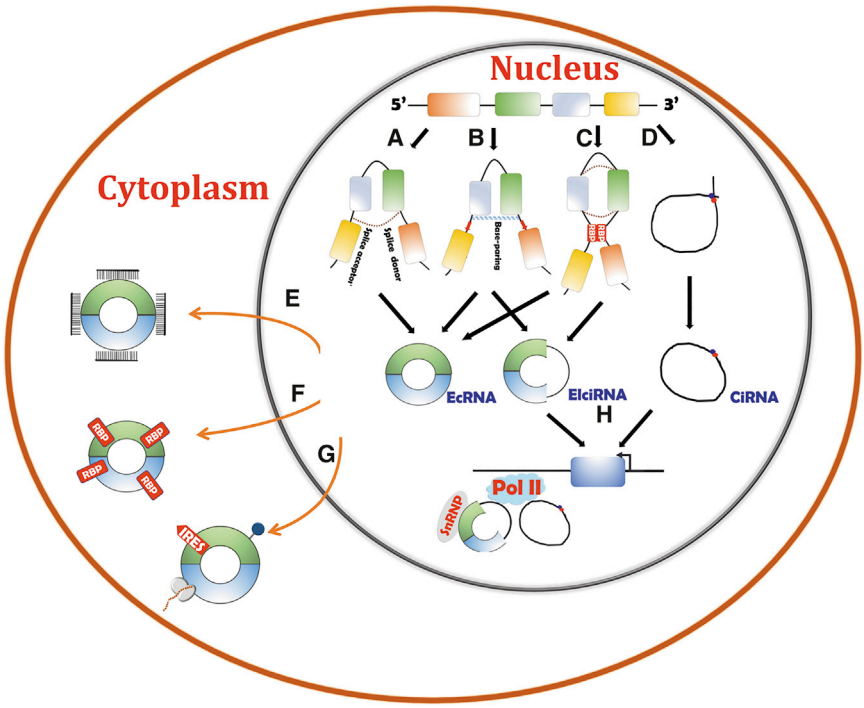

Figure 1. Biogenesis models and the underlying action mechanisms of circRNAs

(A) Lariat-driven circularization model dependent on the splice donor and acceptor can produce exonic circRNAs (ecRNAs). (B) Intron pairing-driven circularization indicated by the pairing of RNA base motifs (e.g., Alu repeats, red arrows) in introns can generate ecRNAs or exon-intron circRNAs (EIciRNAs). (C) RNA-binding proteins (RBPs) can bridge with pre-mRNAs to produce ecRNAs or EIciRNAs. (D) Circular intronic RNA (ciRNA) is generated by a back-splicing process. (E) circRNAs can bind to miRNAs to influence their function. (F) circRNAs can associate with RBPs to regulate the subsequent pathways. (G) circRNAs can encode proteins assisted by internal ribosome entry site (IRES38100136525000000) elements or m^6A modifications (blue pin-2730572390000000). (H) ciRNAs and EIciRNAs can regulate the expression of parental genes by binding to RNA polymerase II (Pol II).

(MBL) proteins, also participate in the formation of circRNAs.[26,42] The bridging of RBPs with pre-mRNAs is also a pathway for the production of circRNAs (ecRNAs or EIciRNAs)[26,42] (Figure 1C). Zhang et al.[25] proposed a model that described the formation of ciRNAs. In the introns, the GU-rich element near the 5′ splice site and the C-rich element close to the branch will bind together during back-splicing.[25] The other exons and introns are removed by a spliceosome. In this mechanism, only ciRNAs are formed[25] (Figure 1D).

circRNAs have the following common biological properties: (1) high stability: due to their circular structures, circRNAs are more stable than linear RNAs and cannot be cleaved by ribonuclease (RNase).[43] (2) Wide distribution: circRNAs exist in a variety of organisms and all tissues.[38,44,45] Zheng et al.[46] identified ~27,000 circRNAs from six normal tissues and seven cancerous tissues by RNA sequencing. In humans, more than 30,000 circRNAs have been identified.[44,45] (3) Specificity: the expression of circRNAs is tissue-, cell-, and developmental stage-specific.[44,47,48] In heart differentiation, circRNAs have altered profiles at four stages.[48] Rat neonatal hearts have higher levels of overall circRNAs than that in adult rat hearts,[49] indicating the possible roles of circRNAs in the development of heart. (4) Conservation: many circRNAs are conservatively expressed among species,[38,50] whereas a number of circRNAs are specific to species.[19,49,51–53] Werfel et al.[49] determined that only a small number of circRNAs were conserved across humans, mice, and rats. Barrett et al.[53] also confirmed that only ~5%–30% of sequences are homologous among mammals, including mice, humans, rats, and pigs. (5) Altered expression in normal and pathological conditions: studies have identified the differential expression of circRNAs in various diseases.[49,54,55]

The action mechanisms of circRNAs

The wide distribution, expression specificity, and different localization of circRNAs indicate that circRNAs have various biological functions. Recent studies have illustrated that circRNAs can function in different ways.

circRNAs can act as miRNA sponges

circRNAs can absorb miRNAs like sponges with the help of miRNA response elements (MREs).[15,32] Absorption reduces the miRNA levels and thus alleviates the inhibitory effect on the target genes (Figure 1E). circFoxo3 can competitively bind to miRNAs to influence a number of physiological and pathological processes.[56–58] Sry is able to interact with miR-138 through MREs, which might be an important pathway of Sry function.[32] HRCR can directly bind to miR-223, which promotes the pathogenesis of cardiac hypertrophy and heart failure.[27] The absorption of miR-223 by HRCR decreased the activity of miR-223, thereby inhibiting the pathological processes of cardiac hypertrophy and heart failure.[27] Studies have revealed that abundant expression of the circRNAs and/or exceptional binding affinity compared with other mRNA targets might be necessary for circRNAs to suppress miRNA activity by this mechanism.[19,59]

Recently, a special avenue for the downregulation of miRNAs, target-directed miRNA decay (TDMD), has been illustrated.[60–63] TDMD leads to direct degradation of miRNAs rather than transient binding to specific binding sites. This mechanism is triggered by target RNAs such as mRNAs, lncRNAs, and circRNAs that have extensive complementarity with miRNAs.[60] Until now, most known examples of TDMD mechanism, especially endogenous examples, were identified in neuronal cells.[60,61,64] ciRS-7/$CDR1_{as}$ has been found to function as miRNA sponges in heart disease[21] and cancers.[15,16,30–32] In mammalian brain, ciRS-7/$CDR1_{as}$ has been determined to induce degradation

of miR-671 by TDMD.[60] So far, there are few studies on miRNA degradation induced by circRNAs through the TDMD mechanism. However, with the deepening of research, more and more discoveries will be made in this field. TDMD might be one of the mechanisms by which circRNAs could regulate gene expression.

circRNAs can serve as RBP sponges

circRNAs can function by binding to RBPs. circMbl and MBL proteins are generated from the same pre-mRNA.[42] circMbl can absorb MBL proteins to regulate the subsequent physiological processes.[42] circFoxo3 can interact with RBPs such as anti-senescent protein ID-1, transcription factor E2F1, anti-stress proteins FAK, and two G_1-to-S phase transition-related proteins (p21 and CDK2) to promote cardiomyocyte senescence or destroy cell cycle progression[18,65] (Figure 1F).

circRNAs can be templates for protein translation

circRNAs were first found to encode proteins in prokaryotes.[66,67] Recent studies have validated the role of circRNAs in translation.[6,29] circRNAs cannot recruit ribosomes due to the lack of a 5′ cap and 3′ polyadenylated tail. However, they have special initiation pathways with the help of IRES (internal ribosome entry site) and N^6-methyladenosine (m^6A)[68,69] (Figure 1G). circZNF609 and circFBXW7 contain IRES elements and can be translated into proteins that function in myoblast proliferation or glioma suppression.[6,29] Some circRNAs can use m^6A to enable translation.[70] YTHDF3 is an m^6A reader that functions through interacting with circRNAs. YTHDF3 can facilitate the recruitment of translation initiation factors to drive the initiation of circRNA translation.[70] Another recent study found that circβ-catenin has been determined to be the template for the translation of a functional protein. The protein is an isoform of β-catenin and could promote tumor progression in liver cancer by activating the Wnt/β-catenin pathway.[71]

circRNAs can serve as transcriptional regulators of the parental genes

circRNAs with introns (ciRNAs and EIciRNAs) are confined to the nucleus. ciRNAs directly bind to RNA Pol II[25] (Figure 1H), whereas EIciRNAs interact with RNA Pol II via U1 small nuclear ribonucleoproteins (snRNPs)[39] (Figure 1H). Ciankrd52 is a circular intronic RNA that has been shown to promote the transcription of the *ANKRD52* gene by binding to RNA Pol II of the pre-mRNA of the *ANKRD52* gene.[25] circEIF3J and circPAIP2 are two EIciRNAs that can each interact with both U1 snRNP and RNA Pol II to regulate the transcription of parental genes.[39]

The origin of circFoxo3

The *Foxo3* gene is the most widely studied member of the forkhead family and is involved in many physiological processes.[72–74] It can regulate stress resistance, cell apoptosis, and the cell cycle, which are key processes of aging.[75] Therefore, it can function in age-related diseases, such as CVD and cancers.[72–74,76] Downregulation of *Foxo3* has been shown to promote cancer development.[72,74] Therefore, *Foxo3* is considered a tumor suppressor gene.[72,74]

circFoxo3 is an ecRNA generated from the second exon of the *Foxo3* gene by back-splicing and consists of 1,435 nt.[24,57] circFoxo3 is abundantly expressed and located in the cytoplasm,[57] consistent with its extensive roles and modes of action in diseases. circFoxo3 participates in a variety of diseases, such as cancers,[24,57] cardiac senescence,[65] heart failure,[77] and neurodegenerative diseases.[78] circFoxo3 functions through sponging miRNAs or RBPs to influence various cancers.[24,36,57,74]

circFoxo3 and cancers

Consistent with the functions of its parental gene, circFoxo3 is also involved in the pathogenesis of a variety of cancers (Figure 2, Table1), including gastric carcinoma (GC),[58] breast cancer,[79] lung cancer,[80] prostate cancer (PCa),[37,81] esophageal squamous cell carcinoma (ESCC),[82] and so on.

Cancers promoted by circFoxo3

GC. GC is one of the most lethal cancers, has the lowest cure rate, and brings a heavy economic burden to society and individuals.[83] Great effort has been made to uncover the underlying mechanisms of GC progression. In recent years, circFoxo3 has been found to play roles in the initiation and development of GC.[58]

Xiang et al.[58] carried out a series of experiments both *in vivo* and *in vitro*. They determined that circFoxo3 could promote the proliferation and migration of GC cells. The circFoxo3 level was increased in GC cells. *In vivo* experiments with GC cell lines showed that circFoxo3 might promote tumor growth. Bioinformatics analyses, AGO2 RNA immunoprecipitation (RIP) assays, luciferase reporter assays, and biotinylated-miR-143-3p RNA pull-down experiments were performed in sequence. The results suggested that circFoxo3 could be the sponge of miR-143-3p to inhibit its activity in GC cells. Furthermore, miR-143-3p was confirmed to target ubiquitin-specific peptidase 44 (USP44), a cell cycle-related protein that participates in tumor progression and metastasis.[84] The downregulation of miR-143-3p by circFoxo3 increased the level of USP44, which in turn promoted tumor cell proliferation. All findings indicate that circFoxo3 can advance the pathogenesis of GC through the miR-143-3p-USP44 axis. The findings provide a possible therapeutic target for GC.[58]

Glioblastoma. Glioblastoma (GBM) is one of the most serious brain tumors in neoplasms of the central nervous system and occurs very frequently.[85] Recently, the underlying mechanisms of circFoxo3 in GBM pathogenesis have been explored.[24]

Zhang et al.[24] found elevated cirFoxo3 expression in human GBM tissues. Compared with human normal glial cells (HEBs), GBM cell lines had upregulated expression of circFoxo3. Knockdown and overexpression of circFoxo3 combined with Cell Counting Kit-8 (CCK-8) and colony formation assays were performed to explore the functions of circFoxo3 in GBM cells. circFoxo3 knockdown inhibited the GBM cell proliferation and invasion. circFoxo3 was critical to the promotion of GBM tumorigenesis and GBM cell invasion. The

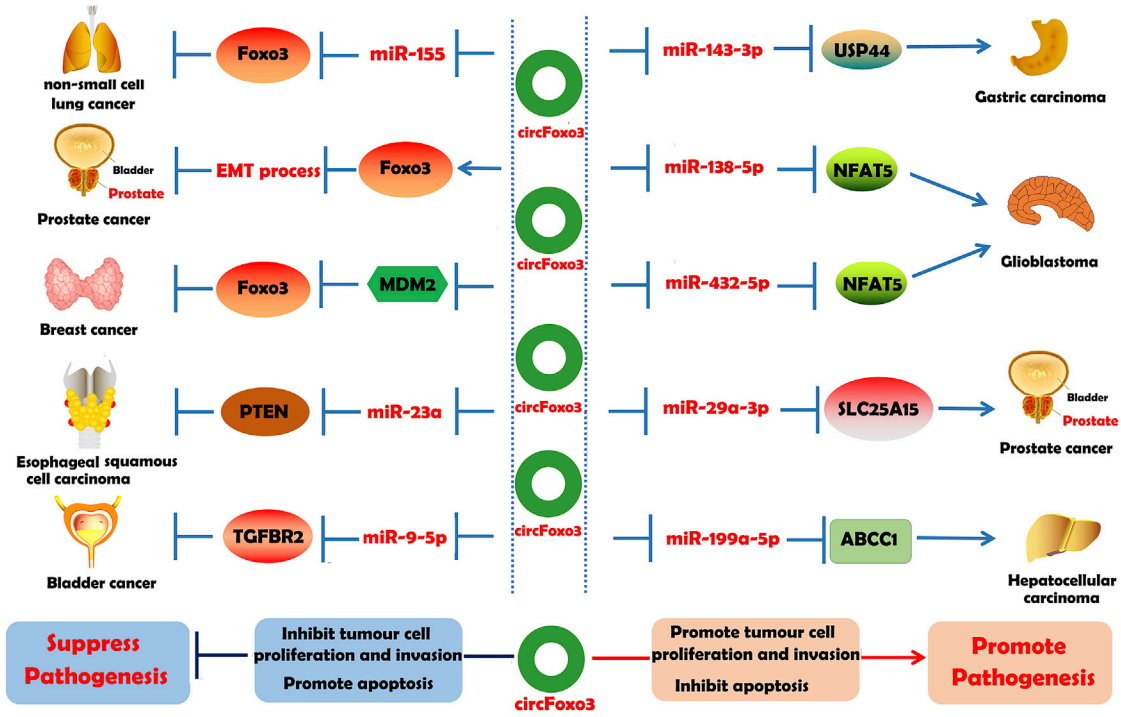

Figure 2. circFoxo3 participates in various types of cancers through different pathways

circFoxo3 can suppress the pathogenesis of non-small cell lung cancer, prostate cancer, breast cancer, esophageal squamous cell cancer, and bladder cancer. On the contrary, circFoxo3 can promote the pathogenesis of gastric carcinoma, glioblastoma, prostate cancer, and hepatocellular carcinoma. In particular, circFoxo3 was reported to have the opposite effect in two studies on prostate cancer.

biotin-coupled miRNA capture and luciferase reporter assay indicated that cirFoxo3 could competitively absorb miR-138-5p/miR-432-5p in GBM cells. Further analyses found that the direct target of miR-138-5p/miR-432-5p was the nuclear factor of activated T cells 5 (NFAT5) protein. NFAT5 is a transcription factor that is dysregulated in tumors, suggesting its role in tumor progression.[86,87] NFAT5 levels were increased and found to be positively correlated with the circFoxo3 levels in GBM.[24] Additionally, the downregulation of circFoxo3 led to decreased levels of NFAT5, which could be reversed by the inhibition of miR-138-5p/miR-432-5p in GBM cells.[24] Thus, circFoxo3 could promote GBM progression through sponging miR-138-5p/miR-432-5p to upregulate NFAT5 expression.[24]

PCa. PCa is an epithelial malignancy and has the highest incidence rate among men over 65 years old.[88,89] The underlying mechanisms of PCa progression are still unclear. Several studies have illuminated the role of circFoxo3 in PCa development.[37,81]

Kong et al.[81] identified that the expression levels of circFoxo3 in both the serum and tissues of PCa patients were higher than those in controls. Silencing of circFoxo3 could suppress PCa cell proliferation, promote PCa cell apoptosis, and inhibit the PCa cell cycle. Bioinformatics analyses, dual-luciferase assays, and pull-down assays revealed that circFoxo3 could bind to miR-29a-3p, which can inhibit PCa cell proliferation and induce apoptosis of PCa cells. Furthermore, solute carrier family 25 member-15 protein (SLC25A15, a protein related to the normal function of mitochondria[90]) was validated to be a target of miR-29a-3p. Downregulation of circFoxo3 could suppress SLC25A15 expression. SLC25A15 expression in PCa tissues was upregulated compared with that in adjacent normal prostate tissues. Overexpression of SLC25A15 significantly suppressed LNCaP-AI cell apoptosis. These results suggested that circFoxo3 might promote PCa development through the miR-29a-3p-SLC25A15 pathway.[81]

Hepatocellular carcinoma. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and causes the highest death rate among all cancers worldwide.[91] There are several reasons for the high mortality rate. HCC is difficult to detect at an early stage. The current treatment is still restricted to surgery, radiotherapy, and chemotherapy, with poor prognosis.[23] Some patients are even resistant to chemotherapy.[23] Adriamycin (ADM) is a common chemotherapy drug for HCC. The ADM resistance of HCC patients severely reduces the efficacy of treatment. circFoxo3 has been identified to take part in the ADM chemoresistance pathway.[57]

Huang et al.[57] revealed increased levels of circFoxo3 in both HCC tissues and HCC cell lines. In addition, the level of circFoxo3 in ADM-resistant HCC tissues was markedly elevated in comparison with that in ADM-sensitive HCC tissues. Overexpression of circFoxo3 greatly promoted tumor cell invasion and growth. Luciferase assays and

pull-down assays indicated that circFoxo3 could bind to miR-199a-5p in ADM-resistant HCC tumor tissues to upregulate ATP-binding cassette subfamily C member 1 (ABCC1), which has been reported to be closely correlated with drug resistance in different cancers.[92] The expression of ABCC1 was higher in HCC tumor tissues than in adjacent tissues. ABCC1 in ADM-resistant HCC tumor tissues was significantly upregulated compared with that in ADM-sensitive HCC tissues. ABCC1 expression could be upregulated by the overexpression of circFoxo3 and inhibited by miR-199a-5p, and vice versa.[57] In conclusion, these findings demonstrate that circFoxo3 could promote ADM resistance in HCC patients via the miR-199a-5p/ABCC1 axis.[57]

Cancers suppressed by circFoxo3

Non-small cell lung cancer. Non-small cell lung cancer (NSCLC) accounts for most lung cancers and is one of the most serious malignant tumors.[93] Despite the great progress made in clinical strategies, only ~15% of patients can survive 5 years after a definite diagnosis.[80] Thus, new therapeutic targets for the diagnosis and prognosis of NSCLC are greatly needed. circFoxo3 is a newly identified promising target that regulates NSCLC development.[80]

Zhang et al.[80] found decreased levels of circFoxo3 in both NSCLC tissues and cells. Experiments in NSCLC cell lines showed that overexpression of circFoxo3 repressed the proliferation and the invasive ability of tumor cells. Bioinformatics analyses and AGO2 RIP assays in NSCLC cells indicated that circFoxo3 could specifically bind to miR-155, which could target the *Foxo3* gene. *Foxo3* mRNA was downregulated in NSCLC tissues and cell lines. Overexpression of circFoxo3 dramatically upregulated *Foxo3* expression. Furthermore, overexpression of miR-155 or knockdown of *Foxo3* abrogated the inhibitory effect of circFoxo3 on tumor cell invasion. Therefore, circFoxo3 can act as a tumor suppressor of NSCLC by promoting *Foxo3* expression by targeting miR-155. circFoxo3 might be a potential target for the diagnosis and prognosis of NSCLC patients.[80]

PCa. In a study by Shen et al.,[37] both low-grade PCa tissues and high-grade PCa tissues were obtained. circFoxo3 exhibited lower levels in high-grade PCa samples than in low-grade prostate cancer and normal prostate tissues. Meanwhile, circFoxo3 showed a decreased level in more invasive cell lines than in less invasive cell lines. In PCa cell lines, circFoxo3 was found to inhibit the viability of PCa cells by enhancing the expression of *Foxo3*. The Foxo3 protein can suppress the function of epithelial-mesenchymal transition (EMT), which participates in metastasis, chemical resistance, and apoptosis resistance. In mice with prostate tumors, circFoxo3 could elevate the chemosensitivity to docetaxel and prolong the survival time of the mice, whereas the knockdown of circFoxo3 had the opposite effects.[37] Therefore, circFoxo3 could suppress the survival, progression, and chemical resistance to docetaxel in prostate cancer by repressing EMT. circFoxo3-, Foxo3-, and EMT-related factors might be possible targets for the exploration of potential therapeutic approaches for prostate cancer.[37]

The result of this article is contrary to that of Kong et al.[81] on the effect of circFoxo3 in PCa.[37] We speculate that the reasons for the opposing results were the different sampling standards and insufficient samples.

Breast cancer. Breast cancer mainly occurs in women.[94] Due to the development of modern clinical diagnosis and treatment methods, breast cancer has well-developed therapies and a very low mortality rate.[94] circFoxo3 has been found to participate in the pathogenesis of breast cancer.[28]

circFoxo3 was downregulated in both breast cancer tissues and breast cancer cell lines. However, the level of circFoxo3 was increased in cancer cells undergoing apoptosis induced by H_2O_2, doxorubicin and cisplatin. The ectopic expression of circFoxo3 was revealed to inhibit tumor growth and prolong lifespan in a mouse model. Furthermore, circFoxo3 could bind to the MDM2 (murine double minute 2) protein. The *MDM2* gene has been determined to be an oncogene. MDM2 is able to polyubiquitinate p53 and Foxo3 to decrease the MDM2 levels independently of the proteasome.[95] Overexpression of circFoxo3 suppressed the binding between MDM2 and Foxo3 and inhibited MDM2-induced ubiquitination of Foxo3, resulting in an elevated level of Foxo3. The increased activity of Foxo3 might promote tumor cell apoptosis.[28] Therefore, circFoxo3 plays an anti-tumor role in breast cancer.[28]

ESCC. Esophageal cancer occurs in the esophagus, which runs from the throat to the stomach. ESCC is one of the most common types of esophageal cancer, causing great pain during eating and high morbidity.[96] However, until now, the incidence and recurrence rates have remained high due to the lack of effective prevention therapeutics.[97] The underlying mechanisms of ESCC pathogenesis need to be clarified to enable the prevention and early diagnosis of ESCC. The role of circFoxo3 in ESCC has been investigated and the mechanism has been illuminated.[82]

circFoxo3 was significantly downregulated in ESCC tissue cell lines compared with that in normal control cells.[82] The expression level of circFoxo3 was shown to be relevant to the tumor node metastasis stage, implying its role in the tumorigenesis of ESCC. Colony formation, CCK-8, and Transwell invasion assays showed that overexpression of circFoxo3 inhibited ESCC cell line proliferation and invasion and provoked ESCC cell apoptosis. According to the bioinformatics screen and a luciferase assay, circFoxo3 was determined to be a sponge of miR-23a. Further analyses indicated that miR-23a could interact with phosphatase and tension homolog (PTEN, a tumor suppressor) to inhibit PTEN expression. PTEN has various regulatory roles, including those in cell proliferation, survival, and motility.[98] Depending on the lipid phosphatase activity, PTEN can exert tumor-suppressor activity.[98] Overexpression of circFoxo3 could increase the expression of PTEN at both on the mRNA and protein levels. All results suggested that circFoxo3 could sponge miR-23a to suppress the development of ESCC via targeting PTEN. The circFoxo3-miR-23a-PTEN signaling pathway may provide possible therapeutic targets and diagnostic biomarkers for ESCC.[82]

Table 1. Roles of circFoxo3 in cancers

Cancer type	Subjects	circFoxo3 expression	Regulatory mechanism	Pathway effect on cancer	References
Gastric carcinoma	cell lines	upregulated	circFoxo3-miR-143-3p-USP44	promotion	58
Glioblastoma	tissues, cell lines	upregulated	circFoxo3-miR-138-5p/miR-432-5p-NFAT5	promotion	24
Prostate cancer	tissues, serum, cell lines	upregulated	circFoxo3-miR-29a-3p-SLC25A15	promotion	81
Hepatocellular carcinoma	tissues, cell lines	upregulated	circFoxo3-miR- 199a-5p-ABCC1	promotion	57
Non-small cell lung cancer	tissues, cell lines	downregulated	circFoxo3-miR-155-Foxo3	suppression	80
Prostate cancer	low-grade PCa tissues and high-grade PCa tissues	reduced level in high-grade PCa samples in contrast to low-grade prostate cancer and normal prostate tissues	circFoxo3-Foxo3- EMT process	suppression	37
Breast cancer	tissues, cell lines	downregulated	circFoxo3-MDM2- Foxo3	suppression	28
Esophageal squamous cell carcinoma	tissues, cell lines	downregulated	circFoxo3-miR-23a-PTEN	suppression	82
Bladder cancer	tissues, cell lines	downregulated	circFoxo3-miR-9-5p-TGFBR2	suppression	101
	tissues, cell lines	downregulated by miR-191-5p	miR-191-5p-circFoxo3	promotion	103
Clinical potential					
Glioblastoma	plasma	upregulated	–	diagnosis	108
Acute myeloid leukemia	bone marrow	downregulated	–	prognosis	36

Bladder cancer. Bladder cancer is a common cancer that most frequently occurs in older people, especially men.[99] The majority (70%) of patients with bladder cancer can be diagnosed at an early stage.[100] However, the recurrence rate is high. Therefore, follow-up examinations after remission are essential. An in-depth illustration of the underlying mechanisms can facilitate clinical treatment and prognosis.

Li et al.[101] found a regulatory pathway of circFoxo3 in bladder cancer. circFoxo3 in bladder cancer tissues was significantly reduced compared with that in adjacent normal tissues. The overexpression of circFoxo3 could inhibit the proliferation, migration, and invasion of bladder cancer cells. Further analyses determined that circFoxo3 competitively absorbed miR-9-5p, which could target transforming growth factor β receptor 2 (TGFBR2).[102] The TGFBR2 levels were also decreased in bladder cancer tissues. The upregulation of circFoxo3 inhibited the activity of miR-9-5p and promoted the expression of TGFBR2. These data suggest that circFoxo3 might suppress bladder cancer through the miR-9-5p-TGFBR2 axis.[101]

In another study, the inhibitory effect of circFoxo3 on bladder cancer was blocked by miR-191-5p.[103] Wang et al.[103] revealed the role of circFoxo3 in urothelial carcinoma, the most common kind of bladder cancer. circFoxo3 expression was obviously reduced both in bladder cancer tissues and cell lines. Overexpression of circFoxo3 could promote apoptosis of bladder cancer cells and markedly decrease the viability of bladder cancer cells, indicating its anti-tumor role. miR-191-5p has been classified to be related to various solid tumors.[104,105] miR-191-5p could bind to the 3′ untranslated region (UTR) of circFoxo3. Further analysis determined that miR-191-5p could suppress apoptosis of bladder cancer cells by inhibiting circFoxo3,[103] implying the promotion effect of the miR-191-5p-circFoxo3 pathway in the progression of bladder cancer.

Clinical significance of circFoxo3

Due to their special properties, such as high stability, wide distribution, time-dependent specificity, and dynamic expression in normal and pathological conditions, circulating circRNAs have great potential as non-invasive biomarkers for the diagnosis and prognosis of different cancers.[106,107] Recent studies have also illustrated the diagnostic and prognostic value of circFoxo3 in cancers[36,108] (Table 1). Chen et al.[108] illuminated the potential role of circFoxo3 in the diagnosis of GBM. The circFoxo3 level was increased in the GBM plasma. Risk score analysis and receiver operating characteristic (ROC) analysis indicated that circFoxo3 might be a promising biomarker for GBM diagnosis.[108] Zhou et al.[36] found the prognostic role of circFoxo3 in acute myeloid leukemia (AML). AML is a kind of blood cancer that begins in the bone marrow. circFoxo3 levels were downregulated in mononuclear cells isolated from the bone marrow of AML patients, and circFoxo3 had considerable diagnostic potential for AML.[36] Follow-up clinical data showed that patients with higher circFoxo3 levels might have longer survival times than patients with low circFoxo3 levels after treatment. All data suggest that circFoxo3 might be a diagnostic and prognostic biomarker for AML patients.[36]

In addition to the role as a biomarker, circFoxo3 also could also be a promising therapeutic target for cancers. circFoxo3 functions

in cancer development mainly by sponging miRNAs that influence many genes. All factors in the pathway might be used as targets for drug design. Therefore, the application of circFoxo3 as a novel therapeutic target is a promising method for cancer treatment.

Concluding remarks and future perspectives

The extensive and powerful role of circFoxo3 has attracted attention from researchers worldwide, leading to many advances. circFoxo3 has been revealed to function by binding to miRNAs or RBPs. circFoxo3 participates in cancers mainly via acting as a miRNA sponge. The circFoxo3-miRNA-mRNA (protein) regulatory pathway enables circFoxo3 to influence the pathogenesis of various cancers through different miRNA-mRNA (protein) axes. circFoxo3 has great clinical value in the diagnosis, prognosis, and therapeutic intervention of cancers. However, some problems should be solved prior to clinical use.

First, the numbers of samples were insufficient in most studies, which might cause deviation of the experimental results. Larger cohorts are needed in the future. Second, there is a lack of standardized methodologies to measure various effects, which might lead to inconsistencies in similar studies. Generally accepted methodologies should be explored. Third, despite the findings, the exact underlying mechanisms of circFoxo3 in various cancers are still unclear and need further investigation. Lastly, there is no uniform nomenclature system: the same circRNA might have different names in studies from different laboratories, which can lead to misjudgment or omission in the follow-up analyses. For example, circFoxo3 has also been named circFOXO3, circ-Foxo3, circ-FOXO3 or circ-FoxO3 in different studies. In-depth studies should be performed to solve these problems.

In summary, circFoxo3 is intimately involved in the pathogenesis of cancers, which provides a novel avenue for the diagnosis, prognosis, and therapeutic intervention of cancers in the future. With the advancement of technology and the cooperation of all researchers, we have faith in the realization of the clinical application of circFoxo3 in cancer treatment.

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AUTHOR CONTRIBUTIONS

L.Z. searched the original articles and drafted the manuscript. Y.W. revised the manuscript. Y. Zhang and Y. Zhao discussed the clinical problems and pathological discoveries. P.L. and L.Z. conceived the idea and framework of the review and performed the final proofreading. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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