

Circular RNAs: An emerging type of RNA in cancer

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Abstract

Circular RNAs (circRNAs), a novel type of widespread and diverse endogenous non-coding RNAs (ncRNAs), which are different from the linear RNAs, form a covalently closed continuous loop without 5' or 3' polarities. The majority of circRNAs are abundant, conserved and stable across different species, and exhibit tissue/developmental-stage-specific characteristics. They are generated primarily through a type of alternative RNA splicing called “back-splicing,” in which a downstream splice donor is joined to an upstream splice acceptor through splice skipping or direct splice. Recent studies have discovered circRNAs function as microRNA sponges, binding with RNA-associated proteins to form RNA–protein complexes and then regulating gene transcription and translation into polypeptides. Emerging evidence indicates that circRNAs play important roles in the regulation of the development and progression of multiple cancers by serving as potential diagnostic and predictive biomarkers involved in tumor growth and invasion and providing new strategies for cancer diagnosis and targeted therapy. In this review, we briefly delineate the diversity and characteristics of circRNAs and discuss the highlights of the biogenesis of circRNAs and their potential functions in tumor.

Keywords

circular RNAs, gene transcription, microRNA sponge, non-coding RNA (ncRNA)

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Introduction

Following small RNAs (microRNAs, miRNAs) and long non-coding RNAs (lncRNAs), circular RNAs (circRNAs) are gradually recognized as a new type of endogenous non-coding RNA (ncRNA) displaying an important role in gene expression.¹ CircRNAs form a covalently closed loop structure, typically devoid of the terminal structures (5' caps and 3' polyadenylated tails),² which keep themselves resistant to exonuclease R and more stable than linear RNAs. In the early 1970s, circRNAs were first found in some plant viroid, and then in Hepatitis δ virus and some yeast mitochondrial and so on.^{3,4} Unfortunately, although the existence of circular transcripts has been known for several decades, they are considered as molecular flukes or artifacts of aberrant RNA splicing.⁵ Recently, novel bioinformatics and RNA deep sequencing

technology have allowed comprehensive studies of circular RNA species, confirming that circRNAs are not an unexpected product of RNA transcription derived from the exon or intron of genes and are stably expressed in human, mouse, fruit fly, nematode, and other biological cells.^{1,6–8} Unlike linear RNAs, circRNAs are generated by non-sequential back-splicing of pre-messenger RNA (mRNA) transcripts, related with reversed complementary

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sequences including inverted repeated Alu pairs and exon skipping and RNA-binding protein (RBP).^{6,9} Functionally, circRNAs, as the miRNA sponges, regulate related gene expression and translating peptides.^{1,6,10–12} Moreover, circRNAs are implicated in regulating cell proliferation and invasion in cancers, such as colon cancer, gastric cancer, and esophageal cancer, and may provide novel strategies for cancer diagnosis and therapy.^{13–16}

Discovery and characteristics of circRNAs

With the development of RNA sequencing and biological information technology, thousands of circRNAs across species from Archaea to human have been discovered, occupying a large proportion of the RNA family. Jeck et al.⁸ have identified >25,000 distinct RNA species in human fibroblasts and Memczak et al.¹ distinguish about 2000 circRNAs in human, 1900 in mice, and 700 in nematode. circRNAs are extraordinarily enriched in human and mouse brain, well conserved in sequence, and sometimes even detected in *Drosophila* brains.¹⁷ In human peripheral whole blood, circRNAs have higher expression than corresponding linear mRNAs, indicating that circRNAs may be used as biomarkers in standard clinical blood samples.¹⁸

Bioinformatic analysis reveals several shared features of circRNAs. The abundance of circRNAs exceed that of associated linear mRNAs showing more stable property than linear mRNAs due to their resistance to exonuclease R.¹⁹ They are highly conserved and predominantly cytoplasmic and possess tissue-specific characteristics.¹⁹ Intronic circRNAs are primarily located in the nucleus in eukaryotes participating in regulation of gene expression at the transcription or post transcription level.²⁰ Intriguingly, some circRNAs harbor miRNA binding sites (miRNA response elements [MREs]) and attenuate endogenous miRNA-mediated transcriptional repression through target mimicry.^{1,8}

Biogenesis of circRNAs

Recent studies have shown the biogenesis of circRNAs through back-splicing differs from the canonical splicing of linear RNAs. They are divided into exonic circRNAs, intronic circRNAs, and exon-intron circRNAs. Jeck et al.⁸ put forth two models

about the origination of circRNAs, termed as lariet-driven circularization and intron-pairing-driven circularization. It is demonstrated that exon circularization depends on flanking intronic complementary sequences and alternative formation of inverted repeated Alu pairs causing alternative circularization, and resulting in multiple circular RNA transcripts produced from a single gene.²⁰

In addition, RBPs serve as activators or inhibitors of the processing of circRNAs in some conditions. It is shown that the production of over one-third of abundant circRNAs are dynamically regulated by the alternative splicing factor, Quaking (QKI), and the addition of QKI motifs are sufficient to induce de novo circRNA formation from transcripts that are normally and linearly spliced.²¹ However, double-strand RNA-editing enzyme ADAR1 antagonizes circRNA expression by melting stems structure.²² The detailed mechanism of the formation of circRNAs remains elusive.

Functions of circRNAs

CircRNAs have important functions in gene regulation from the transcription or post-transcription level, then affecting the level of gene expression.

CircRNAs as miRNA sponges

Emerging evidence shows that a large number of circRNAs as miRNA sponges negatively regulate miRNAs. Human circRNA cerebellar degeneration-related protein 1 transcript (CDR1) acts as a miR-7 sponge by binding with miRNA effector complexes and positively associates with miRNA target site Argonaute in a miR-7-dependent manner, involved in suppressing miR-7 activity and increasing miR-7 target gene expression level.¹ Similarly, the testis-specific circRNA, sex-determining region Y (Sry), serves as the miR-138 sponge,²³ suggesting that circRNAs as the miRNA sponges are a common phenomenon.²⁴

CircRNAs as gene transcription and expression regulators

Besides regulating miRNAs, some circRNAs are predicted to function as robust post-transcriptional regulators of gene expression, and bind and regulate linear RNA transcription and protein expression.²⁵ Some circRNAs are circularized with introns “retained” between exons; we term them

exon-intron circRNAs (EIciRNAs). EIciRNAs are predominantly localized in the nucleus, interact with U1 snRNP, and enhance transcription of their parental genes.¹¹ The circRNA circMbl and its flanking introns contain conserved muscleblind (MBL) binding sites, which are specifically bound by MBL. Meanwhile, the MBL levels strongly affects circMbl biosynthesis via the MBL-binding sites, suggesting that circRNAs function in gene regulation by competing with linear splicing.⁶ In addition, circular intronic RNA (ciRNA) ci-ankrd52, largely accumulating to its sites of transcription, associates with elongation Pol II machinery and acts as a positive regulator of Pol II transcription. Knockdown of ci-ankrd52 leads to the reduced expression of their parent genes.¹⁰ Above all, we come to a conclusion that exonic circRNAs (ecircRNAs) may have regulatory functions as miRNA sponges in the cytoplasm, while ciRNAs and EIciRNAs seem to exert transcriptional regulation in the nucleus.

CircRNAs and cancer

CircRNAs are viewed as large species of transcripts in eukaryotic cells. The evolutionary conservation of circularization conceals certain important functions and recent works have suggested that circRNAs play crucial roles in multiple cellular processes in the initiation and development of diseases such as atherosclerosis,²⁶ Alzheimer's disease,²⁷ and many other ones. Growing evidence shows that circRNAs have critical functions in tumor (Table 1) and serve as the huge diagnostic and therapeutic potentials for cancer.

The correlation between circRNAs and miRNAs

CircRNAs are involved in fine-tuning the level of miRNA-mediated regulation of gene expression. In particular, their interaction with tumor-related miRNAs indicates a great significance in tumor. Ghosal et al.⁴⁵ have analyzed human disease-associated miRNA data collected from 174 diseases including cancer, found that circRNAs interact with these disease-associated miRNAs via the Gene ontology (GO) enrichment analysis, and identified enriched genes associated with particular biological processes including 22 stimulus

response genes and 43 cell cycle-related genes in breast cancer. Furthermore, Circ2Trait, a first comprehensive database of potential association of circRNAs with human diseases is built up.

CircRNAs as miRNA sponges in cancer

It has been shown that miR-7 have tremendous effects on the development of a variety of cancers, such as breast cancer, hepatocellular carcinoma, cervical cancer, Schwannoma tumor, tongue cancer, lung neoplasm, gastric cancer, and colorectal cancer.²⁸ ciRS-7 as a miR-7 sponge strongly quenches the activity of mir-7 via increase of the expression of miR-7 target oncogenes and decrease of the tumor suppression genes involving in lung cancer-associated biological processes and prognosis.⁴⁶ In addition, viral oncogene E6/E7 is associated with miR-7 overexpression in HPV-positive HeLa cell line.⁴⁷ Another well-known miRNA sponge is circSry, which contains miR-138 binding sites and functions as a miR-138 sponge.²³ Cir-ITCH act as a miRNA sponge of miR-7, miR-17, and miR-214 in esophageal squamous cell carcinoma and as sponges of miR-7 and miR-20a in colorectal cancer.^{16,36} CircZEB1.5, circ-ZEB1.19, circZEB-1.17, and circZEB1.33 as the miR-200 sponge are implicated in the suppression of the lung cancer progression.³⁷

CircRNAs regulation of cancer-related signaling pathways

The ciRS-7/miR-7 axis is probably involved in cancer-associated biological processes via downregulating oncogenic gene expression, such as EGFR and XIAP, as well as inhibiting the tumor-suppressor gene expression, such as KLF4 in cancer.²⁸ Through sequestering and inhibiting miR-7 activity, ciRS-7 promotes the initiation and development of cancer, such as cervical cancer and hepatocellular carcinoma.^{23,28,32} Additionally, miR-7 indirectly upregulates E-cadherin and results in reduced epithelial to mesenchyme transition (EMT), participating in tumorigenesis and cancer progression.⁴⁸

Aberrant regulation of the Wnt signaling pathway is considered as a prevalent theme in cancer biology. It has been reported that cir-ITCH is involved in tumor formation and chemosensitivity through regulation of miRNA mediated Wnt/ β -catenin signaling pathway via ubiquitination and degradation of phosphorylated Dvl2.^{16,36}

Table 1. CircRNAs associated with human tumor.

CircRNA	Gene symbol	Gene position	Function	Disease
CDR1as/ciRS-7	CDR1	chrX:139865339–139866824	miR-7 sponges ¹	Neuroblastomas ²⁸ Astrocytoma ²⁸ Lung carcinomas ^{28,29} Cervical cancer ²³ Breast cancer ^{28, 30} Schwannoma tumor ³¹ Hepatocellular carcinoma ³² Gastric carcinoma ³³ Tongue cancer ³⁴ Colorectal cancer ³⁵ Esophageal carcinoma ¹⁶
Cir-ITCH	ITCH	chr20:33001547-33037285	miR-7,miR-17,miR-214 sponges miR-7,miR-20a sponges	Esophageal carcinoma ¹⁶ Colorectal cancer ³⁶
circ-ZEB1.5 circ-ZEB1.19 circ-ZEB1.17 circ-ZEB1.33 circMYLK	ZEB1 MYLK	chr10:31749965-31791437 chr10:31749965-31750166 chr10:31661946-31750166 chr10:31749965-31791437 chr3:123366070-123368041	miR-200 sponges	Lung cancer ³⁷
circ-Foxo3 circ-KLDHC10 hsa_circ_0007374 hsa_circ_0006229 hsa_circ_002059 hsa_circ_0001649 hsa_circ_0005075 has_circ_001988 hsa_circ_100855 hsa_circ_104912	FOXO3 KLDHC10 AZINI TNS3 KIAA0907 SHPRH EIF4G3 FBXW7 – –	chr6:108984657-108986092 chr7:129760588-129762042 chr8:103846416-103852051 chr7:47384352-47385954 chr1:155891165-155895634 chr6:146209155-146216113 chr1:21377358-21415706 chr4:153332454-153333681 – –	Enhance Foxo3 expression Upregulated Upregulated Downregulated Potential biomarker Potential biomarker Potential biomarker Potential biomarker Potential biomarker	Breast cancer ³⁹ Colorectal cancer ⁴⁰ Colorectal cancer ⁴¹ Colorectal cancer ⁴¹ Gastric carcinoma ¹⁵ Hepatocellular carcinoma ⁴² Hepatocellular carcinoma ⁴³ Colorectal cancer ¹⁴ Laryngeal carcinoma ⁴⁴ Laryngeal carcinoma ⁴⁴

CircRNAs as potential biomarkers in cancer

Most circRNAs are abundant and conserved across species and exhibit tissue/developmental-stage-specific property. Importantly, circRNAs are stably expressed in saliva, blood, and exosomes as the promising biomarkers for diagnosis, prognosis, and therapeutic response for cancer patients. circRNA_100855 is upregulated and circRNA_104912 is downregulated in laryngeal squamous cell cancer tissues (LSCC) tissues, and their expressions are significantly related with tumor stage and neck nodal metastasis, suggesting an important role in the tumorigenesis of LSCC.⁴⁴ Similarly, circ_002059, significantly downregulated in gastric cancer, is correlated with distal metastasis, TNM stage, gender, and age, implying a potential novel biomarker for the diagnosis of gastric carcinoma.¹⁵ Other circRNAs have been reported aberrantly expressed in human cancers such as hepatocellular carcinoma and colorectal

carcinoma,^{14,42–43} indicating the critical role of circRNAs in physiological and pathological processes of cancer.

Conclusion

Previously, circRNAs, as a recently discovered RNA type, were thought to be “scrambled” exons without known functions. With the potential functions of circRNAs as miRNA “sponges” in human diseases, they are a hotspot in the field of RNA research. CircRNAs are mainly focused on their expressing profiles, characteristics, biogenesis, and functional mechanisms in various diseases including cancer. These findings will disclose the physiological and pathological processes of the vast majority of circRNAs as new biomarkers and potential therapeutic targets in cancer. Developing circRNA-based therapeutic strategies is expected to be applied to clinical practice in cancer as early as possible.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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