Review

Circular RNAs: Characteristics, function and role in human cancer

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Summary. Circular RNAs (circRNAs) are universal and diverse endogenous noncoding RNAs (ncRNAs) that are widely found in eukaryotic cells. They form a covalently closed, continuous stable loop structure without 3' or 5' tails, which are different from linear RNAs. As new members of the ncRNAs, circRNAs' formation, function, and mechanism are attracting increased research attention. CircRNAs play important roles in all kinds of cancer and may be potential novel biomarkers and therapeutic targets for cancer treatment through their function as microRNA (miRNA) molecular "sponges", RNA-binding protein (RBP) sponges, protein translators, and gene transcription regulators. In this review, we introduce the formation and function of circRNAs, and summarize the biological effects of circRNA in tumorigenesis and progression, providing evidence for the potential use of circRNAs in the diagnosis and clinical treatment of cancer.

Key words: CircRNA, RNA binding protein, Cancer progression

Introduction

Messenger RNAs (mRNAs) that encode proteins account for only about 2% of the genome in eukaryotic cells, while the majority of the remaining RNAs (75-90%) are non-coding RNAs (ncRNAs) (Atianand and

Fitzgerald, 2014). Studies have shown that ncRNAs regulate gene expression levels during physiological and developmental processes, and have important biological functions. Current research on ncRNAs, such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) has focused on their relationship with certain diseases and physiological processes, indicating that they have potential as biomarkers and therapeutic targets in clinical practice. In recent years, with the development and application of RNA sequencing (RNA-seq) technology, scientists have identified a new type of endogenous noncoding RNA: Circular RNAs (circRNAs), CircRNAs were first discovered in plant-infected Viroids and Sendai virus by Kolakofsky and Sanger, respectively, in 1976 (Kolakofsky, 1976; Sanger et al., 1976). Thereafter, other researchers found circRNA structures in animal cells and fungal yeasts (Hsu and Coca-Prados, 1979; Arnberg et al., 1980; Matsumoto et al., 1990); however, in subsequent decades, although some circRNAs were identified in individual genes of mammalian cells, they were thought to result from byproducts of pre-mRNA alternative splicing or initially regarded as viral genomes (Cocquerelle et al., 1993). This situation remained unchanged until 2012, when Salzman et al. (2012) conducted a comprehensive and systematic report on circRNAs, and people began to understand the reality of circRNAs. Studies had showed that circRNAs have differentiation and tissue-specific expression patterns (Rybak-Wolf et al., 2015; Kristensen et al., 2018b), in this review, the characteristics of circRNAs, their mechanism of formation and function, and their role in tumors are analyzed.

Molecular characteristics and formation of circRNAs

Molecular characteristics of circRNAs

A study showed that more than 10% expression genes are capable of producing circRNAs (Salzman et al., 2012). Despite having no 5' end cap and 3' end poly(A) tail, circRNAs have the following important characteristics: (1) These circular molecules have a closed ring structure, and are therefore not easy to digest using exonucleases; (2) circRNAs are widely expressed in the body, and their expression levels are sometimes more than 10 times that of linear RNA from their respective host genes (Salzman et al., 2012); (3) most circRNAs have highly conserved sequences; (4) most circRNAs are located in the cytoplasm, while a small number of intron-derived circRNAs are located in the nucleus (Zhang et al., 2014); (5) most circRNAs comprise exonic sequences, while a few of them are formed via intron cyclization (Salzman et al., 2013; Guo et al., 2014); (6) Some circRNAs are rich in miRNA response elements (MREs), derived from exons; and (7) circRNAs play a regulatory role at the level of transcription or post-transcription.

Formation mechanism of circRNA

The currently discovered circRNAs can be divided into three categories according to their origin: (1) exonic circRNAs (ecircRNAs) derived from exons (Salzman et al., 2012), which account for over 80% of identified circRNAs. Jeck et al. (2013) identified two models of ecircRNA formation: Lariat-driven circularization, and intron-pairing-driven circularization. Researchers demonstrated that circRNA molecules are mostly generated by back-splicing, where downstream exons are spliced to upstream exons in a reverse order (Wilusz and Sharp, 2013), leading to a circular transcript. Besides, heterogeneous nuclear ribonucleoprotein L (HNRNPL) could also regulate circular RNA formation via back splicing (Fei et al., 2017); (2) circular intronic RNA (ciRNA) (Zhang et al., 2014): Intron-derived circRNAs are dependent on consistent GU-rich sequence near the 5' tail of the pre-mRNA that prevents the RNA from branching and degrading the C-rich element. Errichelli et al. (2017) suggested that the RNA-binding protein FUS regulates circRNA biogenesis by binding the introns flanking the back-splicing junctions. Numerous studies have demonstrated that reverse complementing intron regions (such as Alu elements) play an important role in the generation of circRNA. Aktaş et al. (2017) found that the loss of DHX9 leads to an increase in the number of circular-RNA-producing genes and amount of circular RNAs, translational repression of reporters containing inverted-repeat Alu elements, and transcriptional rewiring of susceptible loci; (3) exon-intron circRNA (ElciRNA) composed of exons and introns (Salzman et al., 2013). Studies have proved that exon cyclization depends on the substitution on flanking intronic complementary sequences and

Table 1. CircRNAs in major malignant tumorigenesis and progression processes.

Type of cancer	Name	Expression/Function in cancer	Sponge miRNAs	Author
Gastric cancer	Circrna_100269	Downregulated	miR-630	Zhang et al., 2017a,b
	Circ_LARP4	Tumor suppressor	miR-424-5p	Zhang et al., 2017a,b
	CircPVT1	Oncogene	miR-125	Chen et al., 2017
	Circ_0047905	Oncogene	-	Chen et al., 2017
	Circ_0138960	Oncogene	-	Chen et al., 2017
	CircRNA7690-15	Oncogene	-	Lai et al., 2017
Esophageal cancer	Circ_0067934	Oncogene	-	Xia et al., 2016
Hepatocellular carcinoma	Circ-ZKSCAN1	Tumor suppressor	-	Yao et al., 2017a,b
	CDR1as	Oncogene	miR-7	Xu et al., 2017
Breast cancer	Circ-ABCB10	Oncogene	miR-1271	Liang et al., 2017
	Circ_103110	Upregulated	-	Lü et al., 2017
	Circ_104689	Upregulated	-	Lü et al., 2017
	Circ_104821	Upregulated	-	Lü et al., 2017
	Circ_006054	Downregulated	-	Lü et al., 2017
	Circ_100219	Downregulated	-	Lü et al., 2017
	Circ_406697	Downregulated	-	Lü et al., 2017
Lung cancer	Circ_0013958	Oncogene	miR-134	Zhu et al., 2017
	Circ_100876	Upregulated	-	Yao et al., 2017a,b
Bladder cancer	Circrna MYLK	Oncogene	miR-29a-3p	Huang et al., 2016
	circTCF25	Oncogene	miR-103a-3p;miR-107	Zhong et al., 2016
Glioma	cZNF292	Oncogene	-	Yang et al., 2016
	circ-FBXW7	Tumor suppressor	-	Yang et al., 2018
Hematological malignancies	circ_0004277	Downregulated	-	Li et al., 2017
Colorectal cancer	ciRS-7	Oncogene	miR-7	Weng et al., 2017
	circCCDC66	Oncogene	-	Hsiao et al., 2017

alternative formation of inverted repeated Alu pairs, which could be helpful in determining the production rate of circRNAs. Usually, introns that surround the exons are spliced out; however, in some cases, they are retained, and were thus named retained-intron circRNAs or ElciRNAs (Li et al., 2015). Recently, studies demonstrated that the effect on circRNA abundance is dependent on intronic Quaking (QKI) binding motifs. The addition of QKI binding motifs can induce circRNAs to be produced by genes that did not undergo cyclization (Conn et al., 2015).

The mechanism of circRNAs

CircRNAs act as miRNA molecule "sponges"

Researchers found that circRNAs contain a large number of miRNA response elements (MREs), which can compete with endogenous RNA (acting as competing endogenous RNAs, ceRNAs) through binding with miRNAs and inhibiting their functions. For example, ciRS-7 (circular RNA sponge for miR-7) contains more than 70 selectively conserved miRNA target sites, and was associated with Argonaute (AGO) proteins in a miR-7-dependent manner (Hansen et al., 2013); hsa_circ_001569 was identified as a sponge for miR-145 and could inhibit miR-145, which leads to the upregulation of *E2F5* and *FMNL2*, which are the targets of miR-145 (Xie et al., 2016). Studies also revealed that circTCF25 overexpression could promote cell proliferation and migration ability, either *in vitro* or *in vivo*, by downregulating miR-103a-3p/-107 expression and increasing *CDK6* expression (Zhong et al., 2016). However, bioinformatics analysis showed that a small number of circRNAs have a large number of miRNA binding sites, and that many circRNAs contain only a small number of MREs; therefore, circRNA-mediated inhibition of miRNA activity as a common phenomenon remains controversial (Guo et al., 2014). CircRNAs, miRNAs, and ceRNAs form regulatory networks to maintain a balance and regulate the body's life activities, which have become the focus of research in recent years.

CircRNAs regulate gene transcription

Li et al. (2015) found that ElciRNAs enhance gene transcription and trans-regulate gene expression by interacting with small nucleoside RNA and RNA polymerase II in the U1 nucleus at 300 bp upstream of the gene transcription initiation site. Ashwal-Fluss et al. (2014) found that the second exon of the splice factor MBL can be cyclized to form a circRNA that competes with the linear splicing of the pre-mRNA and affects the formation of linear RNA to regulate related gene expression.

The interaction between circRNAs and proteins

MiRNA effector Argonaute (AGO) could be degraded by binding to circRNAs such as CDR1as and SRY (Hansen et al., 2011; Memczak et al., 2013), which in turn inhibits mRNA translation; circPAIP2 binds to RNA polymerase II complex and affects the final regulation of transcription by altering the enzyme activity (Li et al., 2015). In addition, circRNAs also participate in the regulation of multiple physiological processes by interacting with proteins. For example, circ-Foxo3 can inhibit the function of CDK2 and induces cell cycle arrest through the circ-Foxo3-p21-CDK2 ternary complex (Du et al., 2016). The results suggested that the interaction of circRNA molecules with proteins could also be used to develop treatments for HIV infection.

CircRNAs are involved in protein translation

Most circRNAs are composed of exons and are mainly present in the cytoplasm, so they may be loaded into ribosomes and translated into polypeptides *in vivo* (Pamudurti et al., 2017). Legnini et al. (2017) reported that Circ-ZNF609, which contains an open reading frame spanning from the start codon, associates with heavy polysomes, and could be translated into protein in a splicing-dependent and cap-independent manner. Besides, Yang et al. (2017) showed that N6-methyladenosine (m6A), which was the most abundant base modification of RNA, promotes efficient initiation of protein translation from circRNAs. Recently, studies found that a circRNA has a translational function in human osteosarcoma cells, but its translation efficiency is very low. Recently, some researchers have demonstrated that circRNA could also be translated into functional proteins. Abe et al. (2013, 2015) found that RNA can be efficiently translated in cellular *Escherichia coli* translation systems and in living human cells, and abundant protein products are produced by rolling circle amplification (RCA) mechanisms.

CircRNAs in major malignant tumorigenesis and progression processes

CircRNA and gastric cancer

CircRNA is an important regulator in tumorigenesis and progression of gastric cancers. CircRNA_100269 is downregulated in gastric cancer and could suppress gastric cancer cell growth through a novel pathway by targeting miR-630 (Zhang et al., 2017). Circ_LARP4 could inhibit gastric cancer cell proliferation and invasion by sponging miR-424-5p and regulating LATS1 expression (Zhang et al., 2017). It was also reported that circPVT1 (Chen et al., 2017), circ_0047905, circ_0138960, and circRNA7690-15 might function as oncogenes in gastric cancer tumorigenesis (Lai et al., 2017). Thus, these results provided evidence that circRNAs might have potential as biomarkers for the diagnosis and prognosis of gastric cancer.

CircRNAs and esophageal cancer

Xia et al. (2016) reported that circ_0067934 was overexpressed in esophageal squamous cell carcinoma (ESCC) tumor tissues compared with paired adjacent normal tissues, and was associated with differentiation and TNM stage of ESCC. In addition, through *in vitro* silencing of hsa_circ_0067934 using siRNA, researchers found that hsa_circ_0067934 downregulation inhibited ESCC cell proliferation and migration, and induced cell cycle progression arrest, suggesting that the circRNA might have potential as a new biomarker and therapeutic target for ESCC.

CircRNA and human hepatocellular carcinoma

Studies revealed that the *ZKSCAN1* mRNA and circ-ZKSCAN1 cooperated closely in inhibiting human hepatocellular carcinoma (HCC) cell growth, migration, and invasion. Circ-ZKSCAN1 overexpression might predict a good prognosis of HCC (Yao et al., 2017a,b). Xu et al. reported that the *CDR1as* was overexpressed, while the miR-7 expression level was decreased in HCC, indicating a negative correlation between them. *CDR1as* in hepatocellular carcinoma cells is a carcinogenic sequence that promotes the proliferation and metastasis of HCC cells by adsorbing miR-7 (Xu et al., 2017).

CircRNAs and breast cancer

Researchers identified that circ-ABCB10 was significantly overexpressed in breast cancer tissues, and could promote breast cancer tumorigenesis through sponging miR-1271 (Liang et al., 2017). Lü et al. demonstrated that circ_103110, circ_104689, and circ_104821 expression levels were upregulated in breast cancer tissues, whereas the expression level of circ_006054, circ_100219, and circ_406697 were downregulated (Lü et al., 2017). They also demonstrated that different circRNAs might have different functions in breast cancer; however, they are all important in breast cancer tumorigenesis because they participate in the cancer-related pathways and sponging of certain miRNAs.

CircRNAs and lung cancer

Zhu et al. studied the function of circ_0013958 in non-small cell lung cancer (NSCLC), and found that it could sponge miR-134, and upregulated oncogenic cyclin D1, thus playing a pivotal role in the development of NSCLC. The authors suggested that circ_0013958 could be used as a potential non-invasive biomarker for the early detection and screening of lung cancer (Zhu et al., 2017). Yao et al. also revealed that circ_100876 was significantly overexpressed in NSCLC tissues compared with adjacent nontumorous tissues, and its expression level was associated with lymph node metastasis and tumor stage of NSCLC (Yao et al., 2017a,b), suggesting that the circRNA was tightly connected with lung cancer tumorigenesis and progression.

CircRNAs and ovarian cancer

Studies on circRNAs in gynecological cancer are comparatively limited. Bachmayr-Heyda et al. (2015) demonstrated that ovarian cancer cell proliferation is related to the overall expression abundance of circRNAs. Ahmed et al. (2016) demonstrated that circRNAs might participate in ovarian cancer development, progression, and metastases through fine-tuning the regulatory balance of miRNAs. Given that the networks of circRNAs, miRNAs, and mRNAs are complex, a single circRNA could regulate a series of downstream genes by sponging different miRNAs. The authors also observed co-expression of circRNAs and downstream gene targets that are highly enriched for cancer associated pathways, indicating that circRNAs might act as ovarian cancer drivers.

CircRNAs and hematological malignancies

Bonizzato et al. (2016) reported that circRNAs from genes such as JAK2, PAX5, IKZF1, ETV6 and EBF1 are prevalently present in hyperdiploid leukemia compared with normal leukocyte samples. Li et al. (2017) showed that lower hsa_circ_0004277 expression level was found in the AML newly diagnosed group than the ctrl group, and was very likely to be a diagnostic biomarker or therapeutic target in AML.

CircRNAs and glioma

Studies from Yang et al. (2016) showed that cZNF292, an important circular oncogenic RNA, plays a critical role in the progression of human glioma tube formation by influencing glioma cell proliferation and cell cycle progression via the Wnt/β-catenin signaling pathway. Besides, endogenous circRNA circ-FBXW7 functions as a tumor suppressor in glioma through encoding a functional protein FBXW7-185aa, and its expression level was positively associated with glioblastoma patient overall survival (Yang et al., 2018).

CircRNAs and colorectal cancer

Recently, Hsiao et al. (2017) found that CircCCDC66 expression was higher in colon cancer than in polyps, and was associated with poor prognosis. CircCCDC66 involved in multiple pathological processes, such as cell proliferation, migration and invasion through modulating relevant oncogenes. Weng et al. (2017) showed that the overexpression of ciRS-7 in colorectal cancer cells led to the blocking of miR-7 and resulted in a more aggressive oncogenic phenotype through permitting the inhibition of miR-7 and subsequent activation of EGFR and RAF1 oncogenes.

CircRNAs and other cancers

Along with the above cancer types, studies have investigated circRNAs in other cancers. For example, Sand et al. (2016) found 23 high expression and 48 low expression circRNAs in basal cell carcinoma, with a total of 354 miRNA binding sites, which might play a role in the development and progression of basal cell carcinoma. In bladder cancer, Huang et al. found that the expression of circRNA MYLK and lncRNA H19, as an endogenous lncRNA, caused the upregulation of *DNMT3B, VEGFA,* and *ITGB1* through binding to miRNA-29a-3p (Huang et al., 2016). Zhong et al. (2016) showed that the over-expression of circTCF25 could promote bladder cancer proliferation and migration through miR-103a-3p/-107/CDK6 pathway. Recently, Okholm et al. (2017) reported that abundant circRNAs, for example, circHIPK3 and circCDYL, possess multiple biological features, and might serve as prognostic biomarkers in non-muscle-invasive bladder cancer. These emerging roles of circRNAs provide opportunities in basic research to reveal their complex biological crosstalk, their role in carcinogenesis, and their efficacies as biomarkers for cancer diagnosis and prognosis.

Problems and prospects

The discovery of circRNAs enriches our understanding of eukaryotic transcription groups and their important roles in organisms are gradually being recognized, especially in cancer, thus circRNAs have quickly become a focus of RNA research. However, fundamental problems still existed in this field, for example, the template switching and rolling circle amplification during reverse transcription; a lack of thorough examination of the tumor cell content in the studied samples by an experienced pathologist is often lacking in studies of circular RNAs in cancer; their susceptibility to become posttranscriptionally modified; a common standard for reporting and naming circRNAs is lacking; the expression of circRNAs does not often correlate well with the expression of host gene linear expression (Kristensen et al., 2018a), which needs further research in the future.

The distribution, conservation, and tissue specificity of circRNAs indicate that they have potential as novel tumor diagnostic markers. At present, a number of databases for circRNAs have been established to calculate and predict their expression, and to construct networks of circRNAs-miRNAs-mRNAs, for example, circBase (Glažar et al., 2014), circRNADb (Chen et al., 2016), AtCircDB (Ye et al., 2018), CSCD (Xia et al., 2017) and so on. The establishment and application of these databases will promote the study of circRNAs in tumor.

Although the mechanism of the formation and function of circRNAs and their detailed functions in tumors remain unclear, with the continuous progress in molecular biology technology, it is believed that within a few years, the functions of many circRNA in disease will be determined, leading to their development as new diagnostic markers and potential targets for tumors, providing new perspectives and directions for the diagnosis and treatment of tumors.

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