

Circular RNAs: Fundamental mechanisms in tumor metastasis and detection strategies (Review)

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Abstract. As a class of endogenous non-coding RNAs, circular RNAs (circRNAs) are produced by an event known as back-splicing. Although circRNAs were initially considered to be the product of abnormal splicing events, increasing evidence has shown their vital role in the development of various diseases, especially malignant tumors. Tumor metastasis is the leading cause of tumor deterioration and cancer-associated mortality, and involves biological changes to malignant cells. A number of circRNAs can mediate tumor progression, especially tumor metastasis. Furthermore, due to their unique structural features, circRNAs are highly stable in body fluids, and have temporal and tissue specificity, making them potentially ideal non-invasive biomarkers for sensitive monitoring of tumor changes. The present review focuses on circRNAs associated with tumor metastasis and discusses their functional mechanisms. Furthermore, the current review summarizes advances regarding circRNAs as biomarkers and detection strategies.

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1. Introduction

Circular RNAs (circRNAs/circs) are a type of single-stranded and covalently closed non-coding RNA (ncRNA), which lack 5'caps and 3'poly(A) tails. These properties make them more structurally stable to escape digestion by RNA ribonucleases such as RNase R and exonuclease (1). CircRNAs were first identified in 1976 (2) and were initially considered to be products of mis-splicing (3); therefore, they were ignored and even treated as 'junk'. However, with the development of high-throughput technology, circRNAs have gradually been recognized to be of vital importance in various diseases (4), including nervous system disorders (5), cardiovascular disorders (6), immune diseases (7), metabolic diseases (8) and cancer (9,10).

Metastasis occurs through a multi-step biological process termed the invasion-metastasis cascade, which involves the spread of cancer cells to anatomically distant organs and their subsequent adaptation to the microenvironment there (11). There are four steps implicated in this process: i) Local invasion from the primary sites through the surrounding extracellular matrix (ECM) and stromal cell layers; ii) perfusion into the vascular lumen; iii) traveling and survival in the bloodstream; and iv) infiltration into distant organs and the restarting of proliferation (11,12). The relentless growth of the tumor after it metastasizes from the primary site results in rapid deterioration of the disease. Therefore, despite improvements in diagnosis, surgery and care, most cancer deaths are due to metastasis occurring after tumors have become resistant to conventional treatment, leading to increased mortality (13). In addition, metastasis is often detected at late stages for a number of patients with no signs of metastasis at the time of initial diagnosis. These metastases may exhibit an organ-specific pattern of spread (14). For example, breast cancer (BC) and prostate cancer (PCa) often metastasize to the bone, which can occur years or even decades after the apparent success of primary treatment (15). Consequently, it is necessary to study the mechanism of tumor metastasis, and its early detection and prevention.

Since circRNAs may be involved in various aspects of tumor metastasis, the present review focuses on the specific mechanism of circRNAs affecting tumor metastasis and the

potential of circRNAs as biomarkers. In addition, the present review summarizes the detection methods of circRNAs with the ultimate purpose of preventing cancer metastasis earlier and reducing the mortality of patients with cancer metastasis.

2. Biogenesis and characteristics of circRNAs

CircRNAs are specific circular ncRNAs, which are mainly produced by selective splicing and widely expressed in eukaryotic organisms. CircRNAs formed by exon back-splicing (circularization) were originally identified in the 1990s (16,17). Studies have confirmed that circRNAs are mainly produced from the splicing of mRNA-precursors (pre-mRNAs), and the mechanism underlying the circularization of circRNAs differs from the traditional splicing manner used to produce linear RNA (18,19). CircRNAs can be divided into three main types based on the diversity of splicing: Exonic circRNAs (EcRNAs), exon-intron circRNAs (EIciRNAs) and intron-derived circRNAs (ciRNAs). The specific formation mechanism of circRNAs has been summarized in numerous reviews (20-22). Back-splicing results in the downstream 5' splice site (donor) and the upstream 3' splice site (acceptor) coming close together and gives rise to EcRNAs. More rarely, if introns are retained in the circle, EIciRNAs are produced (16,19,23-25). The process of ciRNA formation is slightly different, and it depends on a common motif that contains a 7-nt GU-rich element near the 5' splice site and an 11-nt C-rich element close to the branchpoint site (26). Currently, there are three recognized mechanisms of circRNA formation: Exon-skipping, intron pairing and RNA-binding protein (RBP) interactions (Fig. 1) (27-29). EcRNAs are the most abundant and well-documented. Notably, several studies have reported on the formation of circRNAs; however, the biogenesis of circRNAs remains unclear.

3. Mechanisms of action of circRNAs in tumor metastasis

CircRNAs are widely present in eukaryotes and have a variety of functions. At present, the most commonly known functions of circRNAs are to sponge microRNAs (miRNAs/miRs), interact with RBPs, regulate parental genes and participate in translation. Based on these mechanisms, circRNAs can affect tumor metastasis in numerous ways, such as regulating tumor metastasis through exosomes, the tumor microenvironment (TME), epithelial-mesenchymal transformation (EMT) and metabolism (30,31). The effects of these mechanisms on tumor metastasis are discussed in the present review (Fig. 2).

Common mechanisms of action of circRNAs in tumor metastasis

miRNA sponges. miRNAs, another class of key ncRNA molecules 20-25 nucleotides long, predominantly regulate post-transcriptional gene expression in eukaryotes, primarily by directly binding to the untranslated regions of mRNA targets to influence protein synthesis (32). Hansen *et al* (33) first proposed the definition of miRNA sponges in 2013. Target mRNAs harbor specific miRNA response elements (MREs), enabling miRNAs to inhibit target mRNA function or promote mRNA degradation by binding to these elements. Notably, each miRNA can regulate multiple target

genes, while multiple miRNAs often synergistically modulate the expression of a single gene (34). ciRS-7, also known as CDR1as or CDR1NAT, was the first identified circRNA with the function of sponging miRNAs. This circRNA covers >70 selectively conserved miR-7 target sites, and inhibits the biological activity and function of miR-7 (33). The existence and importance of ciRS-7 as a miR-7 sponge has been reported in various types of cancer (35). Su *et al* (36) reported that ciRS-7 increased the invasion and migration of non-small cell lung cancer (NSCLC) through the miR-7/NF- κ B subunit axis. Additionally, Zhang *et al* (37) demonstrated that depletion of ciRS-7 could upregulate miR-7 expression, thereby inhibiting the metastasis of laryngeal squamous cell carcinoma. More circRNAs have been verified to act as miRNA sponges. Some circRNAs may have a variety of MREs, allowing them to target multiple miRNAs at the same time, and thus, regulate multiple mRNAs. However, some circRNAs involved in tumor metastasis as miRNA sponges may not actually function through the proposed mechanisms due to the absence of binding sites or the presence of only a few binding sites for miRNAs. Furthermore, the circRNA-to-miRNA ratio must be taken into account (38).

RBP interactions. RBPs are a group of highly conserved proteins that serve a key role in the post-transcriptional modulation of gene expression. In particular, RBPs participate in the processing of intracellular pre-mRNAs, mRNA output and localization to different subcellular regions in the cytoplasm (39). Increasing evidence has indicated that aberrant expression and function of RBPs are associated with cancer metastasis (40,41). Wang *et al* (42) summarized how RBPs affect tumor metastasis, including through ncRNA processing, alternative splicing, alternative polyadenylation, RNA stability, RNA localization, translation regulation and N⁶-methyladenosine (m⁶A) modification. Therefore, interactions between circRNAs and RBPs may regulate tumor metastasis through some of these mechanisms. For example, by interacting with flanking introns, eukaryotic translation initiation factor 4A3 promotes circSTX6 production, triggering the metastasis of bladder cancer (BCa) cells (43). In addition, circUSP1 can affect USP1 and vimentin expression by stabilizing human antigen R (HuR), thus promoting the metastasis of gastric cancer (GC) (44).

The m⁶A modification is a prevalent internal modification in eukaryotic RNAs, and there is accumulating evidence indicating that the m⁶A modification is involved in the regulation of RNA processing, which is closely related to the occurrence of multiple human diseases, especially cancer (45,46). Notably, m⁶A modifications are modulated by 'writers', 'erasers' and 'readers'. Among them, m⁶A methyltransferases, such as METTL3/14/16, RNA binding motif protein 15/15B, zinc finger CCCH-type containing 3, vir like m⁶A methyltransferase associated, Cbl proto-oncogene like 1, and WT1 associated protein, are the 'writers'. Demethylases, including FTO and ALKBH5, are the opposite of 'writers' and are known as 'erasers'. 'Readers', including YTH N⁶-methyladenosine RNA binding protein (YTHDF1/2/3, YTHDC1/2, insulin like growth factor 2 mRNA binding protein (IGF2BP)1/2/3 and heterogeneous nuclear ribonucleoprotein A2/B1, are recognized by m⁶A-binding proteins (47). In terms of the role of RBPs as m⁶A readers, the recognition of m⁶A-modified RNAs

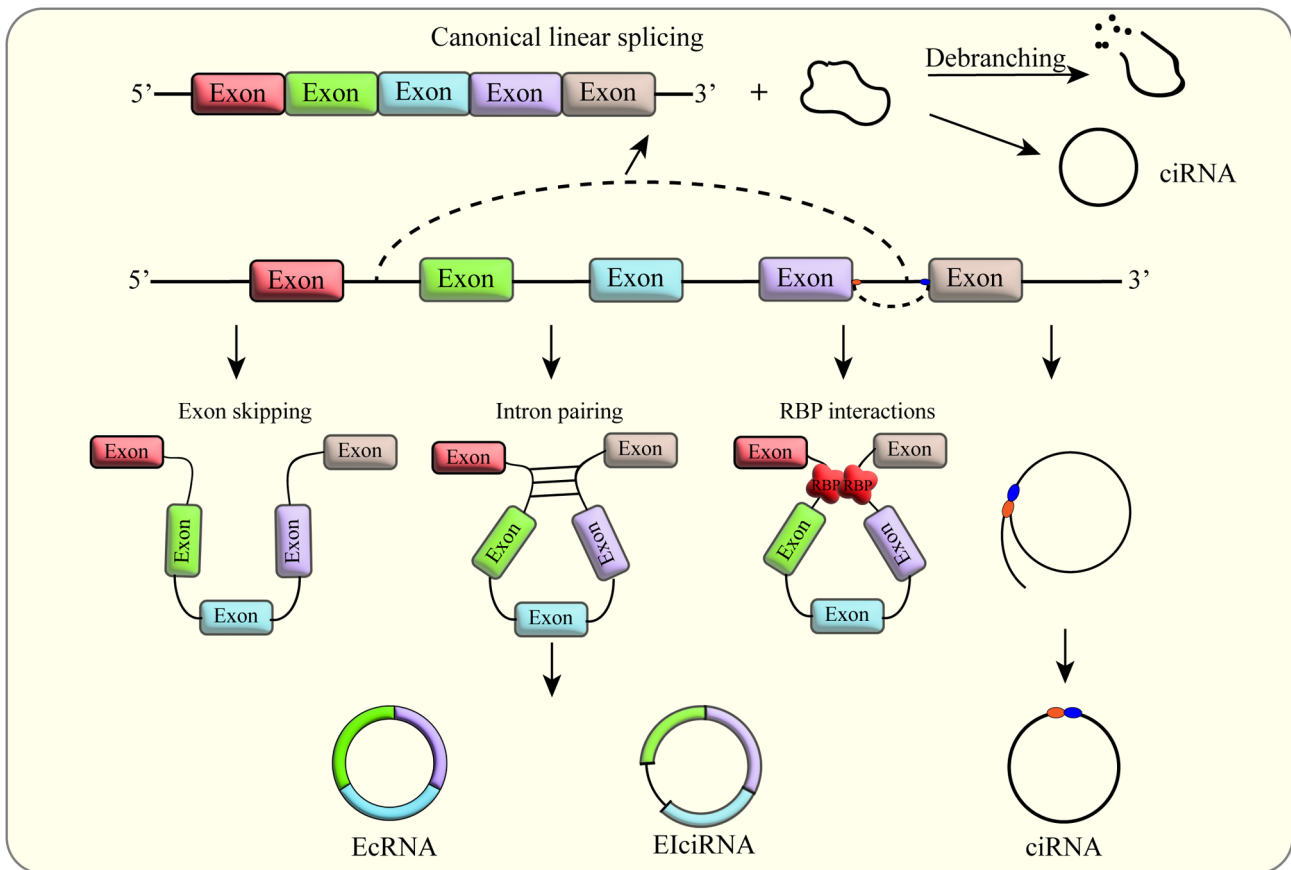


Figure 1. Biogenesis of circular RNAs. EcRNAs and EIciRNAs are conventionally formed in three ways: Circularization caused by exon skipping, intron pairing-driven circularization and RBP interaction-mediated circularization. ciRNA forms a lariat structure through splicing by relying on a conserved motif, thereby evading debranching. ciRNA, intron-derived circular RNA; EcRNA, exonic circular RNA; EIciRNA, exon-intron circular RNA; RBP, RNA-binding protein.

mediated by IGF2BP1 is partly disturbed by circPTPRA through its interaction with the KH domains of IGF2BP1 (48). CircMETTL3, upregulated in an m⁶A-dependent manner by its host gene METTL3, has been identified to promote the metastasis of BC via the circMETTL3/miR-31-5p/CDK1 axis (49). Ruan *et al* (50) also reported on the diverse function of circMETTL3 in triple-negative BC (TNBC) metastasis. As a key factor in m⁶A modification, METTL3 is a target of miR-34c-3p, and METTL3 is inhibited by the interaction between circMETTL3 and miR-34c-3p, suppressing tumor metastasis of TNBC. In addition, there is evidence that m⁶A modification triggers the translation of circRNAs mediated by the interaction with YTHDF, YTHDC2 and eukaryotic translation initiation factor 4 γ 2 (51). Taken together, circRNAs serve a crucial role in tumor metastasis by interacting with RBPs. Therefore, targeting of RBPs may prevent metastasis or improve the prognosis of patients with metastasis.

Translation into proteins/peptides. Although circRNAs are a type of ncRNA, due to the absence of a 5' end cap and a 3' end poly(A) tail, a few reports have revealed that some circRNAs have translation potential. As early as 1986, Wang *et al* (52) observed that a single-stranded circRNA derived from the hepatitis δ viral genome had an open reading framework (ORF), start codon AUG and stop codon, making the translation of a 215-amino acid (aa) polypeptide possible. Typically, the presence of at least one ORF serves as a primary prerequisite for

protein-encoding capacity, while internal ribosome entry site sequences are also critical determinants of translational potential (53). More studies have investigated this mechanism. For example, Hwang and Kim (54) summarized the mechanisms of circRNA translation that have been studied thus far. The coding function of circRNAs has been demonstrated in the metastasis of various tumors, supported by clinical samples and sequencing data from circRNAs in cell lines. Li *et al* (55) identified that circEIF6 encodes a novel peptide, EIF6-224aa, which is associated with the oncogenic effects of circ-EIF6. As for the mechanism, EIF6-224aa directly interacts with myosin heavy chain 9 (MYH9), which is widely considered to be an oncogene in BC, and reduces MYH9 degradation by inhibiting the ubiquitin-proteasome pathway and subsequently activating the Wnt/ β -catenin pathway, leading to the metastasis of TNBC. In addition, using bioinformatics data, Li *et al* (56) selected a circRNA, circTRIM1, with high expression in doxorubicin-resistant TNBC and revealed that it can encode a new protein referred to as TRIM1-269aa. TRIM1-269aa is a carcinogenic factor that interacts with myristoylated alanine rich protein kinase C substrate to influence its translocation, thereby activating the PI3K/AKT/mTOR pathway and ultimately promoting the metastasis of TNBC cells. Although the mechanism of action of circRNA-encoded proteins is still unclear, it has been widely confirmed that circRNA-encoded proteins serve an important regulatory role in tumor metastasis.

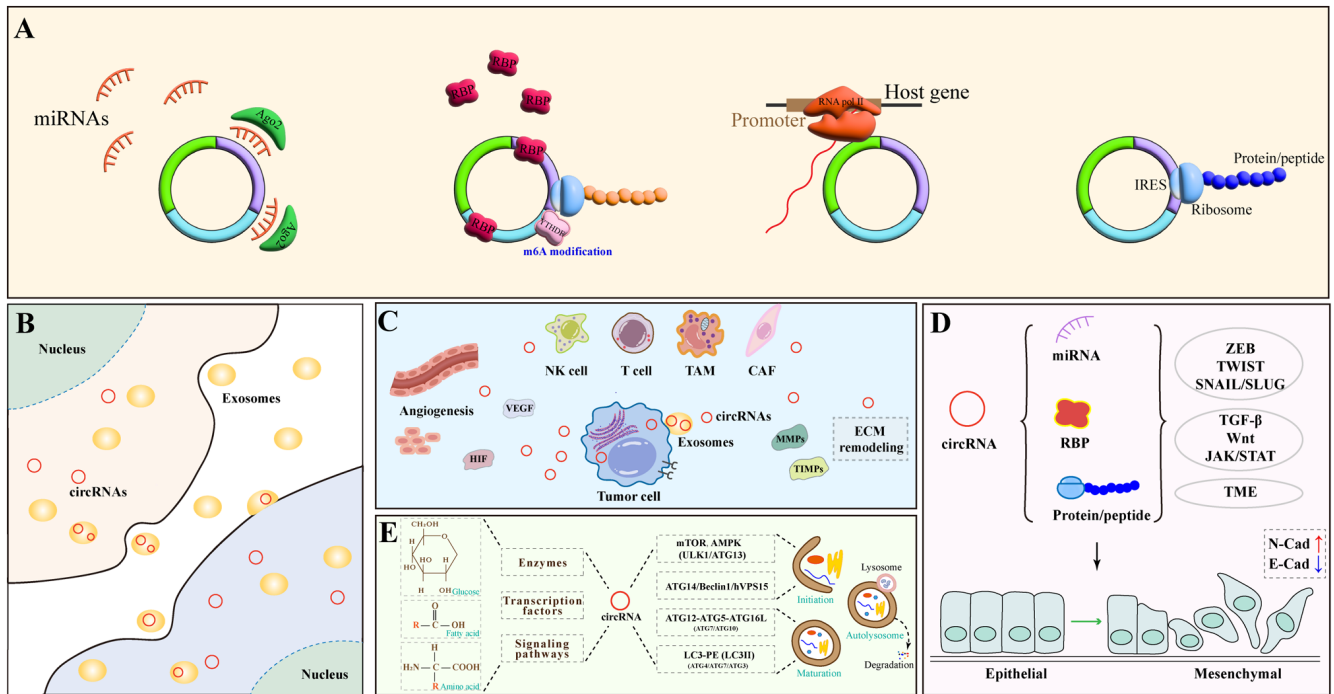


Figure 2. Functions of circRNAs. (A) CircRNAs function mainly through four direct mechanisms of action: Sponging miRNAs, interacting with RBPs, regulating host genes and coding proteins or peptides. (B) CircRNAs can be encapsulated into exosomes and serve a role in regulating tumor metastasis through transport and release. (C) CircRNAs interact with endothelial cells, immune cells, tumor-derived fibroblasts and ECM in the tumor immune microenvironment to affect immune function, and then affect tumor progression. (D) CircRNAs can regulate transcription factors such as ZEB and TWIST, signaling pathways such as the TGF- β and Wnt pathways, and the tumor immune microenvironment through various direct action modes, influencing the epithelial-mesenchymal transition process. (E) Metabolism-related circRNAs regulate tumor metastasis by influencing glucose, fatty acid and amino acid metabolism, and autophagy processes through enzymes, transcription factors and signaling pathways. Ago2, argonaute RISC catalytic component 2; AMPK, AMP-activated protein kinase; ATG, autophagy related protein; CAF, cancer-associated fibroblast; circRNA, circular RNA; E-Cad, E-cadherin; ECM, extracellular matrix; HIF, hypoxia-inducible factor; hVPS15, vacuolar protein sorting 15; IRES, internal ribosome entry site; JAK, Janus kinase; LC3-PE, microtubule-associated proteins 1A/1B light chain 3-phosphatidylethanolamine complex; m⁶A, N⁶-methyladenosine; miRNA, microRNA; N-Cad, N-cadherin; NK, natural killer; RBP, RNA-binding protein; RNA pol II, RNA polymerase II; TAM, tumor-associated macrophage; TIMP, tissue inhibitor of metalloproteinase; TME, tumor microenvironment; TWIST, twist family bHLH transcription factor; ULK1, unc-51 like autophagy activating kinase 1; YTHDF, YTH domain-containing family protein 1; ZEB, zinc finger E-box binding homeobox.

Regulation of host genes. Previous studies have shown that circRNAs can affect tumor metastasis by regulating host genes. There is a special link between circRNAs and their parental genes. circRNAs are derived from the circularization of host genes, and circRNAs can, in turn, regulate these host genes in various ways (57), mainly through the subsequently described mechanisms.

i) **miRNA sponges.** Li *et al* (58) reported that circITGA7 binds to miR-370-3p to antagonize its suppression of neurofibromin 1, which is a well-known negative regulator of the Ras pathway, and revealed that circITGA7 inhibited metastasis of colorectal cancer (CRC) cells by suppressing the Ras signaling pathway and promoting the transcription of ITGA7. Zhou *et al* (59) identified differentially expressed circRNAs in lung adenocarcinoma (LUAD). CircENO1 (hsa_circ_0000013) was validated to be upregulated in LUAD tissues and cell lines by performing circRNA sequencing. Mechanistically, circENO1 regulated glycolysis via the miR-22-3p/ENO1 axis to regulate migration in LUAD cells.

ii) **Regulation of mRNA stability.** CircRNAs affect the stability of their host gene mRNA by recruiting or sponging RBPs, which can interact with mRNA to regulate their stability. For example, Zhao *et al* (60) reported that circ_0075804 derived from E2F transcription factor 3 (E2F3) pre-mRNA

improved the stability of E2F3 mRNA by combining heterogeneous nuclear ribonucleoprotein K, and thus, facilitated the progression of retinoblastoma. Additionally, Zhang *et al* (61) found that a novel circRNA, circ_0006401 generated from collagen type VI $\alpha 3$ chain (col6a3), encodes a novel 198-aa functional peptide, which promoted the stability of col6a3 mRNA to promote the metastasis of CRC.

iii) **Regulation of the transcription of host genes.** Feng *et al* (62) screened upregulated circRNAs in PCa using microarray analysis. Subsequently, circ_0005276 (circXIAP) was selected for further study, and it was shown that circXIAP interacted with the fused in sarcoma protein to activate the transcription of XIAP, leading to PCa metastasis.

iv) **Regulation of parental proteins.** Hu *et al* (63) demonstrated that upregulated circGSK3 β interacted with the GSK3 β protein to inhibit its activity and then acted on the metastasis of esophageal squamous cell carcinoma. In addition, it has been shown that more circRNAs can simultaneously regulate the function of their host genes through various mechanisms, thus affecting tumor metastasis (64). The research that has been conducted on circRNA-mediated regulation of host genes may improve the understanding of the relationship between circRNAs and their host genes, and the role of the relationship in human cancer metastasis.

Other mechanisms of action of circRNAs

Exosomal circRNAs and metastasis. Tumor-derived exosomes serve pivotal roles in several aspects of cancer biology, and it is becoming increasingly clear that exosomes are involved in several processes of the tumor metastasis cascade, including angiogenesis, EMT induction, migration and invasion. By being enveloped in exosomes, circRNAs can be transferred from secretory cells to recipient cells, thereby regulating tumor metastasis (65). Zhou *et al* (66) summarized how exosomal circRNAs affect tumor metastasis: i) Cytoplasmic circRNAs sponge miRNAs and remove the suppression of miRNA-regulated genes, ii) CircRNAs that are transferred to recipient cells can interact with certain proteins through specific binding sites, iii) exosomal circRNAs can regulate the TME via immune system cells. Xie *et al* (67) demonstrated that exosomal circSHKBP1 increased HuR expression and then enhanced VEGF mRNA stability as a miR-582-3p sponge. Subsequently, heat shock protein 90 degradation was inhibited, resulting in the metastasis of GC. Due to the characteristics of exosomes, which can transport various biomolecules between cells, circRNAs can be transported to distant cells to serve their roles, thus promoting tumor metastasis (68). For example, Zhu *et al* (69) selected highly expressed circ_0004277 as a research object via reverse transcription-quantitative PCR (RT-qPCR). This previous study suggested that exosomal circ_0004277 inhibited zonula occludens-1 and promoted EMT in normal perihepatic cells, leading to the metastasis of hepatocellular carcinoma (HCC). In the TME, exosomes, particularly exosomal circRNAs, mediate the interplay between immune cells, including lymphocytes, macrophages, natural killer (NK) cells, dendritic cells and tumor cells (70). Multiple observations have indicated that exosomal circRNAs are crucial regulators of immune escape (70,71). As indicated by *in vivo* experiments, exosomes derived from GC cell lines are absorbed mainly by NK cells and macrophages, thereby contributing to the lung metastasis of GC cells (72,73). Yang *et al* (74) identified that exosome-derived hsa_circ_0085361 (circTRPS1) was associated with the modulation of the intracellular reactive oxygen species balance and CD8⁺ T-cell exhaustion via the miR-141-3p/glutaminase 1 axis, thus inhibiting the metastasis of BCa cells. Furthermore, Chen *et al* (75) identified a link between circRNA and macrophage polarization. Exosomal circFARSA derived from NSCLC cells induced macrophage M2 polarization via PTEN ubiquitination and degradation, which further activated the PI3K/AKT signaling pathway, resulting in the metastasis of NSCLC. There is increasing evidence that tumor-derived exosomes mediate premetastatic niche remodeling to establish a supportive and receptive microenvironment that promotes tumor cell colonization and metastasis. Uptake of these signals in the extracellular environment by target cells triggers a series of changes that contribute to the formation of the premetastatic microenvironment (76). The tumor-promoting effects of exosomal circRNAs indicate that they may be ideal diagnostic biomarkers and therapeutic targets, and this is a current research hotspot.

CircRNAs and the TME in metastasis. i) Cancer-associated endothelial cells (CAEs). As an essential component of stroma in the TME, CAEs are responsible for supporting tumor angiogenesis, which refers to the generation of new blood vessels

necessary for sustained tumor growth and cancer metastasis. VEGF is a critical angiogenic element that also participates in the metastasis of several tumors (77). Therefore, the present review aimed to discuss the progress regarding circRNA-induced regulation of angiogenesis and tumor metastasis. Ding *et al* (78) demonstrated that circPAK2 interacted with IGF2BP2, a mechanism that stabilized VEGFA mRNA. This regulatory process promoted angiogenesis and invasion in GC, thereby emerging as a key factor governing lymph node metastasis in this malignancy. Further research has elucidated that circMYLK accelerated the tube formation of HUVECs and metastasis of BCa. Mechanistically, circMYLK could directly bind to miR-29a and relieve suppression of the target VEGFA to activate the VEGFA/VEGFR2 signaling pathway and promote the EMT process (79). Additionally, circRNAs can absorb miRNAs and activate signaling pathways contributing to tumor angiogenesis, including those involving the hypoxia-inducible factor family, platelet-derived growth factor, TGF- β , tumor necrosis factor- α , IL, fibroblast growth factor and MMPs (80). Additionally, circRNAs can affect lymphangiogenesis and regulate lymphatic metastasis of cancer cells. For example, the novel circRNA circEHBP1 is upregulated in BCa, and is positively associated with lymphatic metastasis and poor prognosis of patients with BCa. CircEHBP1 upregulates the expression of transforming growth factor β receptor 1 (TGFBRI) through miR-130a-3p, activates the SMAD3 signaling pathway and promotes the secretion of VEGF-D, finally driving the lymphangiogenesis and metastasis of BCa (81). Currently, most solid tumors metastasize primarily through lymphatic vessels; however, the specific mechanisms of lymphangiogenesis are still unclear, as are the potential strategies to block it. Therefore, it is worth further exploring the role of circRNAs in lymphangiogenesis and the underlying molecular mechanism.

ii) Immune cells. Immune cells are the most critical component of the TME, and the function of immune cells is closely related to tumor development and metastasis. The interactions among cancer cells, immune cells and released factors may regulate tumor immunity, establishing an environment conducive to tumor development (82). Previous studies have shown that circRNAs interact with immune cells to regulate tumor metastasis.

Tumor-associated macrophages (TAMs) are the most common immune cells in the TME and serve a vital role in the occurrence and metastasis of tumors. Duan *et al* (83) have summarized the interactions between circRNAs and TAMs, improving the understanding of the relationship between circRNAs and TAMs. Notably, studies have shown that circRNAs can induce the M2-like polarization of TAMs. For example, Lu *et al* (84) screened circ_0001142 through bioinformatics methods, and reported that it sponged miR-361-3p and served a vital role in inducing M2 polarization of macrophages, thus promoting BC cell metastasis. Similarly, circSHKBP1 is upregulated in NSCLC tissues and cells, and participates in the metastasis of NSCLC through the miR-1294/pyruvate kinase M2 axis to recruit TAMs and induce M2 polarization (85). Another study has provided novel insights into the relationship between circRNAs and TAMs, and reported that circASAP1 enhanced the pulmonary metastasis of HCC cells. Mechanistic studies have elucidated that

circASAP1 acts as a competing endogenous RNA (ceRNA) for miR-326 and miR-532-5p, the common direct targets of which are MAPK1 and colony stimulating factor 1, and mediates TAM infiltration (86). Although the aforementioned results have indicated that circRNAs can regulate macrophage infiltration and polarization, the ways they affect macrophage polarization are still not fully understood. However, it should be acknowledged that tumor development is related to the M2 polarization of macrophages and circRNAs are closely related to it. Apart from this, various factors in the TME may also serve a role in this process. Therefore, actions need to be taken to continue assessing the mechanism.

Infiltration of immune cells, particularly that of tumor-infiltrating lymphocytes (TILs), is vital in mediating tumor progression (87). As aforementioned, exosomal circRNAs serve an essential role in tumor immunity, and thus, circRNAs may have the potential to regulate lymphocyte function. Weng *et al* (88) identified a positive association between a plasma circRNA (circ_0064428) and TILs, which may affect tumor metastasis. Considering the effect of CD151 on tumor progression, a CD151-derived circRNA, circ_0020710, has been verified to upregulate C-X-C motif chemokine ligand (CXCL)12 expression through miRNA adsorption and to be associated with cytotoxic lymphocyte failure, ultimately leading to the metastasis of melanoma (89). Apart from these, the programmed cell death protein 1/programmed death-ligand 1 (PD-L1) signaling pathway has received increasing attention among all immune checkpoints due to its demonstrated effectiveness as an immune regulator in numerous tumor types, such as BC, CRC, GC and others (90,91). It has been revealed that circRNAs can regulate immune escape, primarily as ceRNAs. PD-1, a protein expressed on the surface of T cells, regulates tumor immune function by interacting with PD-L1 on tumors, and emerging evidence has shown that circRNAs are involved in this process (92). For example, the downregulation of circCPA4 suppresses the EMT and migration of NSCLC cells by sponging let-7 miRNA and decreasing PD-L1 expression. At the same time, NSCLC cells inactivate CD8⁺ T cells in a secreted PD-L1-dependent manner. This may indicate that circCPA4 regulates NSCLC cell mobility and inactivates CD8⁺ T cells in the TME through the let-7 miRNA/PD-L1 axis (93). Li *et al* (94) validated that circRHBDD1, an oncogene, interacted with IGF2BP2, and inhibited its ubiquitination and degradation, while upregulating PD-L1 expression and impeding the infiltration of CD8⁺ T cells, consequently promoting the migration of GC cells.

Chan and Ewald (95) previously discussed studies on the relationship between NK cells and tumor metastasis, suggesting that changes in immune cell function in the TME are part of the process of tumor development. Nevertheless, there are few examples of circRNAs and NK cells interacting to regulate tumor metastasis. Therefore, it is necessary to further explore the corresponding mechanism.

iii) Cancer-associated fibroblasts (CAFs). CAFs are highly versatile in primary and metastatic tumors, and are actively involved in cancer progression by influencing tumor angiogenesis, immunology and metabolism, alongside other components in the TME (96). CircRNAs can be expressed in both normal fibroblasts (NFs) and CAFs. After using high-throughput sequencing, Zheng *et al* (97) selected

circCUL2 as the respondent, which was predominantly expressed in CAFs, and investigated the effect of circCUL2 on pancreatic ductal adenocarcinoma (PDAC). In terms of the mechanism, circCUL2 was shown to function as a miR-203a-3p sponge and to modulate the MyD88 innate immune signal transduction adaptor/NF- κ B/IL6 axis, thus inducing a CAF inflammatory phenotype and further activating the STAT3 signaling pathway to promote the metastasis of PDAC cells. Accumulating evidence has suggested that exosomal miRNAs or miRNAs serve a critical role not only in cancer cells but also in NFs/CAFs by mediating the formation and activation of CAFs (98,99). CircRNAs, as miRNA sponges, regulate the function of CAFs by interacting with miRNAs and ultimately affect tumor metastasis. Notably, Liu *et al* (100) found that CAF-derived cytokines enhanced HCC metastasis by activating the circRNA-miRNA-mRNA axis in tumor cells. Specifically, CAF-derived CXCL11 upregulated circUBAP2 content, which sponged miR-4756 and inhibited interferon induced protein with tetratricopeptide repeats 1/3 expression, subsequently inducing HCC metastasis. Additionally, CAFs can regulate tumors by releasing exosomes, and circRNAs in CAF-derived exosomes can also regulate tumor metastasis through ceRNA mechanisms. For example, a novel circEIF3K/miR-214/PD-L1 axis mediating hypoxia-induced CRC progression via CAFs has been reported, providing a rationale for clarifying the relationship between CAFs and circRNAs (101). Notably, there is complex crosstalk between CAFs and immune cells, and the role of circRNAs in this relationship requires further study to clarify the mechanism of tumor metastasis.

iv) ECM. The ECM is a non-cellular three-dimensional macromolecular network containing structural proteins, surface receptors and cell-matrix proteases, and serves an important role as a tissue barrier for tumor metastasis (102). CD44, a cell-surface glycoprotein contributing to cell adhesion and metastasis, has been shown to enhance the migration of clear cell renal cell carcinoma cells via the circPPP6R3/miR-1238-3p axis (103). Similarly, circFNDC3B, circularized with exons 5 and 6 of FNDC3B, upregulates CD44 expression. In a previous study, RNA immunoprecipitation and pull-down assays demonstrated that circFNDC3B bound to IGF2BP3, followed by an interaction with CD44, which resulted in the malignant metastasis of GC (104). Because of the various matrix components, the ECM continuously undergoes remodeling mediated by several matrix-degrading enzymes to support tumor cell invasion of the basement membrane and stroma, blood vessel penetration and metastasis during tumorigenesis (105). The MMP family appears to serve a vital role in tumor metastasis, and is also inextricably linked with circRNAs (106). For example, in ovarian cancer cells, the protein expression levels of VEGA and MMP-2 are decreased after the depletion of circ-CSPP1, indicating that circ-CSPP1 may affect cell migration by regulating matrix remodeling and angiogenesis (107). In addition, circ_0000064, which is highly expressed in lung cancer cells (A549 and H1229), downregulates MMP-2 and MMP-9 expression after gene depletion, thereby affecting tumor dissemination and invasion (108). It may be concluded that circRNAs participate in the metastasis and invasion of numerous types of cancer by modulating the TME, primarily functioning as matrix remodelers. Therefore, paying attention to how circRNAs affect ECM may be critical in diagnosing

tumor metastasis and could provide novel directions for future research.

CircRNA-mediated EMT in metastasis. EMT is a complex cellular process involved in tumor progression. Cancer cell plasticity is essential for the phenotypic transitions that occur during the metastasis of primary tumors, such as undergoing EMT. EMT is characterized by a reduction of membrane E-cadherin expression and the loss of epithelial cell identity, alongside the acquisition of mesenchymal features and increased N-cadherin expression and migration (109). Thus far, a number of studies have confirmed the potential of circRNAs in regulating EMT. TGFBR1, an essential protein in the TGF- β signaling pathway, is closely related to cell EMT (110). Zhang *et al* (111) screened a novel circRNA (circCACTIN) via microarray assays and revealed binding interactions between circCACTIN and miR-331-3p. Mechanistically, circCACTIN/miR-331-3p binding was shown to improve the stability of TGFBR1, and thus, speed up the EMT process and GC cell metastasis. Similarly, depletion of hsa-circ_0058106, which is upregulated in laryngeal cancer, inhibits the migration and invasion of laryngeal cancer cells, with decreased expression levels of N-cadherin and vimentin, and increased expression levels of E-cadherin (112). In addition to the ceRNA mechanism, circRNAs can regulate EMT by regulating parental genes, as aforementioned. CircENO1 sponges miR-22-3p to affect its host gene ENO1, which affects EMT and tumor metastasis through glycolysis (59). CircFoxo3 has been reported to be involved in various types of cancer, such as BC, GC, PCa and others (113). Shen *et al* (114) elucidated that circFoxo3 inhibited the migration and invasion of PCa cells, which was related to the circFoxo3-induced suppression of Foxo3 and EMT. EMT is a phenomenon often accompanied by tumor metastasis; thus, circRNAs may influence the process of EMT and ultimately affect tumor metastasis through different mechanisms, such as by acting as ceRNAs or in combination with RBPs. Notably, circRNAs appear to be widely altered during EMT and are likely to be regulated by diverse splicing factors (115). Therefore, the relationship between circRNAs and EMT remains to be explored.

Metabolism-related circRNAs and tumor metastasis. During the occurrence and development of tumors, the metabolism of tumor cells will adopt specific patterns to adapt to the rapid growth of tumors, including glycometabolism, lipid metabolism and amino acid metabolism. Previous studies have identified a close relationship between tumor metabolism and metastasis (116,117). First, circRNAs not only affect the abnormal uptake of glucose but also impact glycolysis in various ways, such as by regulating transporters and enzymes, transcription factors and signaling pathways. For example, the AKT/mTOR axis is a crucial process in cancer metastasis that maintains energy homeostasis via energy production, such as the Warburg effect, and blocks catabolic activities (118). Zhang *et al* (119) reported that circNRIP1 could enhance glucose uptake and induce the generation of lactic acid by sponging miR-149-5p, which targets AKT1, which has been confirmed to be a factor promoting metastasis in GC. Second, increased lipid production is a marker of tumor metabolism since cancer cells also need large amounts of fatty acids to make cell membranes. Fatty acid synthase (FASN) is a key enzyme responsible for catalyzing fatty acid synthesis.

CircFARSA relieves the inhibition of the oncogene FASN by sponging miR-330-5p and miR-326, and this regulatory mechanism aligns with the results of *in silico* analysis (120). In addition, studies have shown that circRNAs can regulate amino acid metabolism in various diseases (121,122), while its effect on tumor metastasis still needs to be further explored.

Autophagy is an irreplaceable catabolic process that delivers harmful or inessential biological macromolecules from the cytoplasm to cellular lysosomes for degradation and recycling. Autophagy is required for the turnover of components and energy, and for environmental adaptation (123). Growing evidence has verified that circRNAs can influence cancer progression by affecting autophagy, including roles of activation and inhibition (124,125). In the process of TNBC metastasis, the circEGFR/miR-224-5p/autophagy related (ATG)13/unc-51 like autophagy activating kinase 1 axis serves a critical role through the promotion of transcription factor EB nuclear trafficking (126). Notably, a reduction in circ_0032821, which is markedly upregulated in human GC tissues and cells, promotes the autophagy of GC cells *in vitro* and is involved in the suppression of GC metastasis (127). Furthermore, He *et al* (128) demonstrated that the autophagy-associated circRNA circATG7 elevated the levels of autophagy by regulating ATG7 through two different mechanisms. As circATG7 is nucleoplasmic colocalization, it interacts with miR-766-5p in the cytoplasm to remove its inhibitory effect on ATG7, while binding with HuR protein in the nucleus enhances the stability of ATG7 mRNA. In addition, circPABPN1 blocks the interaction between HuR and ATG16L1 mRNA, thereby decreasing the production of ATG16L1-a gene implicated in autophagy (129). HuR has been demonstrated to act on a number of autophagy-related genes to regulate the occurrence of autophagy (130). Given the binding capacity between circRNAs and HuR, circRNAs may serve roles in regulating the activation or inhibition of autophagy.

4. Effects of circRNAs on various tumor metastases

To date, numerous reports have demonstrated that circRNAs are abnormally expressed in tumor tissues, and emerging evidence has shown that circRNAs serve a critical role in the metastases of tumors. Zhang *et al* (131) summarized the roles of different circRNAs in cancer metastasis, resulting in a clearer understanding of the role of circRNAs. Abnormally expressed circRNAs may regulate the metastasis of different cancers through various mechanisms: They may either promote tumor initiation and progression or protect the body against tumors. For instance, Wang *et al* (132) conducted a mechanistic study on circ6834 and found that circ6834 regulated by TGF- β cannot only expose the function of TXNIP by sponging miR-873-5p, but also bind to AHNK to mediate the degradation of TRIM25, thereby inhibiting the metastasis of NSCLCs. Furthermore, circLARP1B, a mammalian conserved circRNA, interacts with the HNRNP protein to inhibit the stability of the LKB1 gene, thereby affecting lipid metabolism and the progression of HCC metastasis (133). By database screening and validation, hsa_circ_0003692 was found to be downregulated. More importantly, hsa_circ_0003692 inhibited the process of EMT and metastasis of GC by encoding the FNDC3B-267aa protein (134). Overall,

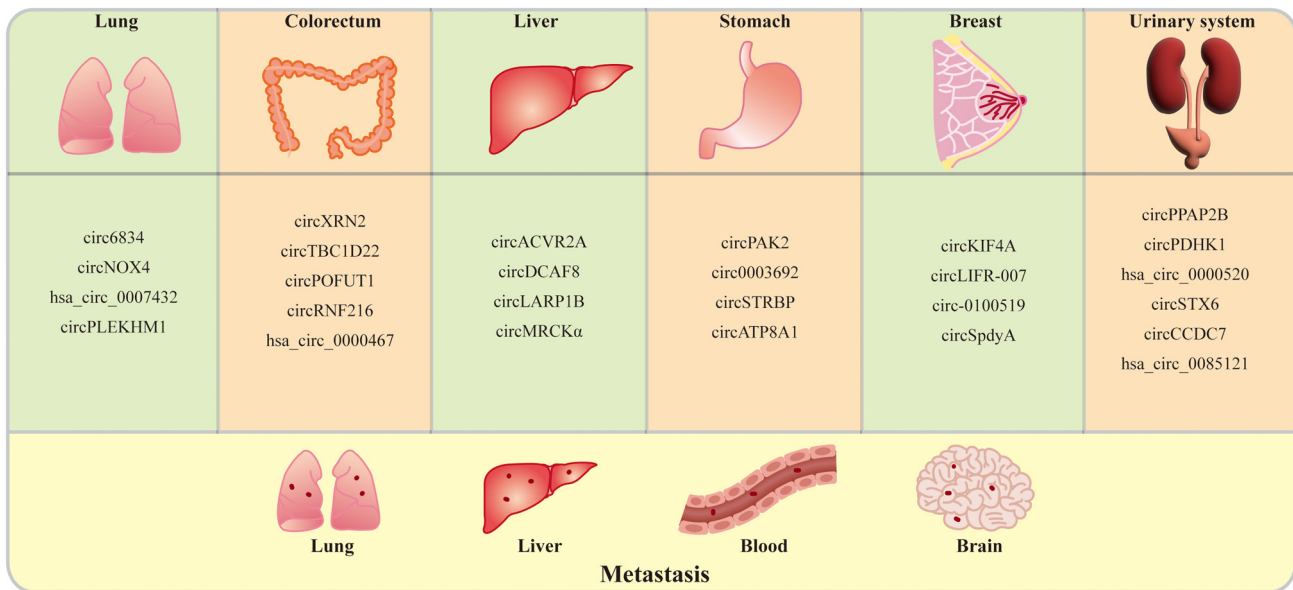


Figure 3. Overview of circRNAs in various types of cancer metastases. CircRNA/circ, circular RNA.

numerous studies have shown that circRNAs are involved in the occurrence and metastasis of various cancers, and metastasis often occurs in sites such as the lungs, liver, blood and brain (Fig. 3). A summary of recent studies on the relationship between circRNAs and various common tumor metastases is shown in Fig. 3 and Table I (135-160). Nevertheless, the role of circRNAs in diagnosing and treating cancers needs to be further studied.

5. CircRNAs as potential biomarkers before tumor metastasis

Most types of cancer lack typical clinical symptoms at onset. If diagnosed at an earlier stage, especially before metastasis, numerous patients with cancer could be treated earlier, resulting in an improved prognosis and quality of life. Furthermore, biomarkers can be detected in the circulation (whole blood, serum, or plasma), excretions or secretions (stool, urine, sputum, or nipple discharge) in a simple, non-invasive and continuously evaluable manner without the need for a biopsy or imaging (161). Therefore, liquid biopsy has been investigated by scientists and oncologists for several years, and circRNAs have been verified as ideal liquid biopsy biomarker candidates because of their tissue- and developmental stage-specific expression, and their abundance in both tumor tissues and body fluids (162). Furthermore, circRNAs lack a 5' end cap and a 3' end poly(A) tail resistant to RNA enzymes, making them more stable in body fluids than linear RNAs for detection.

Several studies have confirmed the potential of circRNAs as metastatic and prognostic prediction biomarkers in different tumors. For example, the plasma levels of hsa_circ_0001785 in patients with BC are markedly decreased after surgery, indicating its potential as a prognostic biomarker. Furthermore, the plasma levels of hsa_circ_0001785 are closely related to histological grade, TNM stage and distant metastasis, which can be used in the staging and grading of BC (163). In CRC, Yang *et al* (164) demonstrated that circPTK2 expression was

high in both CRC tissues and serum. Furthermore, a receiver operating characteristic analysis showed that circPTK2 was a marker of CRC in patients with nodal [area under the curve (AUC)=0.7249] or distal (AUC=0.7865) metastasis. Similar to these findings, the levels of circ_0001178 and circ_0000826 have been shown to be elevated in the tissues of patients with CRC with liver metastasis compared with those without metastasis. Both circRNAs may distinguish patients with liver metastases among samples, and the AUC values were 0.945 for circRNA_0001178 and 0.816 for circRNA_0000826 (165). In addition, given the role of exosomes in promoting tumorigenesis and metastasis, they are ideal diagnostic biomarkers with a unique capacity for carrying and delivering circRNAs (166). Chen *et al* (167) revealed that circPRMT5 was upregulated in serum and urine exosomes from patients with urothelial carcinoma of the bladder, indicating that the levels of circPRMT5 in serum and urine exosomes may be a risk predictor of metastasis. Circ-IARS, which has been shown to be upregulated in pancreatic cancer tissues and plasma exosomes from patients with metastatic disease, is also positively associated with tumor and lymph node metastasis status (168).

The only opportunity for a complete cure in almost all types of cancer relies on a timely diagnosis, especially before metastasis occurs, so that timely treatments can be implemented. There remains a pressing clinical need to identify and develop highly sensitive and specific diagnostic biomarkers that can detect different cancer types and their precursor lesions as early as possible. Due to their unique advantages, circRNAs may provide unlimited opportunities to detect tumors before metastasis.

6. Detection strategies for circRNAs

After determining the complex dynamics of circRNAs during cancer development and their advantages and potential as biomarkers, highly efficient and convenient analysis of circRNAs is required to understand the mechanism of

Table I. Summary of dysregulated circRNAs in common cancer metastasis recently.

Cancer type	CircRNA	Sample source	Mechanism	Target	Role (+/-)	Metastasis	(Refs.)
LC	Circ6834	NSCLC tissues	miRNA sponge, protein binding, EMT	TGF- β /SMAD2 and SMAD3/QKI/circ6834; miR-873-5p/TXNIP; AHNAK/TRIM25	-	Lung	(132)
	CircNOX4	NSCLC tissue-derived CAFs	miRNA sponge, TME (CAFs)	miR-329-5p/FAP/IL-6	-	Lung	(135)
	hsa_circ_0007432	LC tissues, LC cells	Protein binding, TME (M2 polarization of macrophages)	SRSF1/KLF12/IL-8	+	Not mentioned	(136)
	CircPLEKHM1	NSCLC cell-derived exosomes	Protein binding, TME (M2 polarization of macrophages), exosomes	HIF-1A/circPLEKHM1/PABPC1/eIF4G/OSMR/JAK-STAT3	+	Bone	(137)
CRC	CircXRN2	CRC tissues	miRNA sponge, EMT	miR-149-5p/ENC1	+	Lung, liver	(138)
	CircTBC1D22A	CRC tissues	miRNA sponge, autophagy	miR-1825/ATG14	-	Liver	(139)
	CircPOFUT1	CRC tissues, CRC cells	miRNA sponge, protein binding	miR-653-5p/E2F7/WDR66; IGF2BP1/BMI1	+	Liver	(140)
	CircRNF216	CRC tissues	miRNA sponge, TME (CD8 ⁺ T cells)	miR-576-5p/ZC3H12C	-	Lung	(141)
HCC	hsa_circ_0000467	CRC tissues	Protein binding	EIF4A3/c-Myc	+	Lung	(142)
	CircACVR2A	Other tumor tissues and cells	miRNA sponge	miR-511-5p/PI3K-Akt	+	Not mentioned	(143)
	CircDCAF8	HCC tissues	miRNA sponge, exosomes, EMT, angiogenesis	miR-217/NAP1L1	+	Lung	(144)
	CircLARP1B	Metastatic HCC tissues	Protein binding, lipid metabolism	HNRNPDLKB1/AMPK	+	Liver	(133)
GC	CircMRCK α	HCC tissues	Protein binding, encoding protein/peptide, glycolysis, TME (CD68 ⁺ TAMs)	USP22/HIF-1 α /TAMs; circMRCK α -227aa/USP22/HIF-1 α	+	Not mentioned	(145)
	CircPAK2	GC tissues	Protein binding, EMT, angiogenesis, lymphangiogenesis, m ⁶ A modification	YTHDC1/circPAK2/IGF2BPs/VEGFA	+	Lymph node	(78)
	Circ0003692	GC tissues	Encoding protein/peptide, EMT	FNDC3B-267aa/c-Myc/Snail/Slug	-	Liver	(134)

Table I. Continued.

Cancer type	CircRNA	Sample source	Mechanism	Target	Role (+/-)	Metastasis	(Refs.)
BC	CircSTRBP	GC tissues	miRNA sponge, exosomes	miR-1294 and miR-593-3p/E2F2	+	Not mentioned	(146)
	CircATP8A1	GC plasma exosomes	miRNA sponge, TME (M2 polarization of macrophages), exosomes	miR-1-3p/STAT6	+	Not mentioned	(147)
	CircKIF4A	TNBC tissues	miRNA sponge, autophagy	miR-637/STAT3 and p62/Beclin	+	Brain	(148)
	CircLIIFR-007	Metastatic BC tissues	Protein binding	hnRNPA1/YAP/SREBF1 and SNAI1	-	Liver	(149)
	Circ-0100519	BC tissues	Protein binding, TME (M2-like macrophage polarization), exosomes	HIF-1 α /circ-0100519/USP7 and NRF2/NRF2	-	Lung	(150)
Urinary system cancer	CircSpdyA	TNBC tissues	Encoding protein/peptide, lipid metabolism, TME (NK cells)	127aa/FASN/fatty acid synthesis/MICA and MICB	+	Lung	(151)
	CircPPAP2B	Invasive ccRCC cell lines	miRNA sponge, protein binding, m ⁶ A modification	HNRNPC/PTBP1 and HNRNPK; miR-182-5p/CYP1B1	+	Lung	(152)
	CircPDHK1	ccRCC tissues	Encoding protein/peptide	HIF-2A/circPDHK1/PDHK1-241aa/PPP1CA/AKT-mTOR	+	Lung	(153)
	CircSTX6	BCa tissues	miRNA sponge, protein binding	EIF4A3/circSTX6/miR-515-3p and PABPC1/SUZ12	+	Lung	(43)
	hsa_circ_0000520	BCa tissues	Protein binding	QKI/hsa_circ_0000520Lin28a/PTEN/PI3K/AKT	-	Lung	(154)
ESCC	CircCCDC7	PCa tissues	encoding protein/peptide	CircCCDC7-180aa/FLRT3	-	Not mentioned	(155)
	hsa_circ_0085121	PCa tissues	Encoding protein/peptide	CircRNF19A-490aa/HSP90AA1/AR/RNF19A/hsa_circ_0085121/circRNF19A-490aa; and HNRNPF/AR-V7	+	Lung	(156)
	CircPDE5A	ESCC tissues	Encoding protein/peptide	PDE5A-500aa/USP14/PIK3IP1/PI3K/AKT	-	Lung	(157)
	CircPHGDH	PTC tissues	miRNA sponge, glycolysis	miR-122-5p/PKM2	+	Lung	(158)

Table I. Continued.

Cancer type	CircRNA	Sample source	Mechanism	Target	Role (+/-)	Metastasis	(Refs.)
Laryngeal cancer	CircMMP9	Metastatic LSCC tissues	Protein binding, m ⁶ A modification	IGF2BP2/circMMP9/ETS1/TRIM59/PI3K/AKT	+	Not mentioned	(159)
PDAC	CircPNT	KRAS ^{G12D} PDAC tissues	Protein binding, exosomes	Rab5B and CD109/DSCAML1/GFRα1/RET	+	Neural	(160)

BC, breast cancer; BCC, bladder cancer; CAF, cancer-associated fibroblast; ccRCC, clear cell renal cell carcinoma; circRNA/circ, circular RNA; CRC, colorectal cancer; EMT, epithelial-mesenchymal transformation; ESCC, esophageal squamous cell carcinoma; GC, gastric cancer; HCC, hepatocellular carcinoma; LC, lung cancer; LSCC, lung squamous cell carcinoma; m⁶A, N⁶-methyladenosine; miRNA/miR, microRNA; NK, natural killer; NSCLC, non-small cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; PTC, papillary thyroid cancer; TAM, tumor-associated macrophage; TME, tumor microenvironment; TNBC, triple-negative BC; +, promotion; -, inhibition.

circRNAs in cancer deterioration and to promote the application of circRNAs as cancer biomarkers in clinical detection. Nevertheless, limitations of detection methods, such as low sensitivity and specificity, have markedly restricted the research on circRNAs and their application as diagnostic biomarkers (169).

Electron microscopy was first attempted shortly after circRNAs were identified; however, this method cannot easily distinguish circRNAs from RNA lariats, which are by-products of RNA splicing (170,171). Furthermore, genome-wide and site-specific circRNAs are usually detected only by identifying unique back-splice junctions (BSJs) in circRNAs, not by detecting internal splicing patterns (172). Nevertheless, a number of researchers have continuously identified and developed circRNA detection technologies over the past few years. Based on their findings (172-176), the present review summarizes the latest progress and discusses the detection methods of circRNAs.

Northern blotting can indicate the size and annular structure characteristics of circRNAs, without other complex reaction processes, and is regarded as the gold standard for confirming the presence of circRNAs. However, its application is limited due to its low sensitivity, RNA consumption and energy consumption (177,178). RT-PCR and RT-qPCR are the most commonly used methods for validating circRNAs, and RT-qPCR is more advantageous because of the quantification of circRNAs (179,180). Due to the presence of RNase R that enables circRNAs to be exposed to complex samples, the considerable sensitivity, operability and simplicity of this technique make it popular for clinical laboratory testing. However, the strength of RNase R is not strong enough for some linear RNAs, or some circRNAs may not be able to resist digestion due to length and other problems. In addition, the primers designed for this process are only based on BSJs, and the results may be interfered with by rolling circle amplification (RCA) during RT, making this method imperfect and occasionally unable to detect target molecules in complex samples (172,181). The third generation of PCR, droplet digital PCR (ddPCR), is a novel detection technology based on RNA amplification that exhibits greater sensitivity and accuracy, and is widely used in low-abundance nucleic acid tests (182). Since it dilutes the template into independent, non-interacting droplets, ddPCR appears to be more sensitive and accurate than RT-qPCR in quantifying circRNAs, and more suitable for circRNA detection in challenging samples, such as plasma, where circRNAs are present in limited quantities (183). Chen *et al* (184) verified the discrepancy in circRNA quantification by RT-qPCR and RT-ddPCR, which is caused by the rolling RT of circRNAs, with the latter eliminating the impact of RCA and achieving higher accuracy. In addition, ddPCR is more tolerant to PCR inhibitors, avoiding the loss of information in samples to a large extent (185). With the development of high-throughput sequencing, such as RNA-sequencing and microarrays, this has been widely used in the search for different cancer-related circRNAs, allowing for the simultaneous profiling of various circRNAs and the identification of novel circRNAs. However, the problem of false positives remains inevitable (167,186,187). Fluorescence *in situ* hybridization is a universally applicable method that provides subcellular localization information about certain circRNAs using a junction-specific probe (188).

Beyond these common classical methods, studies have identified promising novel techniques of circRNA amplification and detection. For example, Zhang *et al* (189) first developed a specific method for the quantitative detection of circRNAs by designing two DNA probes precisely connected in the BSJ of circRNAs, which allows the distinction between circRNAs and corresponding linear RNA without the help of an exonuclease in the process of PCR. Although this method can only detect one circRNA at a time, which limits the high-throughput screening of circRNAs, the advantages of excluding the influence of linear RNA and by-products of RCA, and the minimum detection limit of 1 fM, are enough to indicate its future potential. Furthermore, Liu *et al* (190) designed an RT-mediated RCA (RT-RCA) process to achieve selective amplification of target circRNAs. In the presence of circular DNA primers designed according to BSJ, circRNAs are amplified with ProtoScript II reverse transcriptase to generate a long single-strand RT-RCA DNA containing numerous repeated sequences. Therefore, it can unfold the hairpin structure of the DNA molecular beacon, which contains fluorophore Fam at the 5' end and quencher Dabcyl at the 3' end, thereby emitting a fluorescence signal. Similarly, Jiao *et al* (191) presented a novel tool, linear DNA nanostructure (LDN), for the efficient assessment of circRNAs. RCA is performed according to the circular DNA template to form a long single strand containing multiple hairpin probe-binding sites, and then hairpin-shaped probes H1 (recognizing the BSJ site) and H2 (containing a fluorophore and quencher pair) are hybridized on the DNA scaffold, making an intact LDN. Notably, in the presence of target circRNA, the circRNA triggers a cascade displacement reaction between two hairpin probes sequentially, leading to the fluorescent signal of H2 being restored and finally lighting up the whole LDN. It is commendable that the target circRNA can be recycled until all LDN systems are lit up, resulting in multiple signal amplifications, which is conducive for detecting low-abundance circRNAs in challenging samples. Dong *et al* (192) designed an effective circRNA detection method based on the thermostatic netlike hybridization chain reaction (HCR), which combines RT-RCA with well-designed netlike HCR (three HCR hairpin probes designed based on BSJ: Trigger, H1 and H2) to achieve dual selection and dual signal amplification. In the presence of target circRNA, the network structure is formed by chain replacement reaction in sequence and the fluorescence signal of the H1 probe is recovered to achieve the specific detection of trace circRNAs. Given that circRNA-miRNA interaction (cmRRI) is commonly involved in regulating tumor progression, a biosensing method that directly allows for analysis of cmRRIs has been developed by Jiao *et al* (193). A biotin-modified DNA probe is designed according to BSJ and then connected to streptavidin-modified magnetic beads. When the target circRNA interacts with miRNA, miRNA acts as a primer to trigger the RCA reaction and then captures numerous fluorescence signal probes, ultimately achieving direct, sensitive and specific detection of cmRRIs. In addition, Zhang *et al* (194) first established an ultrasensitive and specific method for the detection of circRNAs with stem-loop primers (SLPs) via loop-mediated isothermal amplification-induced double exponential amplification. Since SLPs only recognize and bind BSJs, only the presence of target circRNA can trigger the formation of a

double-stem loop DNA structure. It is not only free from the use of RNase R, but also excludes the effects of linear RNA and rolling loop amplification products, which is considered to be a relatively sensitive method to detect circRNAs at present.

Current circRNA detection technologies face significant limitations. At the sample preparation stage, low-abundance circRNAs are often lost during extraction due to the existence of linear RNAs, and RNase R digestion may inefficiently process certain circRNA subtypes, potentially leading to erroneous results or incomplete enrichment. Traditional detection methods, such as qPCR, struggle with primer specificity due to sequence variations and RT biases due to the circular structure of circRNAs, whereas high-throughput sequencing is hindered by the inability of short-read technologies to resolve isoforms, the high costs and immature analytics of long-read methods, and the need for deep sequencing to overcome linear RNA background noise. Data analysis is challenged by species-limited databases, inconsistent algorithm outputs and high false-positive rates, while clinical applications are constrained by the low abundance of circRNAs in body fluids, as well as the lack of sensitive and cost-effective single-cell analysis tools, and are further limited by the absence of standardized laboratory protocols.

Innovative technologies are emerging to address these issues: i) Geometry-based platforms combined with fluorescence techniques enable ultra-sensitive, amplification-free detection with high specificity for live-cell imaging and liquid biopsies; ii) CRISPR-Cas13a integrated with droplet digital technology provides a method with favorable sensitivity for portable clinical screening; and iii) machine learning-driven algorithms are enhancing circRNA annotation accuracy and functional prediction (195-197). Unlike fluorescence analysis, surface-enhanced Raman scattering (SERS) is a nano-scale optical phenomenon mainly driven by electromagnetic mechanism, which has the advantages of ultra-high sensitivity, narrow peak width, low background signal and intrinsic chemical fingerprint information (198,199). Numerous studies have applied SERS to detect miRNAs, realizing signal amplification and label-free detection (200,201). Additionally, the detection of DNA methyltransferase (202) and exosomes (203) in human serum can be realized by SERS signal amplification strategies; however, the detection of circRNAs using this method is rare and still relatively novel. Therefore, as a potential detection method, SERS is expected to be applied to the detection of tumor biomarkers in the future, urgently requiring further in-depth research.

7. Conclusions and prospects

The role of circRNAs in the progression of various diseases, especially cancer metastasis, is becoming more clearly understood as the specific mechanism is being widely explored. Among them, the most studied circRNAs can act as molecular sponges of miRNAs, alleviating the inhibition of downstream target genes; however, several circRNAs contain little or almost no MREs. Therefore, the function of circRNAs in this mechanism remains largely undetermined. Furthermore, other functional pathways of circRNAs have been unclear to date, and their specific mechanism requires further discussion. After understanding the mechanism of action of circRNAs in

tumor metastasis, it is possible to use circRNAs as targets for treatment and to assess prognosis. Studies have shown that nanomaterials can be used to deliver circRNA-interfering drugs to inhibit carcinogenic circRNAs, demonstrating that they have potential in cancer treatment (204,205).

Diagnosis and intervention before tumor metastasis remain the best ways to prevent tumor progression as much as possible, reduce cancer mortality and improve the quality of life. Due to the unique advantages of circRNAs, they are expected to be ideal tumor biomarkers. However, their application is limited due to their low abundance or the low sensitivity and specificity of detection technology. How to distinguish them from linear RNAs or other by-products, how to accurately quantitate them, how to make the detection process simple and rapid, how to achieve high-quality detection at a low cost, and how to integrate multiple disciplines and achieve clinical application are the current challenges and urgent issues that remain to be solved.

Attempts to identify the physiological and pathological roles of circRNAs in tumor metastasis and progression remain critical. The identification of more unknown functions and applications of circRNAs will aid the diagnosis and treatment of cancer before and after metastasis.

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Authors' contributions

XD wrote and revised the manuscript. YC and TZ conceived the idea. YJ, HD, QR and HS collected current literature and participated in the revision of the manuscript. Data authentication is not applicable. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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