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Evaluation of the Regulatory Role of Circular RNA in Tumors and Its Application Value

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Abstract:

Cancer is a major challenge to public health in China. Its incidence and mortality rates are increasing year by year, and traditional treatment methods are facing limitations such as drug resistance and side effects. This year's research has found that circRNA plays an important role in tumorigenesis, metastasis and drug resistance by regulating mechanisms such as miRNA interaction networks, gene expression and cell cycles. Its stability and specificity make it a novel biomarker and therapeutic target. However, the standardization of circRNA detection techniques is insufficient, and its role in epigenetic regulation and the tumor microenvironment remains unclear. This article systematically analyzes the generation mechanism, functional diversity (such as molecular sponges, protein interactions, and encoded peptides) of circRNAs, as well as their application strategies in cancer treatment (such as exosome detection) and therapy (such as mesenchymal stem cell delivery), revealing the dynamic network by which they regulate tumor drug resistance and cellular metabolism. The research results provide a theoretical basis for the development of non-invasive diagnostic tools and targeted therapies. In the future, it is necessary to combine multi-group masking techniques and engineered delivery systems to break through the bottleneck of detection technology and explore the synergistic mechanism between circRNA and other ncRNAs, so as to promote the clinical transformation of precision cancer medicine.

Keywords:-Circular RNA; tumor treatment; exosome

I. Introduction

In China, cancer has long threatened human health and the economic burden of treatment is serious. From 1983 to 1987, the cancer incidence rate among men rose from 236.24 cases per 100,000 person-years to 367.57 cases per 100,000 person-years from 2013 to 2017, while the incidence rate among women

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increased from 176.18 cases per 100,000 person-years to 314.39 cases per 100,000 person-years. Among the 32 types of cancer, the age-standardized relative risk of 7 types of cancer in both men and women showed a significant upward trend from 1983-1987 to 2013-2017. Among them, thyroid cancer had the largest increase in age-standardized relative risk in both men and women, with an average annual percentage growth rate of 7.82%, followed by prostate cancer and kidney cancer [1]. The mortality rate shows regional differences. Compared with the United States and the United Kingdom, the incidence of cancer in China is lower. Although the cancer mortality rate in China is lower than that in the UK, it is significantly higher than that in the US [2]. The traditional tumor treatment system includes surgical treatment, radiotherapy and chemotherapy.

95% of the RNA in cells is ncRNA. Among them, circular RNA (circRNA) is a closed ncRNA formed through reverse splicing, and circRNA plays an important role in tumor treatment.

Recent studies have found that RNA molecules with closed circular structures can generate functional peptides through non-classical translation mechanisms. Such bioactive substances play an important regulatory role in the occurrence and development of malignant tumors. Drawing on the principle of antigen-antibody specific binding, a rapid test strip platform suitable for pregnancy testing and respiratory virus screening has been successfully constructed. This technology has the advantages of simple operation and rapid detection, providing the possibility for home self-testing. By screening the translation products of circRNAs specifically expressed in tumor tissues, it is expected to establish a novel early cancer screening system. Compared with traditional nucleotide targets, protein targets have higher molecular specificity. Inhibitors designed for circRNA-derived polypeptides have shown the characteristic of selectively killing tumors [3]. However, this technology still has some shortcomings. For example, the product stability is defective. Approximately 68% of the circRNA translation products have a half-life of less than 2 hours, which seriously affects its detection sensitivity. Its mechanism of action is still unclear. In addition, its standardization system is lacking, that is, similar products have different naming rules in different literatures, resulting in difficulties in data integration. This study mainly focuses on the regulatory role of circRNA in tumors. By further analyzing its mechanism of action, it aims to explore the application value of circRNA in tumor treatment and early diagnosis, etc., providing a comprehensive reference for future research.

II. The Regulatory Reference of circRNA on Tumors

A. The generation mechanism of circRNA

CircRNAs can be classified into four types according to their different sources: exon circRNA, intron circRNA, exon-intron circRNA and mitochondrial circRNA [4]. The generation mechanism of circRNA involves the actions of multiple molecules. Classical mRNA has a linear structure with a 5 ‘cap structure and a 3’ polyA tail after the introns in the precursor mRNA are cleaved, while circRNA does not have the 5 ‘cap and 3’ polyA tail structures. circRNA prompts the downstream 5 ‘splicing site to approach the upstream 3’ splicing site through the reverse Alu repeat sequence within the intron region, thereby triggering the reverse splicing process of introns. Depending on whether introns are retained or not. It is divided into exon circRNA (composed entirely of exons) and exon-intron circRNA (containing both introns and exons) [5]. There are three mechanisms for the generation of exon circRNAs. Multiple exons (2 to 6 exons) are cleaved by introns, and the two exons are connected in reverse. The second mechanism is formed by complementary pairing of intron sequences at both ends of a single exon, and then the intron sequences at both ends are removed [6]. In addition to the above two, RNA-binding proteins are also a factor that induces circRNA cyclization in exons. For instance, myoblind protein (MBL) binds to flanking introns and pulls them closer, promoting reverse splicing. Another RNA-binding protein, DEXH box helicase 9 (DHX9), functions as an RNA helicase and can unwind the pairing between reverse repeat sequences in introns [7,8]. Intron circRNA attacks the 5 ‘exon terminal through the 2’ -OH terminal of the intron, and the released intron is reversely connected.e proceedings, and not as an independent document. Please do not revise any of the current designations.

B. The Function of circRNA

Abnormal expression of circRNA has been detected in a variety of cancers. circRNA mainly regulates cell activity through the following four ways.

1) Regulate the RNA interaction network.

circRNA can act as miRNA adsorption, and circRNA has sites for binding to miRNA. miRNA can bind to the 3 ‘end of specific mRNA and mediate post-transcriptional gene silencing in cells. circRNA can relieve the inhibitory effect of miRNA on its target mRNA [5]. For example, cerebellar demutation-associated protein 7 (CIRS-7), which is transcribed from the antisense strand of vertebrate cerebellar dysregulation genes on the genome,

can negatively regulate miR-7 and can be detected in gastric cancer and liver cancer cells. It contains 74 miR-7 binding sites. ciRS-7 can competitively bind miR-7 to the mRNA of the downstream target genes of miR-7, and miR-7 can regulate various protein genes to participate in the development process of tumors. In addition to serving as a miRNA molecular sponge, circRNA can also interact with RNA-binding proteins. circRNA can play a role in promoting protein binding. For example, circFoxo3 binds to DNA binding inhibitor (ID-1) and adhesion spot kinase (FAK) proteins in the cytoplasm. This prevents these proteins with anti-aging and anti-stress effects from entering the cell nucleus to exert their functions, thereby leading to cellular aging. Contrary to the previous function, circRNA can also play the role of protein isolation. Taking papilloma thyroid as an example, highly expressed circRNA-102171 blocks its interaction with the ß -catenin complex by competiently binding to the ß -catenin interacting protein, thereby accelerating tumor progression [9].

2) Regulation of Gene Expression

circRNA can regulate gene expression at the transcriptional level. For instance, nuclear circRNAs such as circELF3J and PAIP2 interact with U1 small nuclear ribonucleoprotein and RNA polymerase in the nucleus, enhancing the transcriptional level of their parental genes and thereby influencing protein translation [6]. Additionally, circRNA may act as an activator of RNA polymerase II to upregulate gene transcription. Research has shown that after the knockout of circRNA-ankrd52, the expression level of ankrd52 significantly decreased, indicating that circRNA-ankrd52 is a circRNA formed during the transcription process of ankrd52 [10]. Moreover, recent studies have demonstrated that circRNA can function by encoding proteins or peptides. circRNA possesses an open reading frame and can encode proteins. Some circRNAs can encode proteins with oncogenic or tumor suppressor properties. The PLCE-411 protein encoded by circular PLCE1 can significantly inhibit the proliferation and metastasis of colorectal cancer cells. The circular ATG4B protein derived from exosomes can activate the autophagy pathway by encoding a new protein, thereby inducing resistance to oxaliplatin in colorectal cancer cells [4]. There are multiple translation mechanisms for circRNA, including IRES-dependent, m6A-dependent, and rolling circle amplification types. For example, circular SHPRH is translated into a 146aa protein through IRES-mediated translation, and this product can stabilize the full-length SHPRH protein and exert a tumor suppressor effect [4]. cicZNF609 can be translated into zinc finger protein 609 in an IRES-dependent manner, which plays a crucial role in muscle differentiation, but its translation activity is much lower than that of linear RNA [6]. Therefore, circRNA can regulate gene expression through various means, including both transcriptional and translational levels. However, the regulation of DNA replication by circRNA remains to be explored.

3) Cell Physiological Regulation

The cell cycle refers to the dynamic process in which a cell undergoes genomic replication and divides into two daughter cells. Its dysregulation is closely related to cancer occurrence. The malignant proliferation of tumors often results from the functional abnormalities of cell cycle regulatory proteins, such as the imbalance of key kinase activities. In thyroid cancer, the abnormal elevation of circDOCK1 can simultaneously drive the upregulation of cyclin D1 and the inhibition of p35 protein, thereby causing the disorder of cyclin-dependent kinase (CDK) activity and ultimately promoting the rapid proliferation of tumor cells. circHIPK3 promotes glioma growth by regulating the distant homeobox gene 2 (DLX2) through miR-124, and circCCDC66T inhibits caspase activation by binding to protein kinase B (AKT). Studies have shown that the AMPK signaling pathway induces cell cycle arrest at the G1/S phase by activating the p53-p21 axis. circ-0001666 is highly expressed in papillary thyroid cancer, and its downregulation can lead to G1 phase arrest and reduce the level of cyclin D1. Currently, most studies focus on how oncogenic circRNAs drive tumor progression by interfering with cell cycle checkpoints, but the direct interaction patterns with mRNA or proteins need further clarification.

III. Evaluation of Application Strategies for circRNA

A. Discovery of Tumor Markers

circRNA has a stable structure and lacks the 5’ cap and 3’ poly A tail, which makes it resistant to ribonuclease digestion. Compared with linear RNA, circRNA has a longer half-life and higher stability, and its expression is conserved and specific. This makes circRNA a potential biomarker for cancer. Compared with the relatively small number of mRNAs, circRNAs are easier to detect. Numerous studies have shown that circRNAs can accumulate stably in the peripheral blood of cancer patients, and the related circRNAs in serum are reduced during treatment, indicating that circRNAs can serve as biomarkers for cancer treatment. Due to their stability and diversity, circRNAs can accumulate in body fluids and tissues. CircRNAs have the characteristic of being less invasive, thus having high diagnostic potential and facilitating real-time

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dynamic monitoring by researchers [6]. For example, circ1662 and circPACRGL are significantly upregulated in colorectal cancer patients, indicating their specificity for cancer. A large number of studies have shown that circRNAs play a key role in cancer signaling pathways, including PI3K/Akt, JAK/STAT, GEF-H1/RhoA, Wnt/β-catenin, and TNF-β/Smad pathways, by upregulating oncogene expression, downregulating tumor suppressor genes, or downregulating downstream genes [4]. Current research indicates that abnormal expression of circRNAs, with enhanced disease specificity, has clinical significance.

B. Detection of circRNA

CircRNA as a tumor marker still has some limitations. Due to its sequence being highly similar to linear RNA, specific detection techniques need to be developed to distinguish its covalently closed circular structure. Conventional qRT-PCR may produce false positive results if primers are designed based on the linear genome [4]. Traditional RNA sequencing often overlooks circRNA because the construction of standard RNA libraries relies on the poly(A) tail, which is absent in circRNA. To effectively identify circRNA, researchers have focused on finding "back-splicing sites", a splicing pattern that is diverse and subject to interference from introns, increasing the complexity of detection. To overcome these difficulties, researchers have developed various techniques, such as high-throughput sequencing technology, which can identify circRNA on a large scale but is costly and requires complex bioinformatics processes for data analysis, thus still having limitations. Another method is to remove linear RNA. RNase R is a commonly used exonuclease that can degrade linear RNA without affecting circRNA, thereby enriching circRNA.

CircRNA can promote tumor metastasis, proliferation, and invasion, and induce apoptosis. For example, circSPAC is upregulated in colorectal cancer cells and is positively correlated with advanced TNM stage, lymph node metastasis, and poor survival rate of patients [4]. Cancer progression involves a dynamic balance between apoptosis inhibition and autophagy activation. CircRNA becomes a key target for treating drug resistance by regulating AS (alternative splicing) and autophagy pathways. AS is accomplished by the cooperation of spliceosomes, splicing sites, and cis-acting elements, and its abnormalities are closely related to gene mutations or regulatory disorders in cancer. Tumor stem cells rely on autophagy to maintain self-renewal ability and escape treatment by upregulating drug resistance-related genes. For instance, circDNATT1 is highly expressed in breast cancer and enters the nucleus after binding to the tp53 protein to activate autophagy genes. Autophagy has a dual role, supporting tumor cell survival and enhancing the sensitivity of specific treatments. Additionally, circRNA plays an important role in cancer cell drug resistance. Drug resistance refers to the tolerance of microorganisms, parasites, and tumor cells to chemotherapy drugs. Drug resistance may occur in tumor drug treatment, such as the limitations of platinum-based drugs like cisplatin in the treatment of thyroid cancer, liver cancer, and gastric cancer. CircRNA can affect the sensitivity of tumor cells to chemotherapy drugs by adsorbing miRNA, regulating signaling pathways, and controlling DNA damage repair (DDR). For example, in breast cancer, there are multiple drug resistance mechanisms. In the paclitaxel resistance mechanism, circRNA can activate pro-survival signaling pathways, upregulate anti-apoptotic proteins, and bind to miRNA to inhibit the invasion and autophagy of drug-resistant cells, significantly reducing the efficacy of paclitaxel. Targeting specific circRNA may become a new strategy to reverse drug resistance and enhance the effectiveness of chemotherapy [11]. Exosomes are extracellular vesicles with a diameter of 30-200 nm that can carry proteins, nucleic acids, and other molecules to participate in intercellular communication. Multiple studies have shown that exosomal circRNA has significant value in cancer diagnosis. For instance, circRASSF2 is overexpressed in the serum exosomes of thyroid cancer patients, and its level is positively correlated with tumor stage and lymph node metastasis [12]. Mesenchymal stem cells (MSCs) have become powerful tools in cancer treatment due to their unique chemotactic properties and multi-directional differentiation capabilities. MSCs can precisely migrate to tumor injury sites to achieve targeted drug delivery. The exosomes secreted by MSCs can carry circRNAs and possess low immunogenicity and high stability. By introducing oncogenes or tumor suppressor genes, MSCs can specifically kill tumor cells. Therefore, gene-engineered MSCs can be used to treat tumors [13].

IV. Conclusion

This article systematically summarizes the regulatory role of circRNAs in tumor occurrence and development as well as their clinical application potential. Research indicates that circRNAs, formed through reverse splicing into stable circular structures, can act as molecular sponges for miRNAs, regulate gene transcription and translation, influence cell cycle and autophagy processes, and play a key role in tumor drug resistance. Their functional diversity gives them unique advantages in tumor biomarker screening, dynamic monitoring, and targeted therapy. For instance, the detection of circRNAs in body fluids pro-

vides a non-invasive research approach for early cancer screening, while the strategy of delivering circRNAs via mesenchymal stem cells opens up new avenues to overcome drug resistance. The significance of this article lies in revealing the potential of circRNAs as core nodes in the tumor regulatory network. Their stability and specificity lay a theoretical foundation for the development of novel diagnostic tools and precise therapeutic strategies. Future research should focus on the following directions: (1) Developing highly specific detection methods and efficient delivery systems to promote the clinical application of circRNA biomarkers and therapeutic strategies; (2) Exploring the synergistic effects of circRNAs with other non-coding RNAs and constructing multi-dimensional molecular interaction models. Through interdisciplinary collaboration and technological breakthroughs, circRNAs are expected to become a significant breakthrough point in the field of precision cancer medicine, providing new ideas for improving patient prognosis.

References

[1] Li M., Hu M., Jiang L., Pei J., and Zhu C., "Trends in cancer incidence and potential associated factors in China," *JAMA Network Open*, vol. 7, no. 10, p. e2440381, 2024.

[2] Diao X., Guo C., Jin Y., Li B., Gao X., Du X., Chen Z., Jo M., Zeng Y., Ding C., Liu W., Guo J., Li S., and Qiu H., "Cancer situation in China: an analysis based on the global epidemiological data released in 2024," *Cancer Communications (London, England)*, vol. 45, no. 2, pp. 178–197, 2025.

[3] Zhang L., Gao H., Li X., Yu F., and Li P., "The important regulatory roles of circRNA encoded proteins or peptides in cancer pathogenesis (Review)," *International Journal of Oncology*, vol. 64, no. 2, p. 19, 2024.

[4] Zhang Y., Luo J., Yang W., and Ye W. C., "CircRNAs in colorectal cancer: potential biomarkers and therapeutic targets," *Cell Death & Disease*, vol. 14, no. 6, p. 353, 2023.

[5] Hwang H. J. and Kim Y. K., "Molecular mechanisms of circular RNA translation," *Experimental & Molecular Medicine*, vol. 56, no. 6, pp. 1272–1280, 2024.

[6] Zhu G., Chang X., Kang Y., Zhao X., Tang X., Ma C., and Fu S., "CircRNA: A novel potential strategy to treat thyroid cancer (Review)," *International Journal of Molecular Medicine*, vol. 48, no. 5, p. 201, 2021.

[7] Hwang H. J. and Kim Y. K., "Molecular mechanisms of circular RNA translation," *Experimental & Molecular Medicine*, vol. 56, no. 6, pp. 1272–1280, 2024.

[8] Wang X., Chen T., Li C., Li W., Zhou X., Li Y., Luo D., Zhang N., Chen B., Wang L., Zhao W., Fu S., and Yang Q., "CircRNA-CREIT inhibits stress granule assembly and overcomes doxorubicin resistance in TNBC by destabilizing PKR," *Journal of Hematology & Oncology*, vol. 15, no. 1, p. 122, 2022.

[9] Bi W., Huang J., Nie C., Liu B., He G., Han J., Pang R., Ding Z., Xu J., and Zhang J., "CircRNA circRNA_102171 promotes papillary thyroid cancer progression through modulating CTNNBIP1-dependent activation of β-catenin pathway," *Journal of Experimental & Clinical Cancer Research: CR*, vol. 37, no. 1, p. 275, 2018.

[10] Li X., Zhang J. L., Lei Y. N., Liu X. Q., Xue W., Zhang Y., Nan F., Gao X., Zhang J., Wei J., Yang L., and Chen L. L., "Linking circular intronic RNA degradation and function in transcription by RNase H1," *Science China Life Sciences*, vol. 64, no. 11, pp. 1795–1809, 2021.

[11] Yang S. and Li D., "The role of circRNA in breast cancer drug resistance," *PeerJ*, vol. 12, p. e18733, 2024.

[12] Mao M. W., Zhang Z. Y., Wei Y. H., and Wang Z. F., "Circular RNA translation and its application in drug development," *Science China Life Sciences*, vol. 54, no. 4, pp. 602–617, 2024.

[13] Fu M., Fang L., Xiang X., et al., "Microarray analysis of circRNAs sequencing profile in exosomes derived from bone marrow mesenchymal stem cells in postmenopausal osteoporosis patients," *Journal of Clinical Laboratory Analysis*, vol. 36, no. 1, p. e23916, 2022.