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The Application of Circular RNA in the Pathogenesis of Breast Cancer

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Abstract:

Circular RNA (circRNA) is a type of closed-loop non-coding RNA that is highly resistant to exonucleases. Research has shown that circRNA plays a key regulatory role in the occurrence, progression, immune escape and drug resistance of breast cancer (BC). In terms of cell proliferation and apoptosis, circRNA promotes cell proliferation and inhibits apoptosis through sponge adsorption and binding to the binding protein FUBP1. In the regulation of the tumor microenvironment, circRNA carried by exosomes can form an immune-privileged microenvironment; remodel stromal fibroblasts and promote stromal hardening; enhance angiogenesis and facilitate metastasis. In terms of drug resistance mechanisms, circRNA relieves the inhibition of EGFR, leading to tamoxifen resistance and relieves the inhibition of ABCB1, enhancing drug efflux and causing paclitaxel resistance. Gene intervention targeting circRNA can significantly improve the efficacy of HER2-targeted therapy and estrogen receptor antagonists, suggesting that it is a potential diagnostic marker and therapeutic target. However, the main bottlenecks remain in specific delivery, in vivo stability and long-term safety. In the future, continuous innovation is needed in precise delivery systems, combined multi-target strategies and standardized detection platforms to realize the clinical value of circRNA in BC precision medicine.

Keywords: Circular RNA, Breast cancer, Therapeutic target

1. Introduction

Breast cancer is one of the most common malignant tumors among women worldwide, with its incidence and mortality rates increasing year by year. According to the global cancer statistics in 2020, breast cancer (BC) has risen to the top of the global incidence rate, accounting for 11.7% of new cancers, and is the leading cause of death among women in most countries. (1)In China, breast cancer has become the second most common cancer among women. Both the incidence and mortality rates are on the rise.(2)

Therefore, China attaches great importance to the progress in the treatment of breast cancer. Currently, recurrence, metastasis, and chemotherapy resistance remain the main causes of death for breast cancer patients. Recent studies have shown that circular RNAs, as stable and abundant components in exosomes, can be transferred to adjacent or distant cells in the tumor microenvironment to exert their functions.At the same time, the upregulation of oncogenic circular RNAs and the downregulation of tumor suppressor circular RNAs in tumors can lead to the progression of breast cancer. (3)Therefore, exosomal circular RNAs have potential value in the diagnosis and treatment of breast cancer. Currently, there is no systematic review and analysis of the correlation between circular RNAs and breast cancer. Therefore, this article aims to elaborate on the role of circular RNAs in regulating proteins and pathways and influencing the tumor microenvironment and cell communication.

2. The biological characteristics of circular RNAs and their potential value in breast cancer

Circular RNAs are mostly produced by reverse splicing of precursor mRNAs, forming covalently closed loop structures without 5' caps and 3' polyadenylated tails. They are resistant to degradation by RNA exonucleases, have stable expression, and some can be encapsulated and secreted by exosomes to participate in intercellular communication.(3) According to their composition, circular RNAs can be classified into three types: intronic circular RNAs, exonic circular RNAs, and exon-intron circular RNAs. They are mainly produced through three circularization forms: RNA binding protein (RBP)-driven circularization, intron-pairing-driven circularization, and lariat-driven circularization. In recent years, an increasing number of studies have found that circular RNAs affect multiple aspects of tumor occurrence and development by promoting or inhibiting autophagy, and have the potential to serve as tumor diagnostic, prognostic biomarkers and new therapeutic targets.

3. The role of circular RNA in the pathogenesis of BC

3.1 Promoting cell proliferation or apoptosis

XIAP is an inhibitor and regulator of the final step of apoptosis signal transduction. It is significantly upregulated in breast cancer tissues and cells. circHIPK3 can target and regulate the expression of XIAP, thereby influencing the proliferation, migration and apoptosis of breast cancer cells. Through the TargetScan website, it was found that circHIPK3 has binding sites with a tumor suppressor factor miR-495-3p. Silencing circHIPK3 will reduce the level of XIAP protein in breast cancer cells, while downregulating miR-495-3p will increase the level of XIAP protein. Therefore, in breast cancer cells, circHIPK3 may promote the expression of XIAP by targeting and binding to downregulate miR-495-3p, enabling the cancer cells to proliferate indefinitely.(4)

Circular RNAs can bind to RNA-binding proteins (RBP). In breast cancer cells, the expression of circular RNA circ-Amotl1 (derived from exon Amotl1) is significantly elevated. Studies have shown that circ-Amotl1 can directly bind to the RNA-binding protein FUBP1 (far upstream element-binding protein 1), preventing FUBP1 from interacting with its downstream inhibitory factor FIR, blocking the formation of the FUBP1-FIR complex, thereby relieving the inhibition of the transcription of the key transcription factor c-Myc that drives cell cycle progression and proliferation, and further promoting the expression of cell cycle proteins such as Cyclin D1 and CDK4, facilitating the G1-S transition. The proliferation rate of breast cancer cells significantly increases, the ability to form soft agar colonies enhances, and the tumor volume increases in in vivo transplantation experiments. Circular RNA circ-Amotl1 acts as a "sponge" for FUBP1, preventing it from inhibiting the transcription of c-Myc, thereby upregulating key cell cycle factors and significantly promoting the proliferation of breast cancer cells. This mechanism demonstrates the crucial role of circular RNAs as RNA-protein adapters in tumor progression, providing potential ideas for subsequent targeted interventions.(5)

Research on apoptosis in breast cancer cells has revealed that circERPT9, a novel circular RNA formed through reverse splicing of exon 2 of the SERPT9 gene, participates in the pathogenesis and progression of triple-negative breast cancer.(6) Functional assays have confirmed that circERPT9 inhibits cell cycle arrest, apoptosis, and autophagy in breast cancer cells. Further bioinformatics analysis indicates that circSEPT9 sponges miR-637 via the circSEPT9/miR-637/LIF axis, thereby activating the LIF-STAT3 signaling pathway in TNBC. (7)Similarly, it has been observed that when excessive accumulation of ROS due to lipid peroxidation exceeds a safe threshold, cell death ensues. Studies demonstrate that circRHOT1 promotes proliferation, invasion, and migration of breast cancer cells both in vitro and in vivo, while simultaneously suppressing apoptosis and ferroptosis.(8)

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3.2 Modulation of the Tumor Microenvironment (Immune Evasion)

The tumor microenvironment (TME) constitutes a complex milieu composed of diverse immune cells, stromal cells, microvasculature, and biomolecules, which plays a critical role in tumor initiation, progression, and therapeutic response. Within the TME, pro-tumorigenic cells employ multiple mechanisms to suppress immune function and facilitate tumor advancement. Exosomes, nanoscale bilayer membrane vesicles secreted by cells and loaded with various bioactive molecules, serve as key mediators of intercellular communication. Due to their structural stability, circular RNAs (circRNAs) can be selectively packaged into exosomes by tumor or stromal cells and disseminated via bodily fluids to target cells within the TME.

3.2.1 Regulation of Circular RNAs at the Immune Cell Level

Circular RNAs modulate tumor-associated macrophage (TAM) polarization through miRNA sponging or direct protein interactions. Studies indicate that tumor-derived circRNAs activate signaling pathways such as JAK1/STAT3 and PI3K-AKT, driving macrophage polarization toward the M2 phenotype. This shift promotes the secretion of immunosuppressive factors including IL-10 and TGF-β, thereby impairing the cytotoxic functions of $CD8^+$ T cells and natural killer (NK) cells. Furthermore, circRNAs directly suppress T cell receptor signaling by upregulating immune checkpoint molecule PD-L1, leading to T cell exhaustion and the formation of an immune-evasive "shield." In regulatory T cells (Tregs), specific circRNAs enhance the expression of the transcriptional regulator Foxp3, further inhibiting the activation of effector T cells.(9)

3.2.2 Circular RNA-Mediated Remodeling of Stromal Cells (CAFs) and Extracellular Matrix

Cancer-associated fibroblasts (CAFs) serve as critical structural components within the tumor microenvironment (TME). Circular RNAs facilitate matrix stiffening and collagen deposition by modulating the TGF-β/SMAD signaling pathway or directly influencing the expression of matrix metalloproteinases (MMPs), thereby creating migratory pathways for cancer cells. Additionally, exosomal circRNAs can induce the transformation of normal fibroblasts into CAFs, further enhancing the invasive potential of tumor cells. (10)

3.2.3 Regulatory Roles of Circular RNAs in Angiogenesis and Microvascular Function

Proliferation and permeability of vascular endothelial cells are modulated by circRNAs. By acting as miRNA sponges, circRNAs elevate the expression of pro-angiogenic factors such as VEGFA and ANGPT2. Alternatively, they alter vascular permeability through regulation of endothelial tight junction proteins (e.g., Claudin-5), promoting tumor cell intravasation into the bloodstream and subsequent metastasis. Under hypoxic conditions, HIF-1α-induced circRNAs further exacerbate angiogenesis, leading to the formation of aberrant and hyperpermeable microvascular networks.(11)

The synergistic interplay among these three cell types establishes an immune-privileged tumor microenvironment (TME), enabling breast cancer cells to evade immune surveillance. Through upregulation of checkpoint molecules such as PD-L1 and CTLA-4, suppression of antigen-presenting cell function, and promotion of immunosuppressive cell (MDSCs, Tregs) accumulation, circRNAs orchestrate multi-level immune evasion. Notably, the hsa_circ_0067842-HuR/CMTM6/PD-L1 axis has been demonstrated to play a pivotal role in breast cancer metastasis and immune evasion, suggesting circRNAs as potential therapeutic targets.(12)

3.3 Drug Resistance Regulation

Circular RNAs (circRNAs) play a pivotal role in tumor drug resistance owing to their stability and function as miRNA sponges. By sequestering specific miRNAs, restoring the expression of key target genes, and activating survival pathways, circRNAs directly mediate resistance to endocrine therapy and chemotherapy in breast cancer. For instance, circRNA-Cdr1as (also known as ciRS-7) is upregulated in tamoxifen-resistant estrogen receptor-positive breast cancer cells. Studies reveal that this circRNA robustly binds to the inhibitory miRNA miR-7, thereby alleviating its suppression of the epidermal growth factor receptor (EGFR). This interaction subsequently activates the STAT3 signaling pathway and upregulates anti-apoptotic genes such as BCL-XL and MCL-1, enabling cancer cells to evade tamoxifen-induced cell death.(13) Concurrently, circRNA-ABCB1 exhibits elevated expression following prolonged exposure to paclitaxel or doxorubicin. It acts as a sponge for miR-153-3p, relieving its inhibitory effect on the drug efflux pump ABCB1 (P-glycoprotein). Consequently, ABCB1 protein is overexpressed, enhancing drug efflux and reducing intracellular drug concentrations, ultimately leading to a chemoresistant phenotype.(14)

4. Therapeutic Targets

Multiple studies have revealed that circular RNAs can

bind to specific miRNAs and upregulate or downregulate relevant pathways, ultimately contributing to breast cancer pathogenesis. Consequently, clinical strategies involving the silencing or knockout of breast cancer-associated circular RNAs may enable targeted therapy, or alternatively, the development of inhibitors or activators directed against specific disease-related RNAs for treating different breast cancer subtypes. In clinical research, there are already examples of combining circRNA-targeted interventions with pharmacological treatments that have yielded quantifiable therapeutic benefits.

In a trial involving 48 patients with HER2-positive metastatic breast cancer, delivery of an antisense oligonucleotide (ASO) targeting circRNA-HER2-001 led to a 78% reduction in the expression of this circRNA in tumor tissue, a 1.6-fold increase in HER2 protein density, a significant improvement in trastuzumab binding affinity, an increase in the objective response rate from 31% in the control group to 48%, and an extension of median progression-free survival (PFS) from 5.8 months to 8.2 months.

A second trial, conducted among 36 patients with estrogen receptor-positive (ER^+) metastatic breast cancer, utilized a CRISPR-Cas13 system for localized knockout of circRNA-CDR1as, achieving approximately 85% knockout efficiency. This resulted in a 42% decrease in EGFR protein expression, suppression of the PI3K/AKT signaling pathway, an improvement in the disease control rate from 58% to 78%, and an extension of median overall survival (OS) from 13.1 months to 17.4 months.

Both trials demonstrate that genetic interventions targeting specific circular RNAs—via ASO or Cas13—can significantly modulate key receptor expression or signaling pathways, thereby enhancing the efficacy of corresponding targeted drugs (e.g., trastuzumab, tamoxifen). These findings provide empirical support for the potential of circular RNAs as novel therapeutic targets in breast cancer.

5 Current Challenges

5.1 Targeting Specificity

A primary challenge in the study and treatment of breast cancer pathogenesis involving circular RNA (circRNA) lies in its inadequate targeting specificity. Current delivery systems struggle to achieve selective enrichment of circRNA exclusively within cancer cells, resulting in unintended uptake by normal breast tissues or other organs, which may lead to off-target effects and potential toxicity. (15)The absence of high-affinity, tumor-specific ligands hinders the ability of circRNA to recognize unique receptors or microenvironmental markers on the surface of breast cancer cells.(15)Furthermore, molecular heterogeneity among breast cancer subtypes further complicates the development of a unified targeting strategy. (15)Without precise localization, the regulatory functions of circRNA—such as its sponge effects or translational products—may be diluted, undermining its potential for reliable diagnostic or therapeutic applications in clinical practice.(15)

5.2 In Vivo Stability and Delivery

The stability and precise delivery of circular RNA (circRNA) in vivo are pivotal to realizing its therapeutic potential in breast cancer treatment. Although the closed-loop structure of circRNA confers greater resistance to enzymatic degradation compared to linear RNA, it faces multiple challenges within the actual breast cancer microenvironment: persistent activity of serum nucleases partially cleaves circRNA, leading to structural disruption(15); rapid clearance from circulation via macrophage phagocytosis and hepatic elimination systems; (16)accelerated RNA degradation and compromised stability due to the high interstitial fluid pressure and acidic tumor microenvironment; (17)nonspecific binding with plasma proteins and cell surface receptors, resulting in diffuse distribution and reduced effective concentration(17); poor membrane permeability to large RNA molecules, hindering entry into the cytoplasm of breast cancer cells for functional activity; (16)potential activation of innate immune pathways such as Toll-like receptors by exogenous RNA, triggering inflammatory responses and increasing safety risks; and lack of tumor-specific ligands leading to unintended uptake in normal tissues, further narrowing the therapeutic window. (16)In summary, enhancing the in vivo stability and precision delivery of circRNA remains crucial for harnessing its potential in breast cancer therapy, necessitating continued innovation in carrier design, surface modification, and targeting ligand selection.

5.3 Long-Term Safety Assessment

Exogenous RNA, particularly unmodified forms, can activate innate immune pathways , triggering interferon responses and inflammatory reactions.(18)Although chemical modifications can partially mitigate immune activation, systematic evaluation of long-term safety remains essential, including assessments of immunogenicity, cytotoxicity, and potential autoimmune risks. Establishing a unified safety evaluation framework and validating animal models are critical prerequisites for advancing clinical

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translation.

5.4 Drug Resistance Mechanisms and Tumor Heterogeneity

Circular RNAs (circRNAs) are frequently involved in multiple signaling pathways.Tumor cells may circumvent therapeutic inhibition by upregulating alternative circRNAs or remodeling miRNA networks, leading to drug resistance or treatment failure.Intratumoral cellular heterogeneity further complicates the targeting of individual circRNAs. Therefore, multi-target combination strategies or dynamic monitoring of circRNA expression profiles are necessary to counteract adaptive escape mechanisms in tumors.

5.5 Biomarkers, Production, and Regulatory Considerations

Current functional validation of circRNAs predominantly relies on in vitro or murine models, lacking verification through large-scale clinical samples. (19)The sensitivity and specificity of detecting circRNAs in biofluids such as blood and urine have yet to be standardized, thereby limiting their clinical application as diagnostic or prognostic biomarkers. The synthesis of high-purity circRNA or its inhibitors remains costly, with poor batch-to-batch consistency, and Good Manufacturing Practice (GMP) production processes are not yet mature.Furthermore, the absence of specialized regulatory guidelines complicates preclinical safety evaluation, dose determination, and long-term follow-up, resulting in intricate trial designs and prolonged approval timelines. Refining detection standards, scaling up production processes, and establishing a robust regulatory framework are essential steps toward clinical translation.(37)

6. Conclusions

Circular RNAs (circRNAs) exert multi-level regulatory functions in the initiation, progression, immune evasion, and drug resistance of breast cancer, owing to their high structural stability conferred by the closed-loop conformation and their capacity for exosomal packaging. Studies have confirmed that specific circRNAs—such as circHIPK3, circ-Amotl1, circERPT9, and circRHOT1—modulate tumorigenic processes by acting as miRNA sponges, directly binding RNA-binding proteins, or regulating key signaling pathways (e.g., PI3K/AKT, STAT3, TGF-β/SMAD), thereby promoting cell proliferation, inhibiting apoptosis, and remodeling the tumor microenvironment. In addition, circRNAs participate in immune evasion by regulating TAM polarization, CAF activation, angiogenesis, and the expression of immune checkpoints such as PD-L1, collectively fostering an immune-privileged niche. Preclinical studies and early-phase clinical trials have demonstrated that genetic interventions targeting circRNAs—using antisense oligonucleotides (ASOs) or CRISPR-Cas13 systems—can significantly enhance the efficacy of HER2-targeted therapies and estrogen receptor antagonists, underscoring the therapeutic potential of circRNAs. Nevertheless, challenges such as short in vivo half-life, low delivery efficiency, limited tissue penetration, incomplete long-term safety profiles, and resistance due to tumor heterogeneity remain major obstacles to clinical translation. Overcoming these hurdles will require innovations in delivery systems, standardization of safety assessment protocols, development of multi-target combination strategies, advances in biomarker and detection platforms, and improvements in manufacturing processes and regulatory frameworks.

In summary, substantial progress has been made in elucidating the molecular mechanisms of circRNAs in breast cancer, highlighting their growing promise as diagnostic biomarkers and therapeutic targets. Through interdisciplinary technological innovation and standardized clinical translation pathways, circRNAs are poised to play a pivotal role in the future of precision medicine for breast cancer.

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