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Research Progress of circRNAs during Epithelial-Mesenchymal Transition of Hepatocellular Carcinoma

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Abstract: Hepatocellular carcinoma is prone to invasion and metastasis. It often receives a low diagnosis rate in the early stage but has an extremely high mortality rate. Epithelial-mesenchymal transformation (EMT) is a key factor in promoting tumor cell invasion and metastasis. Circular RNA (circRNA) is involved in regulating EMT in hepatocarcinoma cells through multiple pathways, thereby affecting the occurrence and progression of hepatocellular carcinoma. This article mainly reviews the research progress of circRNA related to EMT core transcription factors, circRNA that promotes EMT in liver cancer, and circRNA that inhibits EMT in liver cancer.

Keywords: circRNA; Epithelial-mesenchymal transformation (EMT); Hepatocellular carcinoma (HCC)

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1. Introduction

China is one of the countries with the highest incidence and mortality rates of liver cancer. More than 50% of newly diagnosed and deceased hepatocellular carcinoma (HCC) patients worldwide occur in China, ranking fourth in the incidence of common malignant tumors and second in tumor-related mortality [1,2]. In 2020, there were 905,700 new cases and 830,200 deaths from liver cancer globally, ranking sixth in the incidence of malignant tumors and third in tumor-related mortality. Primary liver cancer is a diverse group of tumors, including HCC, intrahepatic cholangiocarcinoma, and mixed hepatocellular carcinoma-cholangiocarcinoma, with significant differences in etiology, clinical characteristics, histopathology, treatment, and prognosis. Among them, HCC accounts for 85% to 90% of all cases [3].

Currently, although liver ultrasound imaging and serum alpha-fetoprotein (AFP) testing have been widely used for early screening of clinical liver cancer, the sensitivity and specificity of AFP detection are limited. At the same time, the accuracy of the ultrasound examination is greatly influenced by the operator's subjective judgment, and traditional ultrasound examination often struggles to accurately differentiate the nature of liver

focal lesions [4].

The treatment strategy for HCC varies depending on the patient's disease stage, liver function status, and individual differences. Only a few early-stage liver cancer patients can achieve a better prognosis through surgical resection, radiofrequency ablation, or liver transplantation. However, most patients are diagnosed at an advanced stage, limiting treatment options, and the efficacy of traditional chemotherapy and radiotherapy is poor. In recent years, emerging therapies such as targeted therapy and immunotherapy have brought new hope to advanced-stage patients [5].

Therefore, in-depth exploration of the pathogenesis of HCC and the search for novel biomarkers to improve the early detection rate of HCC is of great significance for the early diagnosis and treatment of HCC patients. This not only helps improve patient prognosis and survival rates but also enhances their quality of life.

Circular RNA (circRNA), first discovered in viruses in the 1970s, is a type of RNA with a unique natural closed-loop structure. It lacks the 5'-3' polarity and polyA tail commonly found in linear RNAs, making it more stable and resistant to degradation by RNA exonucleases. However, due to the limitations of early knowledge and technological capabilities, circRNA was initially regarded as a non-coding RNA without biological functions. It was considered to be a byproduct of RNA transcription and splicing processes, which led to a lack of in-depth research on circRNA. In recent years, with the application of high-throughput sequencing technology and bioinformatics analysis in clinical diagnostics and basic research, our understanding of circRNA has undergone profound changes. Nowadays, circRNA is recognized as a group of molecules with diverse biological functions, playing important roles in gene expression regulation, cellular physiology, and disease development. Furthermore, numerous studies have identified circRNAs associated with HCC, suggesting their potential as novel diagnostic markers and targets for drug development in HCC [6].

Based on these prospects, this article aims to provide a comprehensive review of the research progress on circRNA in the context of hepatocellular carcinoma epithelial-mesenchymal transition.

2. Circular RNA

The typical splicing pattern of linear RNA involves joining the 3' end of one exon with the 5' end of an adjacent exon. In contrast, circRNAs are formed by back-splicing, where the 3' end of an RNA molecule is joined to its 5' end, creating a closed-loop structure. This splicing pattern can occur at any position in the genome, including introns, exons, and the antisense strand. Based on their formation mechanisms, circRNAs can be categorized into four types: circular intronic RNAs (ciRNAs), exonic circRNAs (EciRNAs), exon-intron circRNAs (EIciRNAs), antisense circRNAs, and circRNAs derived from intergenic or unannotated genomic sequences (intergenic circular RNAs). EciRNAs consist solely of exonic sequences and represent approximately 80% of all identified circRNAs. They are associated with RNA polymerase II and promote gene transcription, mainly located in the cytoplasm. ciRNAs are composed entirely of intronic sequences and primarily reside in the nucleus. EIciRNAs contain sequences from both exons and introns, and they can interact with small nucleolar ribonucleoprotein U1snRNP to form complexes that further enhance parental gene transcription [7,8].

There are three proposed models for the circular formation of circRNAs: intron pairing-driven circularization, RNA binding protein (RBP)-driven circularization, and lariat-driven circularization [9]. The functions of circRNAs mainly include:

(1) miRNA sponge activity: circRNAs have abundant miRNA binding sites, allowing them to interact with miRNAs and act as molecular sponges that sequester miRNAs, thereby relieving the inhibitory effect of miRNAs on their target genes and increasing the expression levels of these target genes. This

mechanism, known as the competitive endogenous RNA (ceRNA) mechanism, plays a crucial role in cellular growth, differentiation, and disease regulation [10].

(2) Regulation of protein binding: Some circRNAs contain specific binding sites for RNA binding proteins (RBPs) and can act as sponges for these protein molecules. They can inhibit the specific functions of RBPs and the transcription of parental genes by forming RNA-protein complexes [11].

(3) Encoding peptides: Although circRNAs were initially considered non-coding RNAs, recent research has shown that some circRNAs have the ability to encode peptides. These short peptides can be translated by ribosomes in specific cellular environments, participating in cellular regulatory networks.

(4) Influencing transcriptional regulation of host genes: circRNAs can interact with small nucleus ribonucleoprotein U1, affecting the transcriptional regulation of host genes. After U1 binds to circRNA, it forms a complex with RNA polymerase II, which interacts with the transcription factor IIH gene, thereby affecting the activity of RNA polymerase II and influencing the expression of the parental gene [12].

The closed-loop structure of circRNAs allows them to evade degradation by RNA enzymes, enhancing their stability. As a result, circRNAs can be detected in various body fluids such as saliva, blood, and urine. Additionally, circRNAs exhibit high tissue specificity, with ciRS-7 being specifically expressed in brain tissue and rarely expressed in other tissues, making them potential biomarkers for specific diseases [13,14].

3. Epithelial-mesenchymal transition

Epithelial-mesenchymal transition (EMT) is an important cellular biological phenomenon in which epithelial cells gradually acquire the characteristics and functions of mesenchymal cells through molecular regulation (**Figure 1**). Depending on the specific biological context in which EMT occurs, it can be categorized into three distinct types. Type 1 EMT primarily functions during embryonic development, promoting cellular diversity through mesenchymal-epithelial transition (MET). Type 2 EMT plays a critical role in physiological processes such as wound healing, tissue regeneration, and organ fibrosis. Type 3 EMT primarily enhances the invasive and metastatic capabilities of tumor cells, thereby promoting tumor progression [15].

Notably, Type 3 EMT plays a crucial role in the migration, invasion, and distant metastasis of malignant tumor cells. The specific regulatory mechanisms of tumor EMT are not yet fully understood. Multiple signaling pathways, such as Wnt, NK-κB, PI3K, triacylglycerol (TG), and epidermal growth factor (EGF), are involved in the induction of EMT. The occurrence of epithelial-mesenchymal transition in tumor cells often signifies the initiation of the malignant stage of tumor progression [16].

This process is typically accompanied by the downregulation of E-cadherin expression and the upregulation of molecules such as N-cadherin and Vimentin [17]. At the same time, inhibitory transcription factors such as Snail, Slug, ZEB1, ZEB2, and E47 are upregulated, coordinating the expression levels of E-cadherin. Through EMT, tumor cells lose polarity and tight intercellular connections, acquire enhanced invasive and migratory potential, and gradually resemble mesenchymal cells, making them more prone to local infiltration or distant metastasis [18]. Therefore, EMT can mediate the occurrence of invasive, migratory, immune evasion, and therapy resistance characteristics in tumor cells, playing a significant role in HCC metastasis and recurrence. Early detection of changes in relevant indicators associated with EMT is of great clinical significance in guiding patient treatment and improving survival rates.

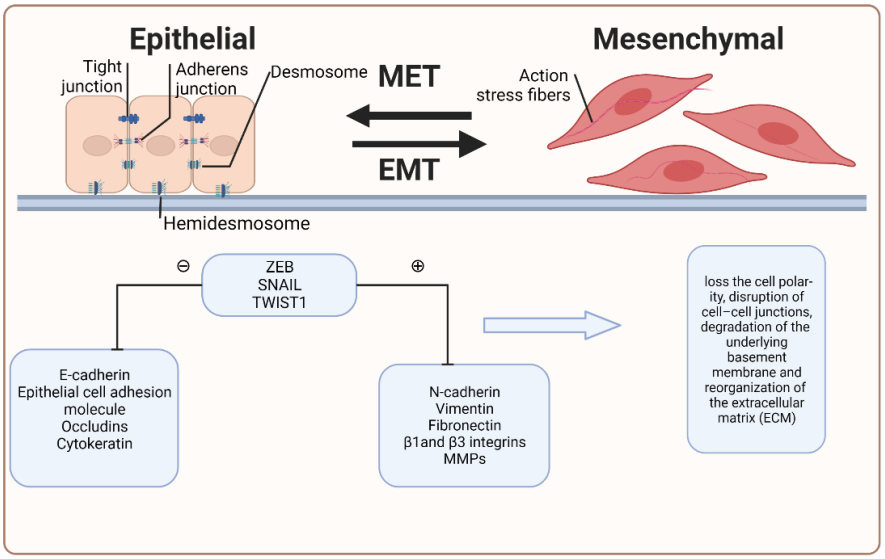

Figure 1. Outline of a typical EMT program

4. circRNA related to epithelial-mesenchymal transition in hepatocellular carcinoma

4.1. circRNA related to EMT-inducing transcription factors

The induction of EMT is initiated by a network of transcription factors (EMT-TFs). Currently, Snail, TWIST, and ZEB are widely recognized as core transcription factors in EMT. These factors can initiate the EMT process by directly or indirectly inhibiting the expression of epithelial markers [19]. Recent studies have reported various circRNAs that regulate the progression of liver cancer by targeting EMT core transcription factors (**Figure 2**).

Previous studies have clearly indicated that the pathogenesis of hepatocellular carcinoma (HCC) is closely associated with the regulatory relationship between has_circ_CYP24A1, miR-506, and Snail2. Specifically, this regulatory mechanism is mainly manifested in the competitive binding of has_circ_CYP24A1 with miR-506, leading to a decrease in the expression level of miR-506, thereby indirectly triggering the high expression of Snail2. This chain reaction rapidly promotes the EMT process in HCC, thereby driving the malignant progression of HCC [20].

has_circ_SEC62 enhances the expression of SNRPA in HCC cells by acting as a sponge for miR-625-5p. SNRPA activates the NOTCH1/Snail pathway both in vivo and in vitro, promoting the epithelial-mesenchymal transition (EMT)-like process in HCC cells, thereby accelerating metastasis [21].

Furthermore, has_circ_KIAA1429 maintains the expression of Zeb1 in HCC by regulating the m6A-YTHDF3-Zeb1 mechanism. This accelerates the EMT process in HCC, promoting the proliferation, migration, and invasion of HCC cells [22].

Twist1 directly binds to Cul2 and its promoter, leading to the abnormal formation of Cul2 circRNA. This promotes the expression of Twist1 through sponge targeting of miRNAs, thereby promoting the EMT process in HCC and enhancing the proliferation, migration, and invasion of HCC cells [23].

Has_circ_IFT80 downregulates the expression of miR-1236-3p, thereby increasing the expression of Twist2 and promoting the proliferation and metastasis of liver cancer cells [24]. These findings suggest that circRNAs can influence the progression of liver cancer by regulating the expression of EMT core transcription factors.

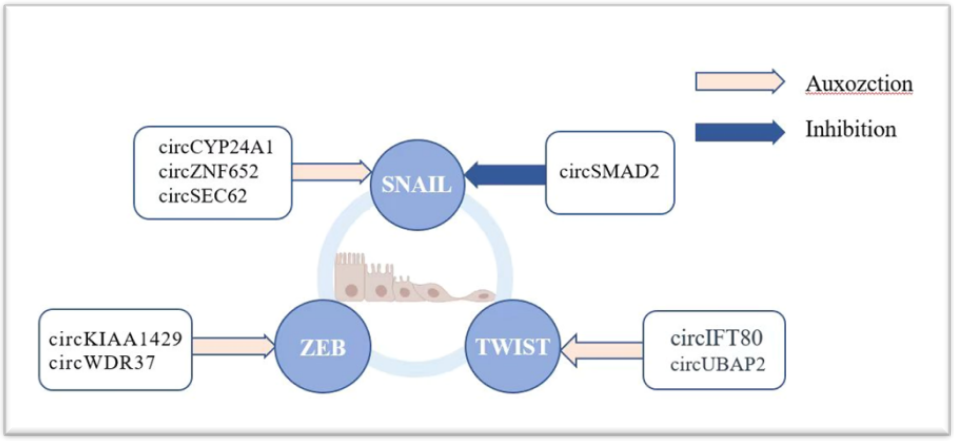

Figure 2. Interaction network between circRNA and EMT-related transcription factors

4.2. circRNA that promotes epithelial-mesenchymal transition in hepatocellular carcinoma

4.2.1. Hsa_circ_0003288 (circBIRC6)

Hsa_circ_0003288 is a circular RNA that has been shown to play a role in HCC. Programmed cell death ligand-1 (PD-L1) is a ligand expressed on the surface of tumor cells that interacts with programmed death receptor-1 (PD-1) expressed on the surface of lymphocytes. This interaction leads to the death of lymphocytes that can kill tumor cells, thereby mediating tumor immune evasion [25]. A previous study indicated that higher expression levels of hsa_circ_0003288 were associated with a poor prognosis in HCC patients [26]. In a study by Xu *et al*., it was found that the expression level of circ_0003288 was significantly increased in HCC, and its overexpression significantly promoted the proliferation and migration of HCC cells, correlating positively with PD-L1 expression levels. The study used RT-PCR to detect the expression levels of hsa_circ_0003288, miR-145, and multiple functional genes and analyzed cell viability, colony formation, apoptosis, cell migration, and invasion using various experimental methods. It was found that hsa_circ_0003288 acts as a sponge for miR-145, upregulating PD-L1 expression levels, and promoting EMT and invasion of HCC through the PI3K/AKT signaling pathway. This suggests its carcinogenic role in the development of HCC. Targeting hsa_circ_0003288 may provide a therapeutic strategy for the treatment of HCC [27].

4.2.2. Has_circ_0001459 (circNEIL3)

Insulin-like growth factor-I receptor (IGF-IR) is a cell surface tyrosine kinase receptor encoded by a gene located on chromosome 15q26.3. Recent studies have found that abnormal activation of IGF-IR can promote the malignant transformation of liver cells and induce EMT through various mechanisms. Akt, activated by IGF-IR, can stabilize slug, a negative regulator of E-cadherin expression. Additionally, IGF-IR may trigger EMT through pathways such as STAT3, FAK, and NF-kB [28,29]. Shen *et al*. found that has_circ_0001459 is significantly upregulated in liver cancer tissues. Dual-luciferase reporter assays confirmed the interaction between has_circ_0001459 and miR-6165, as well as between miR-6165 and IGF-1R. This confirmed that has_circ_0001459 can sponge miR-6165 and induce upregulation of its downstream target IGF-1R, thereby promoting invasion, migration, and EMT of HCC cells and significantly advancing the progression of HCC [30]. This suggests that has_circ_0001459 may serve as a prognostic factor and therapeutic target in hepatocellular carcinoma.

4.2.3. Has_circ_0003998 (circARFGEF2)

Has_circ_0003998 has been found to be highly expressed in non-small cell lung cancer (NSCLC) and breast cancer, promoting cell invasion and migration [31]. Poly(rC) binding protein 1 (PCBP1) is an RNA-binding protein that suppresses tumor formation and metastasis by translational silencing, mRNA alternative splicing, or transcriptional regulation of oncogenes. Importantly, PCBP1 has been reported to be involved in the EMT pathway in cancer, particularly in the TGF-β pathway [32]. PCBP1 can selectively splice the EMT-associated gene CD44v6, thus inhibiting invasion and EMT in HCC.

In a recent study by Song *et al*., it was revealed that has_circ_0003998 acts as a competing endogenous RNA (ceRNA) for microRNA-143-3p, relieving its inhibitory effect on the EMT-related transcription factor FOSL2. Considering that the interaction with RNA-binding proteins (RBPs) is an important part of circRNA function, pull-down experiments and protein mass spectrometry analysis were performed, revealing that has_circ_0003998 can bind with PCBP1 to enhance the expression of CD44v6, thus accelerating EMT progression in HCC. Therefore, the has_circ_0003998/miR-143-3p/FOSL2 axis and the has_circ_0003998/PCBP1/CD44v6 axis were discovered to regulate EMT in HCC. Therefore, has_circ_0003998 holds promise as a novel marker or therapeutic target in liver cancer.

4.2.4. Has_circ_0004277 (circWDR37)

Studies have shown that has_circ_0004277 is overexpressed in colorectal cancer and is involved in the malignant phenotype of colorectal cancer by sponging miR-512-5p to upregulate PTMA expression [33]. ZO-1, a tight junction protein, is not only involved in regulating cell substance transport and maintaining epithelial polarity but is also closely related to the information transmission and regulation of processes such as cell proliferation, differentiation, tumor cell metastasis, and gene transcription [34]. Similarly, significant upregulation of has_circ_0004277 has been detected in HCC cells, leading to the upregulation of N-cadherin and ZEB-1 expression and downregulation of E-cadherin expression at both the protein and RNA levels.

HuR protein consists of three RNA recognition motifs (RRMs) and one flexible hinge region, with RRMs primarily recognizing adenine/uridine-rich elements (AREs) in the 3'UTR of target mRNAs, stabilizing them, and promoting their translation. Recent studies have found that HuR can be secreted by various cells in the human body, but its expression levels are lower in some normal tissues and higher in various malignant tumor tissues, showing an association with poor prognosis. This suggests that HuR is closely related to the occurrence and development of various tumors, and its overexpression can enhance tumor cell proliferation and invasion.

A recent study by Zhu *et al*. demonstrated through *in vitro* and *in vivo* experiments that upregulation of has_circ_0004277 significantly promotes HCC cell proliferation and migration. Furthermore, its overexpression significantly induces N-cadherin and ZEB-1 expression while reducing E-cadherin expression at both the protein and RNA levels, indicating that has_circ_0004277 acts as an important positive regulatory factor in HCC cell growth and exhibits an oncogenic role. Subsequently, target prediction was performed using bioinformatics software, revealing that has_circ_0004277 may block the binding between HuR and ZO-1 mRNA by competitively binding with HuR, thereby inhibiting ZO-1 and stimulating EMT progression. Further experiments confirmed this hypothesis. Additionally, enhanced expression of has_circ_0004277 was observed in HCC cells, tissues, and plasma exosomes [35]. This suggests that it may serve as a potential prognostic marker for HCC patients.

4.2.5. Has_circ_0084922 (circKIAA1429)

M6A modification plays a role in various cellular physiological processes, including mRNA maturation, protein translation, and molecular structural changes, and has become a hot research topic. Increasing evidence

suggests that dysregulation of m6A can lead to various cancers, including HCC [36]. Previous studies have found that hsa_circ_0084922 from KIAA1429 is significantly upregulated in HCC tissues, promoting HCC migration, invasion, and EMT. Conversely, the downregulation of its expression leads to the opposite results. Moreover, Zeb1 has been identified as a downstream target of circKIAA1429, and YTHDF3 enhances the stability of Zeb1 mRNA in an m6A-dependent manner, prolonging the lifespan of Zeb1 mRNA in HCC, thereby accelerating HCC progression. This provides a new potential target for the treatment of liver cancer.

4.3. circRNA that inhibits epithelial-mesenchymal transition in hepatocellular carcinoma

4.3.1. circRNA CDR1as

Cerebellar degeneration-related protein 1 antisense RNA (CircCDR1as) is a circular RNA molecule associated with the occurrence of various cancers and neurodegenerative diseases. It contains over 70 binding sites for miR-7 and can regulate multiple signaling pathways through the sponge effect of binding miR-7, thereby participating in the regulation of tumor growth. For example, miR-7 acts as a tumor suppressor and regulates cell proliferation and various biological processes by triggering signal transduction of growth factors. It is abnormally expressed in various tumors such as colorectal cancer, cholangiocarcinoma, and osteosarcoma, playing an important role in the development of tumors [37]. The study by Yang *et al*. demonstrated that CDR1as changes EGFR signal regulation and cell proliferation by controlling miR-7 expression, proposing a molecular interaction network of the CDR1as/miR-7/EGFR axis in HCC cells [38]. By replacing the miR-7 binding sites in CDR1as with specific base sequences that bind mmu-miR-21 or mmu-miR-130b and transfecting them into Hepa1-6 cells, it was found that the protein levels of Vimentin and N-cadherin significantly decreased compared to the control and negative control groups ($P < 0.01$), while the protein levels of E-cadherin significantly increased ($P < 0.01$). This demonstrated that M-CDR1as can inhibit EMT in mouse Hepa1-6 liver cancer cells by binding and sequestering miR-21 and miR-130b *in vitro* [39].

4.3.2. Has_circ_0000098 (circSLC30A7)

Previous studies have found that ALX4 expression is decreased in HCC tissues, and overexpression of ALX4 inhibits HCC cell proliferation, invasion, and EMT processes [40]. However, the specific mechanism of ALX4 downregulation in liver cancer tissues remains unclear. Further research by Li *et al*. revealed that has_circ_0000098 positively regulates ALX4 expression by competitively binding with miR-1204. Additionally, the study found that the knockdown of ALX4 counteracted the inhibitory effect of downregulated miR-1204 on HCC cell proliferation, migration, invasion, and EMT. Therefore, it can be concluded that the has_circ_0000098/miR-1204/ALX4 regulatory network is involved in the progression of EMT in HCC, and low expression levels of has_circ_0000098 are associated with larger tumor volume and later stage [41]. With further research, has_circ_0000098 holds promise as a reference marker for prognostic prediction in HCC patients.

4.3.3. Has_circ_0008305 (circPTK2)

The PTK2 gene expresses two types of circRNA: circ_0003221 and circ_0008305. Both of these circRNAs are associated with cell proliferation and migration processes in cancer. In NSCLC, has_circ_0008305 targets miR-429/miR-200b-3p and inhibits their activation of the TGF-β-induced EMT pathway through targeting TIF1-γ [42]. In ovarian cancer, circ-PTK2 enhances tumor cell proliferation and migration through a cascade regulatory mechanism involving miR-639 and FOXC1. However, its role in HCC is not yet clear [43]. MiR-92a-3p is a core member of the miR-17-92 cluster, which is overexpressed in human cancers and is believed to be involved in cancer development [44]. The study by Gong *et al*. found that miR-92a-3p acts as an oncogene in HCC by targeting E-cadherin and reducing its expression. Further experiments demonstrated that has_circ_0008305

inhibits the expression of miR-92a as a competing endogenous RNA (ceRNA), upregulating the expression of E-cadherin in liver cancer cells and suppressing the EMT process in HCC (**Table 1**) [45].

Table 1. The EMT-related circRNAs in HCC

Type	CircRNA	Target/pathway
EMT-inducing circRNAs in HCC	circBIRC (hsa_circ_0003288)	circBIRC/miR-145/PD-L1/PI3K/AKT signal pathway
	circNEIL3 (hsa_circ_0001459)	circNEIL3/miR-6165/IGF-1R axis
	circARFGEF2 (hsa_circ_0003998)	circARFGEF2/miR-143-3p/FOSL2 axis circARFGEF2/ PCBP1/CD44v6 axis
	circWDR37 (hsa_circ_0004277)	circWDR37/HuR/ZO-1 axis
	circKIAA1429 (hsa_circ_0084922)	circKIAA1429/m (6)A-YTHDF3/Zeb1 axis
EMT-suppressive circRNAs in HCC	circCDR1as	circCDR1as/miR-21、miR-130b
	circSLC30A7 (hsa_circ_0003288)	circSLC30A7/miR-1204/ALX4 axis
	circPTK2 (hsa_circ_0008305)	circPTK2/miR-92a/E-cadherin axis

5. Conclusion

In summary, a large number of circRNAs are involved in the regulation of EMT in HCC, and they mainly regulate the EMT process through the following mechanisms:

(1) Acting as miRNA sponges: circRNAs can act as miRNA sponges by containing miRNA binding sites, thus binding to miRNAs and inhibiting their regulatory effects on target genes. In liver cancer, certain circRNAs have miRNA binding sites that can bind and inhibit EMT-related miRNAs, thereby releasing target genes and promoting EMT onset and progression.

(2) Interaction with RNA-binding proteins: Some circRNAs can interact with RNA-binding proteins to form circRNA-RBP complexes. These complexes can affect the EMT characteristics of liver cancer cells by regulating the expression of key EMT factors such as transcription factors, cell adhesion molecules, and signaling pathways.

(3) Regulation of transcription levels: Certain circRNAs can interact with transcription factors, affecting their expression and activity. These transcription factors may influence the EMT process in liver cancer cells by regulating the expression of genes involved in EMT.

(4) Regulation of the splicing process: Some circRNAs may be involved in regulating splicing, affecting the splicing forms of EMT-related genes in liver cancer cells. Alterations in splicing can lead to changes in the structure and function of transcript products and thus have an impact on the EMT process.

(5) Regulation of cell cycle and apoptosis: Certain circRNAs influence the onset and progression of EMT in liver cancer cells by regulating the cell cycle and apoptosis.

These circRNAs can regulate the expression of genes associated with the cell cycle and apoptosis and thus influence the EMT characteristics of liver cancer cells. They are mainly distributed in the cytoplasm or stored in extracellular vesicles and can be released into body fluids via the exosome pathway. Monitoring circRNA levels in body fluids is a promising biomarker for assessing early metastasis of liver cancer and plays a crucial role in the diagnosis of the disease.

The relationship between EMT and tumor prognosis is complex and varied, depending on factors such as tumor type, patient characteristics, and treatment strategies. However, previous research has shown that

EMT is associated with malignant features such as invasion, metastasis, and drug resistance in tumors. Studies have shown that cells undergoing EMT have increased migration and invasion capabilities, which can lead to the spread of tumor cells from the primary tumor to distant organs, resulting in the formation of metastatic tumors. In addition, EMT can contribute to drug resistance in tumor cells, thereby reducing the effectiveness of treatment. EMT is also associated with the properties of cancer stem cells (CSCs). CSCs are a small subset of tumor cells that have the potential for self-renewal and multilineage differentiation and are thought to play a critical role in tumor growth, metastasis, and recurrence.

Although further research and validation are needed to understand the relationship between EMT and tumor prognosis, existing evidence suggests that EMT may be an important indicator of poor tumor prognosis. Therefore, the assessment of EMT may have significant implications for determining tumor prognosis and selecting treatment strategies.

6. Outlook

Studies have shown that targeted intervention of circRNAs can effectively interfere with the initiation of EMT, thereby slowing down the invasive and metastatic abilities of liver cancer cells. This strategy may have potential value in preventing hepatocellular carcinoma recurrence and improving patient survival rates. Despite recognizing the importance of circRNAs in the EMT process of liver cancer, the exact regulatory mechanisms remain unclear. Currently, only a limited number of studies have extensively investigated circRNAs in liver cancer cell lines and animal models, and many molecular mechanisms of circRNA regulation of cancer EMT remain to be elucidated. Therefore, it is necessary to further explore the precise role of circRNAs in the regulation of EMT in liver cancer, which will provide a solid basis for their potential as diagnostic and therapeutic targets for cancer. The continued development of advanced technologies will deepen our understanding of the functions and mechanisms of circRNAs, further improving our understanding of biology and its applications in disease mechanisms. Researchers are encouraged to conduct further basic research on circRNAs to uncover their pathological and physiological functions. Additionally, studies on treatment strategies based on circRNAs should be conducted to safely and successfully integrate them into clinical practice.

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Disclosure statement

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