Molecular Therapy
Nucleic Acids
Review

The multifaceted roles of circular RNAs in cancer hallmarks: From mechanisms to clinical implications

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Circular RNAs (circRNAs) represent a distinct class of covalently closed RNA species lacking conventional 5′ to 3' polarity. Derived predominantly from pre-mRNA transcripts of protein-coding genes, circRNAs arise through back-splicing events of exon-exon or exon-intron junctions. They exhibit tissue- and cell-specific expression patterns and play crucial roles in regulating fundamental cellular processes such as cell cycle dynamics, proliferation, apoptosis, and differentiation. CircRNAs modulate gene expression through a plethora of mechanisms at epigenetic, transcriptional, and post-transcriptional levels, and some can even undergo translation into functional proteins. Recently, aberrant expression of circRNAs has emerged as a significant molecular aberration within the intricate regulatory networks governing hallmarks of cancer. The tumor-specific expression patterns and remarkable stability of circRNAs have profound implications for cancer diagnosis, prognosis, and therapy. This review comprehensively explores the multifaceted roles of circRNAs across cancer hallmarks in various tumor types, underscoring their growing significance in cancer diagnosis and therapeutic interventions. It also details strategies for leveraging circRNA-based therapies and discusses the challenges in using circRNAs for cancer management, emphasizing the need for further research to overcome these obstacles.

INTRODUCTION

Circular RNAs (circRNAs) are an evolutionary conserved unique class of covalently closed-loop RNA molecules,[1] initially detected in the Sandai virus[2] and plant pathogenic viroids more than four decades ago.[3] Later studies confirmed their presence in human cancer cell lines.[4,5] CircRNAs predominantly originate from protein-coding genes to a lesser extent from non-coding genes and UTRs, leading to a diverse range of circRNA isoforms.[1,6,7] According to CircAtlas, a database that catalogs circRNA transcriptomes, approximately 413,657 circRNAs have been identified in humans, with 333,856 cataloged as full-length isoforms.[8] Remarkably, circRNAs exhibit a wide size range spanning from approximately 100 to 4,000 nucleotides in length.[9] Various databases and analytical tools have been developed for the computational analysis of circRNAs (Table 1). Comparatively, circRNAs are distinct from linear coding and noncoding counterparts by their atypical structure lacking 5′ to 3' polarity, 5′ caps, and poly(A) tails.[10] Moreover, circRNAs are remarkably stable compared with the other RNA species due to their lack of 3′ end and resistance to Rnase R (digests linear RNA species only).[11] Studies have shown that circRNAs possess extended half-lives exceeding 48 h, in contrast with the median half-life of mRNA of approximately 10 h.[12,13]

CircRNAs are versatile modulators of gene expression, intricately influencing both spatial and temporal gene regulation within the regulatory networks of competitive endogenous RNAs.[14] Notably, the functional roles of circRNAs are linked to fundamental cellular processes, including cell growth, differentiation, cell cycle regulation, proliferation, and apoptosis, thus playing an essential role in maintaining cellular homeostasis.[15–17] Remarkably, circRNAs exhibit tissue-specific, cell-specific, developmental stage-specific, and disease-specific characteristics, underscoring their pivotal role in gene regulation during health and disease.[1] Recently, circRNAs have emerged as key players in the regulatory mechanisms governing various cancer hallmarks, including resistance to therapies. Moreover, the specific expression patterns of circRNAs across different tumor types[18] and stages,[19] detectable in tissues and bodily fluids,[20] highlight their potential as indicators for cancer diagnostics, prognostics, and therapeutic strategies.

BIOGENESIS

The biogenesis of circRNAs is orchestrated by two principal mechanisms: back-splicing and lariat precursor formation. Back-splicing, facilitated by spliceosomal machinery, joins a downstream 5′ splice donor with an upstream 3' splice acceptor, forming exonic circRNAs (ecircRNAs) or exon-intron circRNAs (EIciRNAs) within precursor mRNA introns and exons.[21] Intriguingly, recent cryo-electron microscopy investigations in *Saccharomyces cerevisiae* have elucidated adaptive mechanisms in the spliceosomal complex based on exon length, with longer exons preferentially back-spliced producing

https://doi.org/10.1016/j.omtn.2024.102286.

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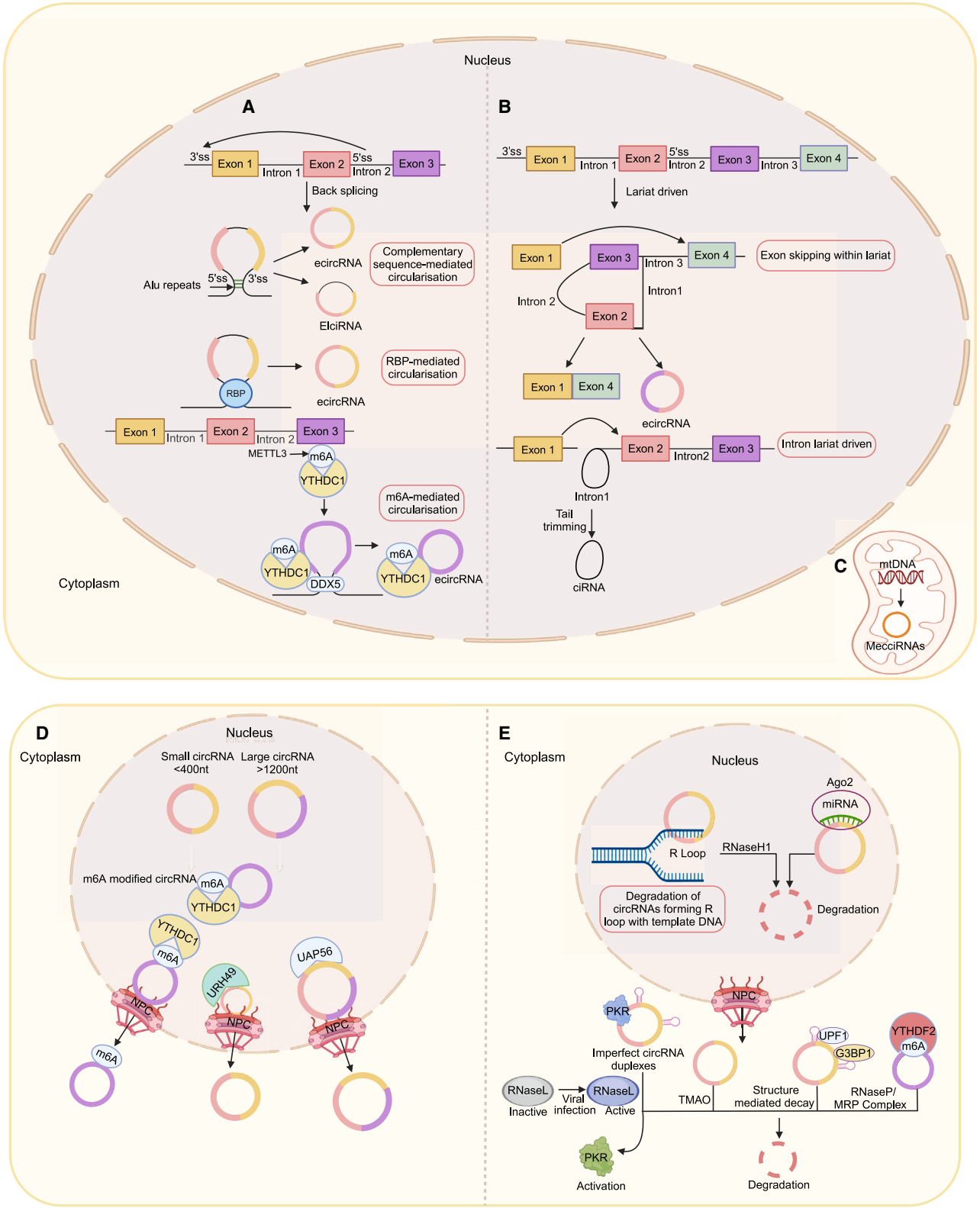
Table 1. CircRNA databases and analysis tools

Database	Utility	URL
CIRCpedia	expression characteristics of circRNAs in cell types/tissues, and disease samples, conservation analysis between humans and mice	http://www.picb.ac.cn/rnomics/circpedia/
circBase	unified circRNAs datasets, expression analysis	http://www.circbase.org
circExp	pre-calculated expression dataset of circRNAs including parental genes	http://soft.bioinfo-minzhao.org/circexp
MiOncoCirc	database of circRNAs from cancer clinical samples	https://mioncocirc.github.io/
CircInteractome	prediction and mapping of RBP and miRNA with circRNAs.	https://circinteractome.nia.nih.gov/
circNET	provide experimentally verified circRNA regulatory networks	https://awi.cuhk.edu.cn/~CircNet/php/index.php
circATLAS	expression pattern, conservation, and functional annotation of circRNAs with regulatory networks and predicted IRES sites	http://circatlas.biols.ac.cn
circRNAdb	provide circRNA genomic information, exon splicing, genome sequence and IRES	http://reprod.njmu.edu.cn/cgi-bin/circrnadb/circRNADb.php
circBank	a comprehensive database and analytical tool for circRNAs	www.circbank.cn
TransCirc	To predict translation potential of circRNAs	https://www.biosino.org/transcirc
riboCIRC	computationally predicted ribosome-associated circRNAs, experimentally verified translated circRNAs, an evaluation of cross-species conservation of translatable circRNAs, a systematic *de novo* annotation of putative circRNA-encoded peptides, including sequence, structure, and function	http://www.ribocirc.com
Circad	comprehensive and updated database of disease associated circRNAs	http://clingen.igib.res.in/circad/
CircFunbase	provide a high-quality functional circRNA resource including experimentally validated and computationally predicted functions	https://bis.zju.edu.cn/CircFunBase/search.php
CSCD2	cancer-specific circRNAs from human cancer and normal tissues/cell lines, predicts potential miRNA-circRNA and RBP-circRNA interactions, potential full-length and open reading frame sequence	http://geneyun.net/CSCD2
Tissue Specific CircRNA Database	tissue-specific circRNAs in human and mouse	http://gb.whu.edu.cn/TSCD
CIRCRNome	contains predicted as well as validated circRNAs across animals and plants	https://clingen.igib.res.in/circRNome/
deepBasev3.0	integrative and interactive display and analysis of the expression, evolution, and functions of various ncRNAs using thousands of high-throughput sequencing data from tissue, tumor, and exosome samples	http://rna.sysu.edu.cn/deepbase3/index.html

circRNAs, while shorter exons spliced canonically.[22] In addition, Alu elements, common *cis*-repetitive sequences in the human genome, influence spliceosomal preferences for circular versus linear RNA processing. Shorter Alu sequences facilitate circRNA formation, whereas longer sequences impede it.[23] Alternative splicing factors such as Quaking (QKI), muscleblind (MBL/MBNL1), RNA binding motif protein 20 (RBM20), and FUS act as RNA binding proteins (RBPs) that interact with sequences adjacent to back-splicing sites, bringing flanking introns into proximity and facilitating circRNA formation.[24] For instance, FUS facilitates the formation of circHIF1A by engaging with its flanking introns, enhancing the growth and spread of triple-negative breast cancer (TNBC).[25] QKI promotes circRNA production by binding to pre-mRNA and bringing circ-forming exons closer together. CircZKSCAN, crucial for hepatocellular carcinoma (HCC), shows a positive correlation with patient survival rates and is positively associated with QKI levels.[26–28] Back-splicing can also be epitranscriptomically facilitated through N6-methyladenosine (m6A) RNA modification. This modification, catalyzed by methyltransferase-like 3, plays a crucial role in RNA metabolism, influencing various processes such as mRNA splicing, 3′ end processing, nuclear export, stability, and translation.[29] m6A modifications in linear RNAs are typically found within coding sequences, 5′ and 3′ UTRs, with enrichment near stop codons and within long internal exons.[30] These modifications influence various stages of mRNA metabolism, including splicing, export, translation, and degradation.[31] In circRNAs, m6A sites are frequently located near back-splicing junctions, crucial for circRNA formation and stability. Additionally, m6A motifs are enriched in RNase R-resistant regions of circRNAs, enhancing their stability and function. Notably, many m6A-circRNAs originate from exons lacking m6A modifications in their linear mRNA counterparts, indicating distinct methylation patterns that likely occur during or after circRNA formation.[30] Intriguingly, m6A modification promotes back-splicing by recruiting the m6A reader protein YTH m6A RBP C1 (YTHDC1) and RNA helicase DEAD-Box Helicase 5 (DDX5). Through its helicase activity, DDX5, unwinds RNA structures near the back-splice junction (BSJ), facilitating the back-splicing process. The interaction between DDX5 and YTHDC1 enhances the recognition and effect of the m6A modification, positioning DDX5 as a pivotal mediator in circRNA biogenesis[32] (Figure 1A). Studies suggest that m6A modifications in circRNAs contribute to cancer progression and other diseases.[33] For instance, in HCC cells resistant to sorafenib,



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the up-regulated circRNA-SORE sequesters miR-103a-2-5p and miR-660-3p, activating the Wnt/β-catenin pathway and enhancing resistance. This up-regulation is linked to increased m6A-mediated stability at a specific adenosine site within circRNA-SORE.[34]

Alternatively, in the absence of complementary intronic sequences, circRNA synthesis predominantly operates through the lariat precursor pathway.[35] This mechanism generates ecircRNAs through exon skipping within the lariat precursor,[35] or intronic circRNAs (ciRNA) through the prevention of intron lariat formation to debranching, facilitated by consensus RNA motifs near the splice sites[36] (Figure 1B). Notably, ecircRNAs are linked through 3′-5′ linkage while intronic lariat circRNAs are linked through 2′-5′ linkage.[37] In addition, mitochondrial circRNAs (mecciRNAs), are produced from the mitochondrial genome. These circRNAs are transcribed from both the heavy (G rich) and light (C rich) strands of mitochondrial DNA (Figure 1C).[38]

CiRNAs and EIciRNAs are primarily localized in the nucleus,[39] whereas ecircRNAs undergo nuclear export in a size-dependent manner. The export of smaller (<400 nt) and larger (>1,200 nt) ecircRNAs is facilitated by RNA helicases Hel25E, URH49 and UAP56 respectively.[40] Intriguingly, circRNAs can also be exported from the nucleus to cytoplasm in an m6A-dependent manner (Figure 1D). For instance, the m6A reader YTHDC1 binds to circNSUN2, facilitating its export to the cytoplasm. Notably, cytoplasmic circNSUN2 promotes colorectal cancer (CRC) liver metastasis by forming a ternary complex with insulin like growth factor 2 mRNA binding protein 2 and High-Mobility Group AT-Hook 2 (HMGA2) proteins.[41]

CircRNAs undergo complex degradation pathways to prevent cellular negative feedback. In the nucleus, RNase H1(degrades RNA-DNA junction) targets circRNAs that form R-loops with template DNA,[42] while microRNAs (miRNAs) in the nucleus degrade them via an argonaute-2-dependent mechanism.[43] In the cytoplasm, m6A-modified circRNAs are cleaved by the RNase-P/ribonuclease for mitochondrial RNA processing (MRP) complex under the guidance of the m6A reader YTH m6A RBP2 (YTHDF2).[44] CircRNA degradation involves structure-mediated RNA decay (SRD), facilitated by the interaction between RBPs UPF1 and G3BP1. UPF1 helicase activity unwinds highly base-paired RNA regions, enhancing G3BP1 proximity and initiating SRD.[45] Additionally, trimethylamine-N-oxide from gut microbiota also facilitates circRNA degradation.[46] During viral infections, circRNAs forming 16- to 26-bp imperfect duplexes bind to dsRNA-activated protein kinase (PKR) to inhibit it, but are subsequently degraded by RNase L, which activates PKR.[47] This detailed degradation cascade highlights the intricate regulation of circRNA stability and function within cells (Figure 1E).

REGULATION

CircRNA biogenesis is intricately regulated by *trans*-acting factors and *cis*-regulatory elements. *Trans*-acting factors include spliceosome machinery, RNA helicases, and RBPs.[48] The spliceosome, an RNA-protein complex comprising small nuclear ribonucleoproteins (snRNPs) U1, U2, U4, U5, and U6, facilitates pre-mRNA splicing. Components of U2 snRNP, like SF(splicing factor)3B1 and SF3A1, are crucial for splice site recognition, and their depletion can shift splicing toward circRNA formation.[49] RNA helicases such as DExH-box helicase 9, eukaryotic translation initiation factor (EIF) 4A3, and adenosine deaminase acting on RNA 1 also play crucial roles in RNA circularization.[48] For instance, EIF4A3 facilitates exon back-splicing to promote circASAP1 synthesis, and its high expression is associated with increased cell tolerance to temozolomide (TMZ) therapy and poorer prognosis in glioblastoma (GBM).[50] For *cis*-regulation, specific *cis*-regulatory elements that bind splicing factors are crucial for circRNA formation. For example, epithelial splicing regulatory protein 1 (ESRP1) binds to GGT-rich sequences in circANKS1B, promoting its formation. In BC, elevated ESRP1 expression correlates with increased circANKS1B levels, which are associated with TGF-β1 mediated cell invasion, metastasis, advanced clinical stages, and reduced overall survival.[51] Notably, cleavage and polyadenylation specific factor 4 (CPSF4), a key component of the 3′-end splicing complex, regulates circRNA formation by binding to polyadenylation signal elements on precursor mRNA, inhibiting the formation of circRNAs. High CPSF4 expression in HCC correlated with reduced circRNA levels and promotes HCC cell proliferation.[52]

MECHANISMS OF ACTION

In the regulatory landscape of gene expression, circRNAs emerged as crucial players, functionally interacting with proteins and nucleic acids across epigenetic, transcriptional, and post-transcriptional layers.[39,53,54] CircRNAs can regulate epigenetic mechanisms by modulating the activity of DNA methyltransferases (DNMTs) and TET1, a DNA demethylase. For instance, FECR1-circRNA binds to the promoter of its parent gene, FLI1, in cis, to recruit TET1 and

Figure 1. CircRNAs metabolism

(A) CircRNAs are back-spliced through Alu repeats forming ecircRNAs or ElciRNAs. RBPs facilitate splice sites proximity, promoting back-splicing. m6A modifications enhance circRNA biogenesis by recruiting m6A reader protein YTHDC1 and RNA helicase DDX5. (B) Lariat precursor-driven biogenesis. In exon skipping, splicing removes exons along with adjacent introns, forming a lariat structure processed into circRNAs. Intron lariat is produced from introns during splicing, which is stabilized and processed into ciRNA. (C) Mitochondrial circRNAs (MecciRNAs) are produced from the mitochondrial genome. (D) YTHDC1 binds to m6A-modified circRNAs facilitating nuclear export. URH49 and UAP56 assist the nuclear export of smaller and larger circRNAs, respectively. (E) CircRNAs forming R-loops with DNA are targeted by RNase H1 in nucleus. Ago2-bound miRNAs cleave circRNAs in nucleus. m6A-modified circRNAs are recognized by YTHDF2 in the cytoplasm, recruit RNase P/MRP complex for degradation. UPF1 unwinds double-stranded regions of circRNAs, facilitating G3BP1 binding and subsequent degradation. Trimethylamine N-oxide (TMAO) induces circRNA degradation, linking metabolism to circRNA stability. Imperfect RNA duplexes formed by circRNAs bind and inhibit PKR, during viral infections, RNase L degrades these duplexes, activating PKR. NPC, nuclear pore complex. RBP, RNA binding protein.

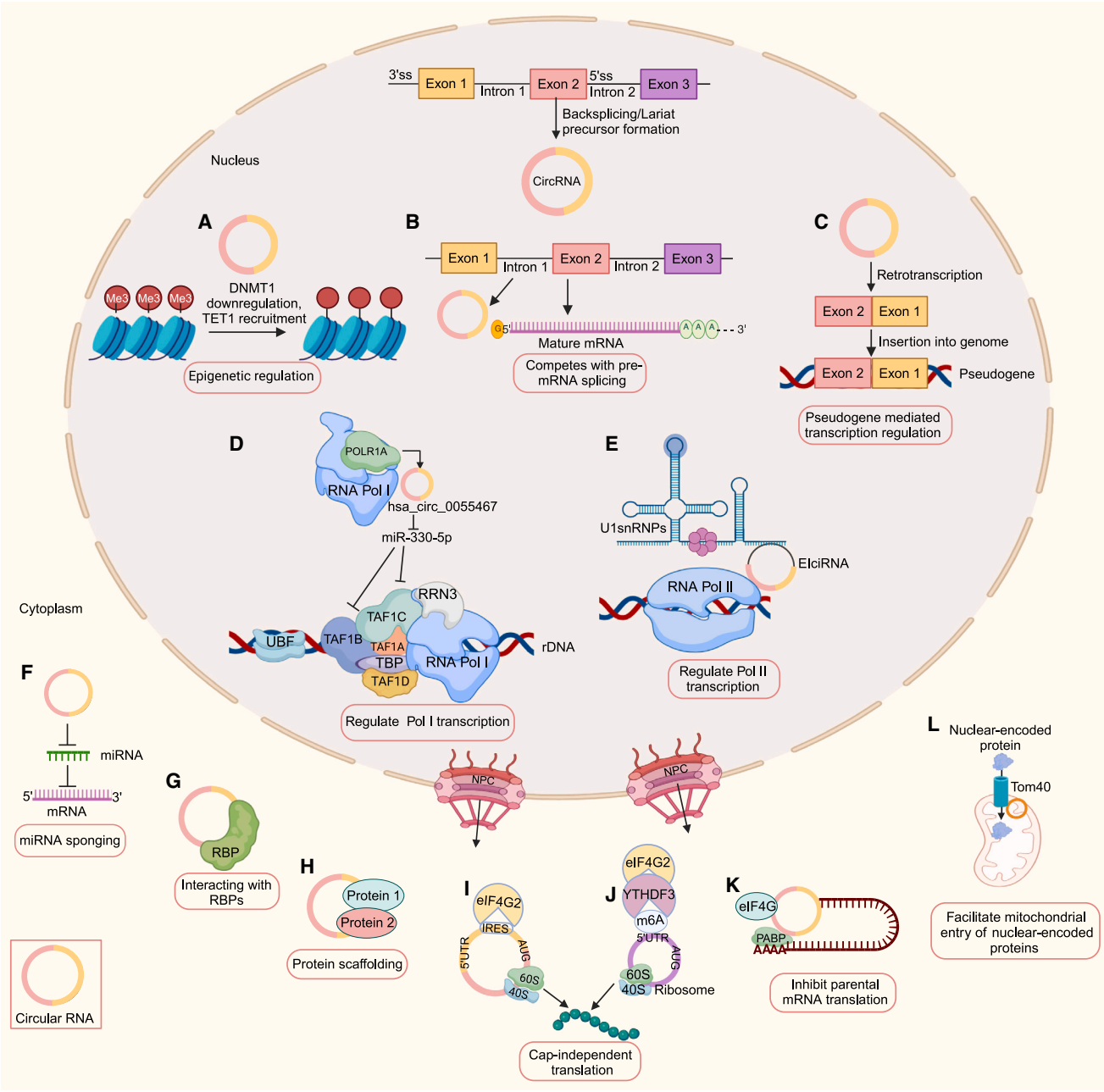

Figure 2. Mechanisms of circRNAs functions

(A) CircRNAs can recruit DNA demethylases or downregulate DNMTs, affecting gene expression. (B) CircRNAs interfere with normal splicing, altering the expression profile of the parent gene. (C) CircRNAs can be reverse transcribed and integrated into the genome. (D) CircRNAs sequester miRNAs that target RNA Pol I machinery, enhancing rRNA synthesis. (E) CircRNAs interact with snRNPs, increasing RNA Pol II transcription. (F) CircRNAs act as sponges for miRNAs, preventing miR-mediated repression of target gene expression. (G) CircRNAs bind to RBPs, affecting RNA stability, localization, and translation. (H) CircRNAs serve as scaffolds, facilitating protein-protein interactions. (I) CircRNAs with IRES can be translated into functional proteins. (J) m6A-modified circRNAs facilitate ribosome recruitment for translation without 5′ cap. (K) CircRNAs interfere with PABP and eIF4G interaction, reducing translation of specific mRNAs. (L) Mitochondrial circRNAs assist in the import and proper folding of nuclear-encoded proteins in mitochondria.

promote DNA demethylation. Simultaneously, it suppresses DNMT1 in trans, a key enzyme required for maintaining DNA methylation[53] (Figure 2A). Additionally, circRNAs can compete with the canonical splicing of their parent transcripts through flanking intronic sequences, thereby introducing an additional layer of complexity to the transcriptional regulation of gene expression[27] (Figure 2B).

Table 2. Mechanism and functions of circRNA-peptide interactions

Tumor type	circRNA	Interactions with proteins	Function
Glioma	CDR1as[66]	CDR1as directly interacts with p53	inhibition of p53 ubiquitination
GC	Circ_CEA[67]	Circ_CEA scaffolds CDK1 and p53 interaction	attenuates p53 functions
Gastric carcinoma	CircRNA LMP2A[68]	circRNA LMP2A directly interacts with KHSRP	accumulation of HIF-1α and up-regulation of VEGFA
LC	CircCIMT[69]	circCIMT directly interacts with glycosylase, APEX1	promotes DNA damage repair through BER pathway
Liver cancer	CircZKSCAN1[28]	circZKSCAN1 directly interacts with FMRP	inhibits Wnt/β-catenin signaling pathway
GBM	circ-E-Cad[70]	circ-E-Cad encoded protein C-E-Cad interacts with EGFR	activates EGFR signaling
GBM	circ-SHPRH[71]	circ-SHPRH encoded protein SHPRH-146aa interacts with SHPRH protein	acts as a protective decoy for SHPRH tumor suppressor protein
GBM	circSMO[72]	circSMO encoded protein SMO-193aa interacts with SMO protein	Hedgehog signaling pathway activation
CRC and HCC	circARHGAP35[73]	circARHGAP35 encoded protein interacts with TFII-I	promotes tumor progression
GBM	CircHEATR5B[74]	circHEATR5B encoded protein HEATR5B-881aa interacts and phosphorylates JMJD5	inhibits glycolysis and proliferation
LUAD	CircASK1[75]	circASK1 encoded protein ASK1-272a.a interacts with Akt1	activates ASK1/JNK/p38 signaling pathway promoting cell death
Endometrial cancer	Hsa-circ-0000437[76]	Hsa-circ-0000437 encoded protein CORO1C-47aa interacts with aryl hydrocarbon receptor nuclear translocator	interferes with the VEGF signaling pathway

JMJD5, Jumonji-C domain-containing protein.

Interestingly, circRNAs that undergo retro-transcription (RNA copied into DNA), can reintegrate into the host genome as processed pseudogenes containing reversed non-colinear exon-exon junctions; however, the implications of this integration on gene regulation remain largely unexplored[55] (Figure 2C). Remarkably, circRNAs can influence basal transcription of RNA polymerase (Pol) I and II. Hyperactive Pol I transcription is a canonical molecular characteristic of cancer cells, intricately linked to various hallmarks of cancer.[56] Recently, we showed that hsa_circ_0055467, originating from the catalytic subunit of Pol I enzyme, POLR1A, suppressed the expression of miR-330-5p, which targets TBP-associated factor (TAF)1B and TAF1C, essential for the initiation of Pol I transcription. Consequently, this enhanced Pol I transcriptional output contributed to malignant phenotypes including accelerated cell proliferation, reduced apoptosis elevated migration, and invasion in lung adenocarcinoma (LUAD)[57] (Figure 2D). Furthermore, Pol II transcription can be influenced by EIciRNA, which can form complexes with U1 snRNPs through RNA-RNA interactions. These EIciRNA-U1 snRNP complexes potentially enhance gene expression by interacting with the promoters of their parent genes[39] (Figure 2E). To date, the ability of circRNAs containing miRNA response elements to sequester miRNAs has been recognized as a predominant mode of circRNA mechanism of action. This post-transcriptional mechanism has been demonstrated to mitigate the repressive effects exerted by miRNAs on their target genes, thus controlling global gene expression (Figure 2F).[14] CircRNAs can also serve as decoys, altering the physiological functions of RNA-binding proteins or act as scaffolds, enhancing protein-protein interactions[58] (Figures 2G and 2H). Remarkably, some circRNAs contain internal ribosome entry sites (IRESs), enabling their translation into functional proteins.[59] These IRES elements facilitate translation by interacting with initiation factors eIF4G2 as an RNA scaffold to recruit 40S ribosomal subunit, leading to the initiation of translation (Figure 2I).[60] Moreover, circRNAs with potential open reading frames and m6A motifs (RRACH, R = A/G, H = U/A/C) can enhance cap-independent translation by directly binding m6A in the 5′ UTR to eIF3, or by interacting with eIF4G2 which recognizes the m6A reader YTHDF3, thus promoting ribosome assembly and translation (Figure 2J).[61] Intriguingly, circRNA-encoded peptides can modulate the activity of the cognate proteins potentially influencing tumor suppressor or oncogenic mechanisms independent of genetic or epigenetic aberrations in DNA.[62] However, their comprehensive roles in tumorigenesis remain largely elusive (refer to Table 2 for interactions between circRNAs-proteins, circRNA-derived peptides-proteins, and their functional impacts in cancer). Additionally, circRNAs can suppress the translation of their parental mRNAs by blocking the association of initiation factors eIF4G and Poly A binding proteins, which are essential for translation initiation (Figure 2K).[63] Several methods have been developed to detect circRNA-protein interactions. The RNA Protection Assay identifies binding regions but requires prior knowledge of interactors.[15] The RNA Pull-Down Assay uses probes to find binding partners, though efficiency can be limited.[39,64] RNA Immunoprecipitation with circRNA sequencing examines interactions, often confirmed by RNase R digestion to avoid false positives.[6,15] Cross-linking immunoprecipitation offers nucleotide-level interaction details, requiring high-quality antibodies and deep sequencing for less abundant circRNAs.[65]

Intriguingly, mecciRNAs, such as mecciND1 and mecciND5, function as molecular chaperones, facilitating the entry of nuclear-encoded

proteins into mitochondria by aiding their folding during import. These mecciRNAs interact with critical components such as translocase of outer membrane 40, highlighting their involvement in the initial stages of protein import at the outer mitochondrial membrane (Figure 2L).[77] Summarily, these diverse mechanisms demonstrate the multifaceted capacity of circRNAs to regulate gene expression and underscore their complex roles in various cellular processes.

CircRNAs IN CANCER HALLMARKS

In 2000, Douglas Hanahan and Robert A. Weinberg[78] outlined the "hallmarks of cancer," pivotal in driving malignancy. These include sustained proliferation, insensitivity to growth suppression, evasion of apoptosis, replicative immortality, angiogenesis, and metastatic capacity. In 2011, this repertoire was further expanded to include cellular energetics deregulation, immune evasion, genome instability, and tumor-associated inflammation.[79] Recent advancements have introduced next-generation hallmarks such as plasticity, microbiome interactions, senescence-associated alterations, non-mutational epigenetic reprogramming, stemness, and drug resistance.[80–83] Collectively, these hallmarks provided a framework for understanding the intricacies of tumorigenesis. Recently, a growing body of studies has conclusively linked aberrant circRNA expression to the initiation, progression, metastasis, and therapeutic outcomes in various types of cancers. Intriguingly, circRNAs originating from genes integral to cancer pathways have been demonstrated to play a crucial role in malignant phenotypes, exerting influence either by modulating their parent genes or by affecting the activity of other effector entities.[57]

In the following sections, we discuss the diverse roles of circRNAs in various hallmarks of cancer.

Sustained proliferation

The constitutive activation of key growth-proliferation signaling pathways such as RAS/RAF/MAPKs, PI3K-AKT, and nuclear factor κB (NF-κB) is a critical oncogenic aberration that drives tumorigenesis.[84] Accumulating evidence suggests that circRNAs can modulate MAPK signaling pathways in cancer.[85] For example, in gliomas, circ-MAPK4 enhanced cell proliferation by up-regulating the MAPK pathway through the sequestration of the miR-125a-3p, a repressor of the p38/MAPK pathway.[86] Similarly, ciRS-7-A decoys miR-7, resulting in the increased expression of miR-7 target genes including epidermal growth factor receptor 1 and RAF1, thus contributing to the hyperactivation of the MAPK pathway in CRC.[87] In gastric cancer (GC), circPIP5K1A up-regulated the expression of the keratin 80 gene by sponging miR-671-5p, this enhanced the interaction of keratin 80 protein with DNA-dependent PKR catalytic subunit, leading to the hyperactivation of the AKT pathway.[88] Remarkably, in HCC, the hsa_circRNA_103809/miR-377-3p/FGFR1 axis seems to have a master regulatory role in promoting the oncogenic Ras/Raf-MEK-ERK signaling pathway.[89] Conversely, some circRNAs can impede growth signaling pathways. For instance, circRHOBTB3 inhibits cell proliferation and G1/S transition by sponging miR-23a-3p, counteracting miR-23a-3p inhibitory effect on the PTEN/AKT pathway in epithelial ovarian cancer.[90] Moreover, circCDK13 plays a tumor-suppressive role by inhibiting the JAK/STAT and PI3K/AKT pathways, resulting in the suppression of cell proliferation, migration, and invasion in liver cancer.[91] Studies have revealed that circRNAs can activate oncogenes, contributing to uncontrolled cell proliferation. For instance, in BC, circ_IRAK3, through the sequestration of miR-603, up-regulated kinesin family member 2A (KIF2A) expression, an oncogene that enhances proliferation. Notably, specific inhibition of circ_IRAK3 restrained cell proliferation, migration, and invasion *in vitro*, and reduced tumor burden *in vivo*.[92] Similarly, in prostate cancer (PC), circABCC4 promoted malignant proliferation by sequestering miR-1182, resulting in the up-regulation of the oncogenic transcription factor FOXP4. Depletion of circABCC4 resulted in anti-tumor phenotypes *in vitro* and *in vivo*.[93] Moreover, circRNAs can accelerate cell cycle progression. For instance, hsa_circ_0016788, by sponging miR-486, upregulates CDK4, thereby promoting the cell cycle in HCC.[94] Remarkably, circβ-catenin derived peptide circβ-catenin-370aa stimulates the Wnt/β-catenin pathway by preventing β-catenin degradation, promoting tumor progression in non-small cell lung cancer (NSCLC).[95] These studies highlight the diverse and context-dependent roles of circRNAs in modulating crucial signaling pathways in cancer.

Evading growth suppressor mechanisms

In cancer, anti-growth/proliferation signals are evaded by disrupting the expression or activity of tumor suppressor genes (TSGs) such as p53, p21, and PTEN.[96] CircRNAs have displayed dual roles, acting as both oncogenic and tumor suppressive, by modulating the expression or function of TSGs across diverse cancer types. In LUAD, the markedly up-regulated circPRKCI functioned as a sponge for miR-545 and miR-589, which dampened the p21-mediated tumor-suppressor effects.[97] Conversely, circMTO1 exhibited a tumor suppressor function in HCC by activating p21 through the sequestration of the oncogenic miR-9.[98] However, in HCC, sequestration of miR-148a by circMRPS35 up-regulated the expression of oncoprotein syntaxin 3, which, in turn, promoted ubiquitin-dependent degradation of PTEN.[99] Conversely, circATRNL1 suppressed miR-23a-3p expression, resulting in the up-regulation of PTEN expression in oral squamous cell carcinoma (OSCC) cells.[100] Furthermore, MDM2-derived circ-MDM2, reduced basal p53 levels, accelerated G1/S to G2/M transition, ultimately leading to hyperproliferation.[101] In contrast, circRNA CDR1as disrupted the p53/MDM2 complex, inhibiting the ubiquitination of the p53 tumor suppressor protein in gliomas.[66] In summary, these intricate regulatory functions of circRNAs in diverse cancers emphasize their crucial role in the mechanisms governing malignant proliferation.

Evasion of apoptosis

CircRNAs modulate apoptosis by interacting with key apoptotic mediators. In BC, circ_IRAK3 sponges miR-603, leading to the up-regulation of anti-apoptotic KIF2A, which inhibits pro-apoptotic Bax and poly adenosine diphosphate-ribose polymerase (PARP), ultimately suppressing apoptosis.[92] Remarkably, in gliomas, the down-regulation of circ-MAPK4 enhanced the proteolytic cleavage of

pro-apoptotic factors, including PARP1, caspase-3, caspase-7, and caspase 9, this effect was attributed to the circ-MAPK4-dependent suppression of miR-125a-3p.[86] Conversely, in GBM stem cells, circLRFN5 promoted ferroptosis by inhibiting the oncogene paired related homeobox 2 (PRRX2), which transcriptionally suppressed GCH1, a ferroptosis inhibitor.[102] Also, circRHOT1 sequestered miR-106a-5p which elevated signal transducer and activator of transcription 3 (STAT3) protein ultimately suppressing ferroptosis in BC, thus underscoring the intricate role of circRNAs in regulating cellular responses to oxidative stress in BC.[103] Moreover, in GC circ_CEA functions as a scaffold between cyclin-dependent kinase 1 (CDK1) and p53 proteins, facilitating CDK1-mediated phosphorylation of p53 at serine 315, which consequently reduced p53 stability, leading to the inhibition of apoptosis by attenuating p53 functions.[67] These findings highlight the diverse and crucial impact of circRNAs on apoptosis-related processes in cancer.

Angiogenesis

CircRNAs play vital roles in regulating angiogenesis and hypoxia across a diverse spectrum of cancers.[104] In CRC, circ-Erbb2 interacting protein (ERBIN), derived from the pro-angiogenic ERBIN gene, targets miR-125a-5p and miR-138-5p expression, resulting in the stabilization of hypoxia-inducible factor 1 alpha (HIF-1α) protein levels, and subsequent activation of the HIF-1α-dependent angiogenic processes.[105] Similarly, circPIP5K1A also activates HIF-1α through sponging miR-600 and promotes angiogenesis in NSCLC cells.[106] In Epstein-Barr virus-associated gastric carcinoma, the circRNA LMP2A specifically interacted with the RNA-binding protein KHSRP, promoting the KHSRP-mediated decay of Von Hippel-Lindau tumor suppressor mRNA leading to the accumulation of HIF-1α and the subsequent up-regulation of the pro-angiogenic factor vascular endothelial growth factor (VEGF)A.[68] In GC, circSHKBP1 promoted angiogenesis by sponging miR-582-3p, resulting in increased expression of the pro-angiogenic factors human antigen R and VEGF.[107] Similarly, in OSCC, circFNDC3B destabilized FUS, through MDM2-mediated ubiquitination, enhancing HIF-1α stability and consequently promoting VEGFA transcription and angiogenesis.[108] In BC, hsa_circRNA_002178 enhanced the expression of the angiogenic COL1A1 by sponging miR-328-3p, thus promoting angiogenesis.[109] Furthermore, hsa_circ_001783 promoted angiogenesis in BC by sponging miR-200c-3p, a known anti-angiogenic miRNA.[110] In glioma stem cells (GSCs), circARF1 up-regulated the oncogenic insulin gene enhancer protein via miR-342–3p sequestration, promoting gliomal angiogenesis through VEGFA-mediated ERK signaling.[111] Intriguingly, Hsa-circ-0000437 encoded peptide CORO1C-47aa, competes with transcription factor TACC3 binding to aryl hydrocarbon receptor nuclear translocator, thereby decreasing VEGF expression, ultimately suppressing angiogenesis in endometrial cancer.[76] In summary, circRNAs intricately modulate pivotal angiogenic mechanisms in cancer.

Invasion and metastasis

CircRNAs promote metastasis by modulating the expression or functions of epithelial-mesenchymal transition (EMT) effectors.[112] In NSCLC, hsa_circ_0020123 has been shown to upregulate the expression of EMT master regulator zinc finger E-box-binding homeobox 1 (ZEB1) via miR-144 sponging, and its stable knockdown remarkably reduced invasion and migration capacities.[113] Also, in NSCLC, circSATB2 promoted cell migration and invasion by sponging miR-326, leading to the up-regulation of Fascin actin-bundling protein 1 oncofetal mRNA-binding protein, a crucial player in cell motility.[114] In OSCC, elevated circIGHG promotes metastasis by sequestering miR-142-5p, resulting in the up-regulation of IGF2BP3 and induction of EMT with increased mesenchymal markers SNAIL, ZEB2, and decreased E-cadherin *in vitro* and *in vivo*.[115] Notably CircFNDC3B functions as a miR-181c-5p sponge, alleviating miR-181c-5p suppression of target genes essential for EMT such as Serpine1, PROX1, ESM1, SNAIL, and ZEB1, thereby promoting EMT-mediated invasiveness in OSCC cells.[108] In HCC, circRNA_10720/Cul2 promoted metastasis by sponging miR-490-5p resulting in the up-regulation of vimentin, and notably, this axis has shown to be controlled by Twist1, a classical EMT transcription factor.[116] Additionally, exosomes derived from MHCC97H cells overexpressing circRNA-100338 exhibit increased MMP9, facilitating basement membrane degradation during metastasis and enhancing the invasive abilities of HCC cells in both *in vitro* and *in vivo*.[117] Overall, circRNA-mediated regulation of EMT-associated factors significantly influences the metastatic properties of cancer cells.

Genome instability and mutation

Genome instability, a precursor of cancer, arises from impaired DNA repair mechanisms that increase oncogenic mutagenesis.[78] In BC, circSMARCA5 disrupts DNA damage repair and drug response by forming non-canonical DNA structures at its parent gene locus. This leads to the up-regulation of a nonfunctional truncated ΔSMARCA5 protein and decreased expression of the functional SMARCA5 protein, which is crucial for chromatin remodeling and DNA repair.[118] Similarly, in LC, circCIMT influences DNA repair by interacting with APEX1, a glycosylase involved in the base excision repair (BER) pathway. Notably, the down-regulation of circCIMT results in a dysfunctional BER pathway during exposure to carcinogens like cadmium.[69] These findings highlight the significant roles of circRNAs in influencing genome stability and mutation dynamics in cancer.

Non-mutational epigenetic reprogramming

Non-mutational epigenetic reprogramming, marked by gene expression changes independent of DNA sequence alterations, facilitates rapid and reversible cellular adaptation and is an established oncogenic mechanism.[119] Recent studies highlight the pivotal role of circRNAs in cancer-related epigenetic reprogramming. For example, in BC, the ecircRNA FECR1, derived from the FLI1 oncogene, regulates FLI1 expression by promoting CpG DNA demethylation at its promoter, facilitated by the down-regulation of DNMT1 and recruitment of the TET1 demethylase.[53] Similarly, in HCC, CircTRIM33-12, which originates from the tumor suppressor TRIM33, enhances TET1 expression by sequestering miR-191, aiding in DNA demethylation.[120] In CRC, hsa_circ_0040809 increases DNMT1 expression

through miR-515-5p inhibition, leading to enhanced DNA methylation of TSGs and furthering cancer progression.[121] These findings highlight the complex roles of circRNAs in modulating epigenetic changes in cancer.

Deregulated cellular energetics

Reprogramming of glycolytic pathways is a major metabolic alteration in cancer, promoting multiple oncogenic phenotypes including metastasis, therapeutic resistance, and stemness.[122] In NSCLC, circSLC25A16 functions as a miR-488-3p sponge, stabilizing HIF-1α and consequently activating the transcription of lactate dehydrogenase A, an enzyme essential for pyruvate to lactate pathway conversion.[123] Moreover, in NSCLC, circ_000667 has been demonstrated to upregulate the expression of signal transducer inhibitor socs2, a negative regulator of glycolysis, by repressing miR-578.[124] Further, circ-ERBB2 promotes aerobic glycolysis in TNBC by sequestering miR-136-5p, thus activating the pyruvate dehydrogenase kinase 4 pathway.[125] Also, in BC, circRNF20 targeted miR-487a, enhancing HIF-1α expression, which transcriptionally activated hexokinase 2, a rate-limiting enzyme of glycolysis.[126] Additionally, circANKRD17 in BC cells accelerated glycolysis by sponging the glycolytic repressor miR-143, relieving its suppression on hexokinase 2 and leading to heightened glycolysis.[127] In OSCC, hsa_circRNA_100290 (circ_SLC30A7) counteracts miR-378a-mediated suppression of GLUT1, a glucose transporter, leading to increased glycolysis and subsequent cell proliferation.[128] Similarly, circMAT2B, up-regulated in HCC, promoted glycolysis-dependent cell proliferation and migration under hypoxic conditions by sponging miR-338-3p and up-regulating pyruvate kinase M2, a key glycolytic enzyme, both *in vitro* and *in vivo*.[129] In GC, the circNRIP1-miR-149-5p interaction modulates AKT1 expression, which further influences the mTOR pathway promoting the aerobic glycolysis and stabilizing HIF-1α, ultimately increasing the translation of the glycolytic enzyme phospho-fructokinase.[130] Mecci-circRNA for translocating phosphor-glycerate kinase 1 (mcPGK1) plays a pivotal role in liver tumor-initiating cell (TIC) self-renewal and metabolic reprogramming. mcPGK1 promotes the translocation of PGK1 cytoplasm to mitochondria, leading to metabolic shift from oxidative phosphorylation to glycolysis through PGK1-PDK1-PDH pathway. This reprogramming activates Wnt/β-catenin signaling and enhances liver TIC function.[131] CircHEATR5B encoded HEATR5B-881aa inhibits glycolysis by promoting the phosphorylation and degradation of the metabolic regulator Jumonji-C domain-containing protein in GBM.[74] The role of circRNAs in reprogramming glycolytic pathways adds complexity to cancer metabolism, highlighting the need to further explore circRNAs as regulators of cancer-associated metabolic alterations.

Avoiding anti-tumor immune response

Tumor cells establish an immunosuppressive tumor microenvironment (TME) to evade anti-tumor immune responses.[132] CircRNAs have been shown to modulate the functions of cells associated with TME.[133] In HCC, circ-MET promoted tumor immune tolerance by up-regulating Snail (an EMT regulator) via miR-30-5p sponging, leading to the activation of the cytokine modulator DPP4, reduced C-X-C motif chemokine ligand (CXCL)10 levels, and hindered CD8+ T cell infiltration in the immunosuppressive TME *in vivo*.[134] Additionally, exosomal CircUHRF1 impedes antitumor immunity in HCC cells by sequestering miR-449c-5p, leading to increased TIM-3 receptor expression in natural killer (NK) cells. TIM-3 pathway has a suppressing effect on NK cell cytotoxicity and reduced tumor infiltration of NK cells, interferon-γ, and tumor necrosis factor-α production, which, in turn, facilitates immune evasion.[135] Conversely, circTRIM33–12 expression positively correlated with NKG2D positive cell numbers in HCC tissues, emphasizing its potential role in enhancing NK cell, $CD8^+$ T cell, and $\gamma\delta^+$ T cell-mediated immune responses against cancer.[120] Moreover, in NSCLC, exosome-released circUSP7 induced T cell dysfunction through the miR-934-SHP2 regulatory axis. Silencing circUSP7 enhanced antitumor immunity by increasing $CD8^+$ T cell populations.[136] Furthermore, circWWC3 enhanced the expression and secretion of IL-4, promoting M2-like polarization of tumor-associated macrophages and elevating programmed cell death ligand 1 (PD-L1) expression, thereby facilitating immune suppression within the TME.[137] In summary, the intricate regulatory interplay between circRNAs and TME is crucial in shaping tumor immunology.

Tumor-promoting inflammation

Tumor-promoting inflammation involves the interplay between infiltrating immune cells and the TME, driven by intrinsic tumor factors and external stimuli, creating conditions favorable for tumor progression.[138] Recent studies demonstrated the crucial role of circRNAs in modulating immune response to promote tumor growth. For instance, in HCC tumors, circASAP1 up-regulation enhanced TAM infiltration by promoting colony-stimulating factor 1 expression via miR-532 and miR-326 sponging.[139] Similarly, in PC, elevated circSMARCC1 levels induce TAM infiltration by up-regulating the pro-inflammatory chemokine CCL20 through miR-1322 sequestration, thereby fostering a pro-inflammatory milieu conducive to tumor progression.[140] These findings highlight the complex interactions between circRNAs and immune cells in modulating tumor inflammation.

Stemness

Cancer stem cells (CSCs) drive tumor initiation, therapeutic resistance, metastasis, and recurrence.[81] circRNAs contribute to cancer stemness by up-regulating stemness factors, maintaining CSCs, and promoting their functions.[141] For instance, circFAM73A, acting as a miR-490-3p sponge, up-regulated stemness transcription factors (Nanog, OCT4, and SOX2) through increased HMGA2 expression, promoting stem cell-like features in GC cells.[142] Similarly, circRNA EPHB4 promoted stemness and proliferation in gliomas by sponging miR-637, leading to increased expression of cancer stemness markers CD133, Oct4, Nanog, and CD44.[143] Another instance is CircPTN, which, by sequestering miR-145-5p, up-regulated the levels of stemness markers (Nestin, CD133, SOX2, and SOX9), promoting GSC stemness and self-renewal.[144] However, certain circRNAs, such as circZKSCAN1, inhibit HCC cell stemness by regulating the RNA-binding protein fragile X mental retardation protein, leading to

inhibition of the downstream cell cycle and apoptosis regulator 1 and the Wnt/β-catenin signaling pathway responsible for promoting stemness.[28] In summary, circRNAs are essential regulators of cancer stemness, offering critical insights into tumor heterogeneity.

Therapeutic resistance

CircRNAs have been implicated in influencing therapeutic efficacy by modulating the expression or activity of key players involved in resistance mechanisms.[145] In OSCC, circANKS1B, derived from the ANKS1B gene, contributed to cisplatin resistance and enhanced metastatic potential through miR-515-5p-TGF-β1 axis.[146] Remarkably, in cisplatin-resistant GC, circAKT3 sequestered miR-198, relieving its inhibitory effect on PIK3R1. This activation of the PI3K/AKT pathway induced BRCA1 gene expression, modulating the DNA damage response and promoting cisplatin resistance.[147] In BC, circ_0001667-mediated repression of miR-4458 up-regulated the expression of transcriptional coactivator NCOA3, consequently, resulting in adriamycin resistance.[148] Moreover, hsa_circ_0000735 suppressed miR-7, causing resistance to docetaxel in PC cells, notably, knockdown of hsa_circ_0000735 rescued docetaxel sensitivity *in vivo*.[149] MecciRNA, cc-COX2 is highly expressed in chronic lymphocytic leukemia (CLL) patients and correlates with poor overall survival. Knocking down mc-COX2 impaired mitochondrial function, decreased CLL cell proliferation, and induced apoptosis. Remarkably, cisplatin, doxycycline, and metformin down-regulated mc-COX2 expression, and their combination with mc-COX2 siRNAs significantly enhanced anti-leukemic effects.[150] Additionally, circRNAs have been shown to influence the efficacy of radiotherapy. In NSCLC, circ_0086720 down-regulated miR-375 expression, leading to increased SPIN1 expression, a gene responsible for promoting radio-resistance by suppressing apoptosis and promoting cell survival pathways.[151] Conversely, Circ_0001287 enhanced radio-sensitivity by sequestering oncomiR miR-21, and up-regulating PTEN in NSCLC cells.[152] Remarkably, circRNAs can also modulate the expression of immune checkpoint inhibitors including CTLA-4, programmed cell death 1 (PD-1), and PD-L1.[133] For instance, circFGFR1 suppressed the expression of miR-381-3p, causing up-regulation of C-X-C motif chemokine receptor 4 expression, resulting in the down-regulation of cytotoxic T lymphocytes and resistance to anti-PD-1 therapy in NSCLC patients.[153] Furthermore, circMET induced resistance to anti-PD1 therapy in HCC by modulating the miR-30-5p/snail/DPP4 axis, decreasing levels of the chemoattractant CXCL10.[134] CircASK1 enhances gefitinib sensitivity by producing ASK1-272a.a, which competes with ASK1 for binding to Akt1. This prevents Akt1-induced ASK1 inactivation and activates the ASK1/JNK/p38 signaling pathway, promoting cell death. Moreover, in gefitinib-resistant LUAD cells, increased YTHDF2-mediated endoribonucleolytic cleavage of m6A-modified circASK1 contributes to its down-regulation, highlighting the role of m6A modification in drug resistance.[75] Thus, circRNAs exert a multifaceted role in cancer therapeutic resistance, impacting chemotherapy, immunotherapy, and radiotherapy resistance through modulation of drug-resistant proteins, miRNA-mediated pathways, and immune checkpoint regulation. Thus, a comprehensive understanding of the intricate roles of circRNAs in therapeutic response can offer critical insights for targeted interventions to overcome therapeutic challenges in cancer treatment.

Microbiome

The human microbiome, an intricate ecosystem of micro-organisms within the body, influences cancer through various mechanisms. Notably, microbial dysbiosis—an imbalance in the microbiome composition and function—has been linked to the development of several cancer types.[154] The pivotal role of circRNAs in linking the microbiome to tumorigenesis is becoming increasingly evident. In GC, *Helicobacter pylori* infection, an etiological factor for stomach cancer, leads to elevated circMAN1A2 expression independently of CagA, a bacterial protein often associated with *H. pylori* infection. This up-regulation of circMAN1A2 promotes GC tumorigenesis by sequestering miR-1236–3p, thereby increasing MTA2 expression, a member of the metastasis-associated family.[155] Similarly, during *Cryptosporidium parvum* infection, ciRS-7, a circRNA originating from the CDR1AS gene, is up-regulated, promoting parasite propagation in human ileocecal adenocarcinoma cells. By acting as a miRNA sponge for miR-1270, ciRS-7 enhances NF-κB signaling, facilitating *C. parvum* infection by suppressing host antipathogen response and apoptosis.[156] In conclusion, these findings highlight the broader significance of circRNAs in mediating the intricate interplay between microbiome and carcinogenesis.

The multifaceted roles of circRNAs across various cancer types create significant challenges in identifying a universal master regulator. For example, CircPIP5K1A enhances cell proliferation in GC by targeting miR-671-5p, thereby activating the AKT pathway,[88] while in LC it promotes angiogenesis by sponging miR-600, leading to HIF-1α activation.[106] Similarly, circMAT2B drives metabolic reprogramming in liver cancer through miR-338-3p and pyruvate kinase M2, while simultaneously promoting immune evasion by inhibiting NK cell activity.[129] These complex and context-dependent functions of circRNAs necessitate detailed investigations to elucidate their regulatory mechanisms and harness their therapeutic potential in clinical settings. In conclusion, circRNAs are central regulatory elements in cancer biology, affecting almost all facets of tumor development and therapeutic response (Table 3). Their broad involvement in key oncogenic processes positions them as potential therapeutic targets and valuable biomarkers for cancer detection and monitoring.

CircRNAs IN CANCER DIAGNOSIS

CircRNAs exhibit distinct expression profiles across various human tissues, showing particularly higher expression levels in organs such as the brain, liver, and heart.[9,157,158] These patterns, documented in the Tissue Specific CircRNA Database (www.gb.whu.edu.cn/TSCD), suggests that their expression is finely regulated in a cell type- and tissue-specific manner, adapting to different cellular environments. Due to their specific expression patterns and stability, circRNAs are gaining recognition as potential biomarkers for cancer diagnostics.[159]

Various methods are currently in use to detect circRNAs. Northern blotting is a highly reliable technique that employs specific probes

Table 3. CircRNAs in hallmarks of cancer

Tumor type	CircRNA	Mechanism of action	Function	Associated hallmarks
Glioma	circ-MAPK4[86]	miR-125a-3p sponging	up-regulation of MAPK pathway	proliferation
CRC	CiRS-7-A[87]	miR-7 sponging	hyperactivation of MAPK pathway	proliferation
GC	circPIP5K1A[88]	miR-671-5p sponging	hyperactivation of AKT pathway	proliferation
Liver cancer	Hsa_circRNA_103809[89]	miR-377-3p sponging	promotes Ras/Raf-MEK-ERK pathway	proliferation
Epithelial ovarian cancer	circRHOBTB3[90]	miR-23a-3p sponging	facilitates PTEN expression	inhibits proliferation
Liver cancer	circCDK13[91]	not addressed	inhibits JAK/STAT and PI3K/AKT pathways	inhibits proliferation
BC	circ_IRAK3[92]	miR-603 sponging	up-regulation of KIF2A oncogene expression	proliferation
PC	circABCC4[93]	miR-1182 sponging	up-regulation of oncogenic transcription factor FOXP4	proliferation
Liver cancer	Hsa_circ_0016788[94]	miR-486 sponging	upregulates cell cycle regulator CDK4	proliferation
NSCLC	circβ-catenin[95]	encodes 370 aa peptide	Wnt/β-catenin pathway	proliferation
LC	circPRKCI[97]	miR-545, miR-589 sponging	inhibits p21	evading growth suppression
Liver cancer	circMTO1[98]	miR-9 sponging	activates p21	tumor suppression
Liver cancer	circMRPS35[99]	miR-148a sponging	degradation of PTEN	evading growth suppression
Head and neck cancer	circATRNL1[100]	miR-23a-3p sponging	up-regulation of PTEN	tumor suppression
Glioma	circ-MDM2[101]	not addressed	reduce basal p53 levels	evading growth suppression
Glioma	CDR1as[66]	binds directly to p53 DBD domain	inhibition of ubiquitination of p53	tumor suppression
BC	circ_IRAK3[92]	miR-603 sponging	up-regulation of KIF2A	suppression of apoptosis
Glioma	circ-MAPK4[86]	miR-125a-3p sponging	cleavage of pro-apoptotic factors	suppression of apoptosis
GBM stem cell	circLRFN5[102]	PRRX2 degradation via the ubiquitin-mediated proteasomal pathway	promotes ferroptosis inhibitor GCH1	inhibition of ferroptosis
BC	circRHOT1[103]	miR-106a-5psponging	suppresses ferroptosis by inhibiting STAT3	inhibition of ferroptosis
GC	circ_CEA[67]	scaffolding CDK1 and p53 interaction	attenuates p53 functions	inhibition of apoptosis
LUAD	circASK1[75]	encoded protein ASK1-272a.a binds to Akt1	activates ASK1/JNK/p38 signaling pathway	promoting cell death
CRC	circ-ERBIN[105]	miR-125a-5p and miR-138-5p sponging	activation of HIF-1α	promotes angiogenesis
LC	circPIP5K1A[106]	miR-600 sponging	activates HIF-1α	promotes angiogenesis
Gastric carcinoma	circRNA LMP2A[68]	interaction with RBP, KHSRP	accumulation of HIF-1α and up-regulation of VEGFA	promotes angiogenesis
GC	circSHKBP1[107]	miR-582-3p sponging	up-regulation of VEGF	promotes angiogenesis
Head and neck cancer	CircFNDC3B[108]	interaction with RBP, FUS	enhances HIF-1α stability and promotes VEGFA transcription	promotes angiogenesis
BC	Hsa_circRNA_002178[109]	miR-328-3p sponging	enhances expression of angiogenic COL1A1	promotes angiogenesis
BC	Hsa_circ_001783[110]	miR-200c-3p sponging	sponges anti-angiogenic miR-200c-3p	promotes angiogenesis
GBM stem cell	circARF1[111]	miR-342-3p sponging	promotes VEGFA-mediated ERK signaling	promotes angiogenesis
Endometrial cancer	Hsa-circ-0000437[76]	encoded protein CORO1C-47aa binds to aryl hydrocarbon receptor nuclear translocator	interferes with the VEGF signaling pathway	inhibits angiogenesis
LC	Hsa_circ_0020123[113]	miR-144 sponging	upregulates the expression of EMT master regulator ZEB1	promotes metastasis
LC	circSATB2[114]	miR-326 sponging	up-regulation of cell motility enhancer FSCN1 protein	promotes metastasis
Head and neck cancer	circIGHG[115]	miR-142-5p sponging	up-regulation of SNAIL, ZEB2, and decreased E-cadherin	promotes metastasis
Head and neck cancer	circFNDC3B[108]	miR-181c-5p sponging	promotes Serpine1, PROX1, ESM1, SNAIL, and ZEB1	promotes metastasis

(*Continued on next page*)

Table 3. *Continued*

Tumor type	CircRNA	Mechanism of action	Function	Associated hallmarks
Liver cancer	circRNA_10720[116]	miR-490-5p sponging	up-regulation of vimentin	promotes metastasis
Liver cancer	circRNA-100338[117]	—	increases MMP9	promotes metastasis
BC	circSMARCA5[118]	form R loop with parent gene	decreased parent gene expression	genome instability and mutation
LC	circCIMT[69]	interact with glycosylase, APEX1	DNA damage repair through BER pathway	decreased genome instability and mutation
Liver cancer	circTRIM33-12[120]	miR-191 sponging	upregulates TET1 expression	non-mutational epigenetic reprogramming
CRC	Hsa_circ_0040809[121]	miR-515-5p sponging	upregulates epigenetic modulator DNMT1	non-mutational epigenetic reprogramming
LC	circ-SLC25A16[123]	miR-488-3p sponging	activation of LDHA transcription	metabolic reprogramming
LC	circ_000667[124]	miR-578 sponging	up-regulation of socs2	inhibits glycolysis
BC	circ-ERBB2[125]	miR-136-5p sponging	activation of PDK4 pathway	metabolic reprogramming
BC	circRNF20[126]	miR-487a sponging	transcriptionally activates Hexokinase 2	metabolic reprogramming
BC	circANKRD17[127]	miR-143 sponging	upregulates hexokinase 2	metabolic reprogramming
Head and neck cancer	circ_SLC30A7[128]	miR-378a sponging	promotes GLUT1	metabolic reprogramming
Liver cancer	circMAT2B[129]	miR-338-3p sponging	upregulates pyruvate kinase M2	metabolic reprogramming
GC	circNRIP1[130]	miR-149-5p sponging	stabilizes HIF-1α	metabolic reprogramming
Liver cancer	McPGK1[131]	chaperone	translocation of PGK1 from cytoplasm to mitochondria	metabolic reprogramming
GBM	circHEATR5B[74]	encoded protein HEATR5B-881aa interacts and phosphorylates JMJD5	degradation of JMJD5	inhibits glycolysis and proliferation
Liver cancer	circUHRF1[133]	miR-449c-5p sponging	suppresses NK cell cytotoxicity and reduced tumor infiltration of NK cells, interferon-γ, and tumor necrosis factor-α production	promotes tumor immune tolerance
Liver cancer	circTRIM33–12[120]	miR-191 sponging	enhances NK cell, $CD8^+$ T cell, and $\gamma\delta^+$ T cell-mediated immune responses	impedes antitumor immunity
LC	circUSP7[136]	miR-934 sponging	induces T cell dysfunction	inhibits antitumor immunity
BC	circWWC3[137]	upregulate IL-4 expression	facilitates immune suppression through M2 macrophage polarization and elevates PD-L1 expression	facilitates immune suppression
Liver cancer	circASAP1[139]	miR-532 and miR-326 sponging	TAM infiltration	tumor-promoting inflammation
PC	circSMARCC1[140]	miR-1322 sponging	TAM infiltration	tumor-promoting inflammation
GC	circFAM73A[142]	miR-490-3p sponging	upregulates stemness transcription factors (Nanog, OCT4, SOX2)	increases stemness
Glioma	circRNA EPHB4[143]	miR-637 sponging	increases expression of cancer stemness-related biomarkers (CD133, Oct4, Nanog, and CD44)	increases stemness
GBM stem cell	circPTN[144]	miR-145-5p sponging	upregulates the levels of stemness markers (Nestin, CD133, SOX2, and SOX9)	increases stemness
Liver cancer	circZKSCAN1[28]	pegulate RBP,FMRP	inhibits Wnt/β-catenin signaling pathway	decreases stemness
Head and neck cancer	circANKS1B[146]	miR-515-5p sponging	upregulates TGF-β1	cisplatin resistance
GC	circAKT3[147]	miR-198 sponging	induces BRCA1 expression	CDDP resistance
BC	circ_0001667[148]	miR-4458 sponging	upregulates the expression of transcriptional coactivator NCOA3	adriamycin resistance
PC	Hsa_circ_0000735[149]	miR-7 sponging	inhibit mir-7	resistance to docetaxel
CLL	Mc-COX2[150]	—	—	resistance to CCCP, doxycycline, metformin

(*Continued on next page*)

Table 3. *Continued*

Tumor type	CircRNA	Mechanism of action	Function	Associated hallmarks
LC	circ_0086720[151]	miR-375 sponging	upregulates SPIN1	promotes radio-resistance
LC	circ_0001287[152]	miR-21 sponging	up-regulation of PTEN to	enhances radio-sensitivity
LC	circFGFR1[153]	miR-381-3p sponging	down-regulation of Tc cells to provide	resistance to anti-PD-1 therapy
Liver cancer	circMET[134]	miR-30-5p sponging	reducing levels of the chemoattractant CXCL10, hindered $CD8^+$ T cell infiltration	resistance to anti-PD1 therapy
GC	circMAN1A2[155]	miR-1236–3p sponging	up-regulation of MTA2	microbiome
Human ileocecal adenocarcinoma	ciRS-7[156]	miR-1270 sponging	NF-κB signaling pathway	microbiome

FSNC1, Fascin actin-bundling protein 1; JMJD5, Jumonji-C domain-containing protein; PPRX2, paired related homeobox 2.

overlapping the BSJ to identify and quantify circRNAs, however, it is labor-intensive and requires a significant amount of circRNA.[160,161] Another hybridization method, fluorescence *in situ* hybridization (FISH), visualizes and quantifies specific circRNAs within cells using probes targeting back-splicing sites.[39,162] This method enables subcellular-level circRNA analysis and gene expression studies. However, the specificity required for probes targeting BSJ sites limits the available FISH probes. RT-qPCR quantifies circRNAs using RNase R to digest linear RNAs and divergent primers (amplify circRNA BSJ sequence not the linear RNA counterpart).[37,163] However, the accuracy may be compromised by artifacts like pseudo-cDNA from template switching and concatemers from rolling circle amplification, potentially leading to overestimated circRNA levels. Droplet digital PCR (ddPCR) significantly advances circRNA quantification over traditional qPCR by providing absolute quantification without reference standards, detecting minute fold changes, and reducing variability between runs.[164] However, ddPCR is often critiqued for being time-consuming and expensive.[165] Advanced methods like circRNA sequencing[166] and circRNA microarrays[167] provide high-throughput options for identifying and measuring circRNA expression levels. Microarray technology profiles multiple circRNAs simultaneously microarray probes immobilized on solid-phase vectors for selective detection and capture, enabling comprehensive annotation of biological functions.[168,169] CircRNA sequencing uses high-throughput sequencing and specialized bioinformatics tools to identify distinctive BSJ sequences, offering valuable insights into expression patterns and alternative splicing events. However, widespread adoption of these techniques is limited by the need for tailored library preparation protocols, computational resources, and the short read lengths of some sequencing platforms, which pose challenges in accurately identifying circRNA sequences spanning the BSJ.[170]

To overcome some of these challenges in circRNA detection, researchers have developed innovative methods. Jiao et al. introduced a simple electrochemical technique using hairpin probes and Duplex-specific nuclease to enhance sensitivity, achieving a detection limit as low as 3.47 fM. This method has been successfully tested in human serum samples, highlighting its clinical potential without requiring RNase R pretreatment.[171] Additionally, Zhang et al. developed a hybrid point-of-care testing (POCT) platform integrating gold nanoflowers/peptide nucleic acid modified carbon fiber microelectrodes with screen-printed electrodes. This platform enhances target-capturing efficiency and allows ultrasensitive detection with minimal sample volumes, achieving a detection limit of 3.29 fM. This method shows high specificity and stability in human serum samples, making it suitable for clinical POCT applications.[172] In the following sections, we discuss the diagnostic potential of circRNAs in various cancer types.

LC

In LC, circRNA-002178, circ-CPA4, and circXPO1 showed increased expression in tumor tissues compared with adjacent normal tissues, suggesting their potential as biomarkers.[166,173,174] Notably, exosomal circRNA-002178, derived from oncogenic long non-coding RNA Ribonuclease P RNA component H1, is highly up-regulated in LUAD and demonstrated a remarkable area under the curve (AUC, a statistical analysis that effectively summarizes test accuracy, ranging from 0 [perfectly inaccurate] to 1 [perfectly accurate], assessed through the trapezoidal rule[175]) value of 0.9967, indicating it as a potential diagnostic biomarker for LUAD.[173] In NSCLC, decreased circ-CPA4 expression levels correlated with a better prognosis, indicating its prognostic potential.[174] Additionally, circXPO1, exhibited a significant inverse correlation between elevated circXPO1 expression and survival outcomes of LUAD patients.[166] Remarkably, up-regulated exosomal circSATB2 demonstrated significant diagnostic potential for LC, with AUC values of 0.616 in LC and normal adjacent tissues and 0.685 in metastatic and non-metastatic LC tissues.[114] Liu et al. discovered the down-regulation of hsa_circ_0086414 and up-regulation of hsa_circ_0005962 in the plasma of LUAD patients compared with healthy individuals. Receiver operating characteristic (ROC) analysis demonstrated that hsa_circ_0005962 has an AUC value of 0.73, while hsa_circ_0086414 has an AUC value of 0.81. Their combined analysis resulted in a notable AUC value of 0.81, underscoring their potential as non-invasive diagnostic biomarkers for LUAD.[176] Recent studies have identified hsa_circ_0077837 and hsa_circ_0001821, as highly discriminating indicators between NSCLC and normal tissues with AUC values of 0.921 and 0.863, respectively.[177] Zhao et al.[178] found hsa_circ_0037515 and hsa_circ_0037516 circRNAs were down-regulated in NSCLC compared with adjacent normal tissues and displayed a promising diagnostic potential, each

with AUC values of 0.81 and 0.82, and their combination achieves an increased AUC of 0.90 with sensitivity and specificity of 0.87 and 0.89, respectively. Additionally, cESRP1 is found to be down-regulated in small cell LC (SCLC) tissues, and its lower expression levels are associated with advanced SCLC stages and reduced patient survival, establishing its role as a prognostic marker.[179] These findings collectively underscore the diagnostic potential of circRNAs in LC.

GBM

A remarkable up-regulation of circASAP1, circPTPRF, and circ_0043278 has been observed in GBM.[50,180,181] Elevated expression of circASAP1 is notably associated with increased resistance to TMZ and poor prognosis in GBM.[50] Additionally, higher levels of CircPTPRF and circ_0043278 correlated with a poor prognosis in III–IV grade glioma patients.[180,181] CircSMARCA5 and circHIPK3 were found to be down-regulated in serum extracellular vesicles of GBM patients. They exhibited strong discriminatory potential for circSMARCA5 and circHIPK3, with AUC values of 0.823 and 0.855, respectively, highlighting their diagnostic potential in GBM.[182] CircASPM expression correlates with tumor grades in glioma, and its Kaplan-Meier survival analysis indicates that its up-regulation is associated with an unfavorable prognosis for GBM patients.[183]

PC

In PC, circ_0086722, circMBOAT2, and circ-0016068 are significantly up-regulated.[184–186] The elevated expression of circ_0086722 is associated with advanced stage, higher Gleason scores (grading system for PC), and poor biochemical recurrence-free survival in PC patients, suggesting its potential as a stratification marker and prognostic marker for PC.[184] Similarly, Shi et al.[185] demonstrated a correlation between the expression of CircMBOAT2 and high Gleason score, and also with advanced stages in PC. In addition, its overexpression has been shown to significantly decrease disease-free survival, indicating its potential as a prognostic biomarker for PC. Furthermore, elevated expression of circ-0016068 has been correlated to tumorigenesis, EMT, and stemness in PC.[186] Conversely, circEPHA3, is significantly down-regulated in high-grade androgen-independent PC tissues and PC cell lines, indicating its potential as a diagnostic biomarker for PC.[187] In a recent study, circATXN10 was shown to be down-regulated in PC compared with normal tissue and exhibited an AUC value of 0.801. Additionally, when combined with up-regulated linear transcript STIL, circATXN10 shows high discriminative capacity with an AUC value of 0.892 between malignant and adjacent normal tissue in PC.[188] These studies emphasize circ-0016068 and circATXN10 as potential diagnostic biomarkers for PC. Further, Hansen et al.[189] identified up-regulated expression of circABCC4 and circZNF577, along with down-regulation of circFAT3, circITGA7, and circATRNL1, in PC tissues, suggesting that this differential expression could be a potential biomarkers panel for PC. In brief, the unique expression patterns of circRNAs are linked to disease severity, progression, and patient outcomes indicating its potential to serve as both diagnostic and prognostic indicators in PC.

BC

In BC, the differential expression of hsa_circ_001783, circWAC, and hsa_circ_0005273 seems to have diagnostic potential.[110,190,191] Hsa_circ_001783 is associated with elevated disease recurrence risk and poor disease-free survival in BC patients. Notably, patients with elevated expression levels of hsa_circ_001783 have exhibited a significantly higher hazard ratio compared with those with low expression levels, indicating its potential usefulness as a prognostic biomarker for BC.[110] In TNBC, circWAC is highly up-regulated and is correlated with resistance to paclitaxel and poor overall survival rates.[190] The highly up-regulated hsa_circ_0005273 seems to have oncogenic functions in BC, the elevated expression of this circRNA has shown to have potential in discriminating BC tumors from adjacent normal tissues.[191] In BC, up-regulation of hsa_circ_103110, hsa_circ_104689, and hsa_circ_104821 demonstrated AUC values between 0.60 and 0.70, while down-regulated has_circ_006054, has_circ_100219, and has_circ_406697 exhibited AUC values ranging from 0.64 to 0.78. These AUC values indicate the potential of circRNAs in distinguishing BC tissues from normal tissues.[192] Moreover, Zeng et al.[51] demonstrated, through Kaplan-Meier curve analysis, that patients with higher expression of circANKS1B exhibit lower overall survival, suggesting it as a promising prognostic biomarker in BC. These findings underscore the diagnostic and prognostic potential of circRNAs in BC, which may have potential implications in molecular diagnostic approaches for BC.

GC

In GC, circNRIP1, circAKT3, circCUL2, circDLG1, circ-0002570, and circDIDO1 showed a distinct differential expression that may have diagnostic relevance.[130,147,193–196] For example, higher expression of circNRIP1 showed a remarkable correlation with lower overall and disease-free survival.[130] ROC analysis highlights the discriminatory potential of circAKT3 and circCUL2, with AUC values of 0.91 and 0.790, emphasizing their diagnostic potential. Elevated circAKT3 expression has been correlated with low disease-free survival, while low circCUL2 expression linked to poor overall survival.[147,193] Further, circDLG1 overexpression has been shown to be associated with a more aggressive tumor phenotype and unfavorable prognosis, particularly in GC patients treated with anti-PD-1 therapy.[194] Circ-0002570 enhances proliferative, migrative, and invasive capabilities, positioning it as a potential biomarker for GC.[195] Conversely, lower levels of circDIDO1 and circMCTP2 are associated with larger tumors, metastasis, and poor prognosis, suggesting their potential as prognostic markers for GC.[196,197] These findings underscore the diagnostic and prognostic potential of circRNAs in GC.

CRC

In CRC, circRNAs showed distinct differential expression patterns, such as down-regulation of hsa_circ_0001666 and hsa_circ_0066351 and up-regulation of hsa_circ_0004585 in CRC tissues.[198–200] Hsa_circ_0001666 showed an inverse relationship with the cancer stage and overall survival of the patients, exhibiting its potential as a prognostic marker.[198] Furthermore, functional correlation analysis of hsa_circ_0066351 indicated a negative correlation with CRC

proliferation and stemness, suggesting its potential for diagnosis.[199] Notably, hsa_circ_0004585 holds promise as a potential diagnostic marker, with a diagnostic accuracy (AUC of 0.731) in CRC tissue samples.[200] These circRNAs can be further validated to exploit them for diagnostic applications in CRC.

In summary, a range of circRNAs demonstrate promising diagnostic potential due to their unique expression profiles and significant associations with cancer progression and prognosis (Table 4). However, despite these promising indicators, the full diagnostic usefulness of circRNAs is yet to be fully realized. Extensive validation and research are necessary to confirm their roles and effectiveness as biomarkers, including large-scale clinical trials to test their reliability and accuracy in a diverse patient population, and the development of standardized assays for detecting and quantifying these circRNAs in clinical settings. Such efforts are crucial for integrating circRNA-based diagnostics into routine clinical practice, potentially leading to earlier detection, better prognostic assessments, and tailored therapeutic approaches for cancer patients.

CircRNA IN CANCER THERAPY

The aberrant expression of circRNAs has been identified as a significant oncogenic factor in a wide array of cancers, underscoring their crucial role in tumorigenesis. Furthermore, the dysregulated expression of circRNAs correlates with tumor progression, metastasis, and resistance to therapy.[201] Therefore, modulating the expression of circRNAs holds therapeutic promise. However, to fully understand their functional roles and therapeutic potential, it is essential to manipulate circRNA expression using both gain-of-function and loss-of-function approaches.

Several strategies are used to modulate circRNA expression effectively. For gain-of-function studies, circRNA overexpression is typically achieved by using vectors that contain the mature circRNA sequence flanked by Alu sequences.[202] This design facilitates the natural back-splicing process when introduced, effectively increasing circRNA levels in cells (Figure 3A). In contrast, loss-of-function approaches use RNA interference technologies, including short hairpin RNA, small interfering RNA (siRNA), and the CRISPR-Cas13 system,[203] to specifically target BSJs of circRNAs and reduce their activity within the cellular environment (Figure 3B). Furthermore, CRISPR-Cas9 technology offers a precise tool for circRNA knockout studies either by excising the genomic regions responsible for circRNA production such as specific exonic or intronic regions involved in back-splicing (Figures 3C and 3D). Beyond genetic manipulation, circRNAs can also be targeted at the protein interaction level. They function as aptamers or scaffolds, interacting with specific proteins to alter their activity. CircRNAs can bind to and modulate the function of key regulatory proteins (Figure 3E), or they may encode peptides that target and modify the activity of both catalytic and regulatory protein subunits, impacting crucial cellular pathways (Figure 3F).

The delivery of circRNAs plays a pivotal role in their therapeutic applications. Several delivery methods such as liposomes,[204] exosomes,[205] lipid nanoparticles,[206] gold nanoparticles,[16] through electroporation, and other methods[207] have been successfully tested predominantly *in vitro* systems for the delivery of circRNAs (Figure 3G). Lipid-based carriers such as liposomes and lipid nanoparticles offer biocompatibility and protection against degradation, while exosomes mimic natural delivery mechanisms, potentially reducing immunogenic responses. Gold nanoparticles are favored for their biocompatibility and precise control of overdosing and release. Electroporation provides a direct, physical method to enhance cellular uptake by temporarily increasing membrane permeability. Below are some recent research efforts focused on both enhancing and suppressing circRNA levels to explore their therapeutic potential in various cancer types.

Zhang et al.[208] used CRISPR-Cas9 genome editing technique to suppress circRNA expression effectively. By deleting the intronic complement sequence (ICS) from the flanking introns of circGCN1L1, they successfully eliminated its expression while preserving the transcription of the linear GCN1L1 mRNA. Intriguingly, excising a single ICS was sufficient to completely halt circGCN1L1 production without impacting the associated linear mRNA.[208] Furthermore, Piwecka et al.[209] leveraged CRISPR-Cas9 to excise the CDR1as locus, generating mutant mice devoid of CDR1as expression. This approach capitalized on the robust back-splicing of the CDR1as precursor RNA, which effectively obscured the detection of any linear transcripts.[209] Remarkably, Du et al.,[16] in their *in vivo* studies, demonstrated that delivering the circFoxo plasmid via gold nanoparticles could promote apoptosis in tumor cells and inhibit tumor growth in B16 (mouse skin tumor) models. Recent studies have leveraged the CRISPR-Cas13d system to selectively silence mature circRNAs, demonstrating enhanced reliability compared with traditional RNA interference methods and reducing false positives. This precise approach has identified therapeutically relevant circRNAs in liver cancer and showcased the potential to improve the efficacy of multi-kinase inhibitors such as sorafenib.[210] In LUAD, targeted inhibition of circXPO1 in a mouse patient-derived xenograft model dramatically reduced tumor growth, suggesting its potential as a therapeutic target for LUAD treatment.[166] In SCLC, cESRP1 was found to be down-regulated, and overexpression of cESRP1 suppressed multi-drug chemoresistance by sponging miR-93-5p, inhibiting TGF-β pathway in cells derived from drug-resistant xenograft tumors compared with the control cohort.[179] Additionally, in LUAD, reconstitution of hsa_circ_0046264 significantly reduced tumor burden in nude mice, underscoring its therapeutic potential.[211] Furthermore, Rama et al.[212] constructed a synthetic circRNA, circ-21 having seven multiple tandem binding sites to sponge oncogenic miR21. This circ-21 counteracted the oncogenic functions of miR-21, offering a potential therapeutic approach for LC.

In GBM, Zhou et al.[180] demonstrated that the elevated expression of circPTPRF increased tumor burden and resulted in reduced survival in mice. In a study led by Li et al.,[186] the reduced expression of circ-0016068 led to decreased BMI-1 expression and suppressed EMT in xenograft tumors. The attenuation of circ-0016068 also impeded tumor growth in a PC mouse model, underscoring its potential as a

Table 4. Diagnostic potential of circRNAs in various cancers

Tumor type	circRNA	Role/feature	Source	Expression pattern	Diagnostic accuracy	Pathway	Mechanism of action
LC	circRNA-02178[173]	diagnostic biomarker	tissue, plasma and exosomes	up	AUC- 0.9967	PDL1	miR-34 sponging
	circ-CPA4[174]	prognostic marker	tissue	up	–	PDL1	let-7 miRNA sponging
	circXPO1[166]	diagnostic and prognostic biomarker	tissue	up	–	IGF2BP1-CTNNB1 axis.	binds to IGF2BP1
	circSATB2[114]	diagnostic biomarker	tissue and exosomes	up	AUC-0.660	FSCN1 expression	miR-326 sponging
	Hsa_circ_0086414[176]	diagnostic biomarker	plasma and tissue	down	AUC-0.81	–	differential expression
	Hsa_circ_0005962[176]	diagnostic biomarker	plasma and tissue	up	AUC-0.73	–	hsa-miR-1265 sponging
	Hsa_circ_0077837[176]	diagnostic biomarker	tissue	down	AUC-0.921	–	differential expression
	Hsa_circ_0001821[177]	diagnostic biomarker	tissue	up	AUC-0.863	–	differential expression
	Hsa_circ_0037515[178]	diagnostic biomarker	tissue	down	AUC-0.81	pulmonary surfactant homeostasis	differential expression
	Hsa_circ_0037516[178]	diagnostic biomarker	tissue	down	AUC-0.82	pulmonary surfactant homeostasis	differential expression
	cESRP1[179]	prognostic marker	tissue	down	–	TGFβ	miR-93-5p sponging
GBM	circASAP1[50]	prognostic marker	tissue and TMZ-resistant GBM cells	up	–	MEK1/ERK1–2 signaling	miR-502-5p sponging
	circPTPRF[180]	diagnostic and prognostic biomarker	tissue	up	–	YY1	miR-1208 sponging
	circ_0043278[181]	diagnostic biomarker	tissue	up	–	Wnt-β-catenin	miR-638 sponging
	circSMARCA5[182]	diagnostic biomarker	tissue, serum and exosomes	down	AUC-0.823	IGFBP2 and NRAS	miR126-3p and miR515-5p sponging
	circHIPK3[182]	diagnostic and prognostic biomarker	serum and exosomes	down	AUC-0.855	cell migration and angiogenic potential	differential expression
	circASPM[183]	prognostic marker	tissue	up		E2F1	miR-130b-3p sponging
PC	circ_0086722[184]	prognostic marker	tissue	up	Gleason score >7	STAT5A	miR-339-5p sponging
	circMBOAT2[185]	prognostic marker	tissue	up	Gleason score >7	PI3K/Akt pathway	miR-1271-5p sponging
	circ-0016068[186]	diagnostic biomarker	tissue	up	–	EMT	miR-330-3p sponging
	circEPHA3[187]	diagnostic biomarker	tissue	down	–	BMP2	miR-513a-3p sponging
	circATXN10[188]	diagnostic biomarker	tissue	down	AUC-0.801	–	differential expression
	circABCC4[189]	diagnostic biomarker	tissue	up	AUC-0.71-0.78	FOXP4 expression	miR-1182 sponging
	circZNF577[189]	diagnostic biomarker	tissue	up	AUC-0.64	–	differential expression
	circFAT3[189]	diagnostic biomarker	tissue	down	AUC-0.82-0.86	–	differential expression
	circITGA7[189]	diagnostic and prognostic biomarker	tissue	down	AUC-0.75-0.81	–	differential expression
	circATRNL1[189]	diagnostic biomarker	tissue	down	AUC-0.78-0.85	–	differential expression

(*Continued on next page*)

Table 4. *Continued*

Tumor type	circRNA	Role/feature	Source	Expression pattern	Diagnostic accuracy	Pathway	Mechanism of action
BC	Hsa_circ_001783[110]	prognostic biomarker	tissue	up	–	–	miR-200c-3p sponging
	circWAC[190]	prognostic biomarker	tissue	up	–	PI3K/AKT	miR-142 sponging
	Hsa_circ_0005273[191]	diagnostic biomarker	tissue	up	–	YAP1-Hippo signaling	miR-200a-3p sponging
	Hsa_circ_103110[192]	diagnostic biomarker	tissue	up	AUC-0.63	Hippo and WNT signaling pathway	differential expression
	Hsa_circ_104689[192]	diagnostic biomarker	tissue	up	AUC-0.61	Hippo and WNT signaling pathway	differential expression
	Hsa_circ_104821[192]	diagnostic biomarker	tissue	up	AUC-0.60	Hippo and WNT signaling pathway	differential expression
	Hsa_circ_006054[192]	diagnostic biomarker	tissue	down	AUC-0.71	tyrosine kinase signaling pathway and developmental process	differential expression
	Hsa_circ_100219[192]	diagnostic biomarker	tissue	down	AUC-0.78	tyrosine kinase signaling pathway and developmental process	differential expression
	Hsa_circ_406697[192]	diagnostic biomarker	tissue	down	AUC-0.64	tyrosine kinase signaling pathway and developmental process	differential expression
	circANKS1B[51]	prognostic biomarker	tissue	up	–	TGF-β1/Smad signaling for EMT	miR-152-3p and miR-148a-3p sponging
GC	circNRIP1[130]	diagnostic and prognostic biomarker	tissue, plasma and exosomes	up	–	AKT1/mTOR pathway	miR-149-5p sponging
	circAKT3[147]	prognostic biomarker	tissue	up	AUC-0.91	PI3K/AKT	miR-198 sponging
	circCUL2[193]	diagnostic and prognostic biomarker	tissue and serum	down	AUC-0.790	ROCK2 expression	miR-142-3p sponging
	circDLG1[194]	diagnostic biomarker	tissue	up	–	CXCL12 to promote cell progression	miR-141-3p sponging
	circ-0002570[195]	diagnostic biomarker	tissue	up	–	VCAN expression	miR587 sponging
	circDIDO1[196]	prognostic biomarker	tissue	down	–	PRDX2 downstream pathways	degrades PRDX2 protein
	circMCTP2[197]	prognostic biomarker	tissue	down	–	MTMR3 expression	miR-99a-5p sponging
CRC	Hsa_circ_0001666[198]	prognostic biomarker	tissue	down	–	Wnt/β-catenin	miR-576-5p sponging
	Hsa_circ_0066351[199]	prognostic biomarker	tissue	down	–	AMPK, EMT	miR-27a-3p and miR-379-5p sponging
	Hsa_circ_0004585[200]	diagnostic biomarker	tissue, plasma	up	AUC-0.731	–	differential expression

AUC, area under curve; Down, down regulation of circRNA expression; FSNC1, Fascin actin-bundling protein 1; Up, up-regulation of circRNA expression.

promising therapeutic target for PC therapy.[186] In GBM, Gao et al.[70] demonstrated circ-E-Cad, as an oncogenic circRNA, encodes a protein called C-E-Cad, a variant of E-cadherin, which activates EGFR independently of EGF ligand by interacting with EGFR. Their in-vivo studies established that anti-C-E-Cad treatments could enhance the effectiveness of EGFR-targeting therapy. Zhang et al.[71] demonstrated that both circ-SHPRH and its encoded protein SHPRH-146aa are down-regulated in GBM. SHPRH-146aa acts as a protective decoy for its cognate protein, SHPRH, a tumor suppressor protein, from degradation. In GBM, Wu et al.[72] showed that SMO-193a.a., a protein encoded by circSMO, critical for Hedgehog signaling, drives tumorigenesis and can act as a potential target for GBM treatment.

In a study by Zhang et al.,[103] circRHOT1 was found to be up-regulated in BC, and this up-regulation caused inhibition of ferroptosis by sponging miR-106a-5p, a miRNA that promotes ferroptosis by targeting STAT3 mRNA in BC cells. Depletion of circRHOT1 has reduced tumor burden in nude mice, highlighting its potential as a therapeutic target in BC.[103] In BC, circNOL10, has been shown to have an inhibitory effect on the JAK2/STAT5 pathway. Ectopic expression of circNOL10 significantly inhibited cell proliferation, invasion, and metastasis *in vitro* and reduced tumor burden *in vivo*. Targeting the circNOL10/miR-767-5p/SOCS2 pathway may have therapeutic implications in BC therapy.[213] Furthermore, the heightened expression of circDIDO1 significantly impeded the growth and metastasis of GC in murine models, suggesting its promise as a potential therapeutic target for GC treatment.[196] In cisplatin-resistant GC cells, the increased expression of CircMCTP2 heightened sensitivity to cisplatin by sequestering miR-99a-5p, thereby increasing the expression of the target gene, myotubularin-related protein 3 (MTMR3). Knockdown of MTMR3 reversed the impact of circMCTP2 on cell behavior. *In vivo*, circMCTP2 mitigated cisplatin

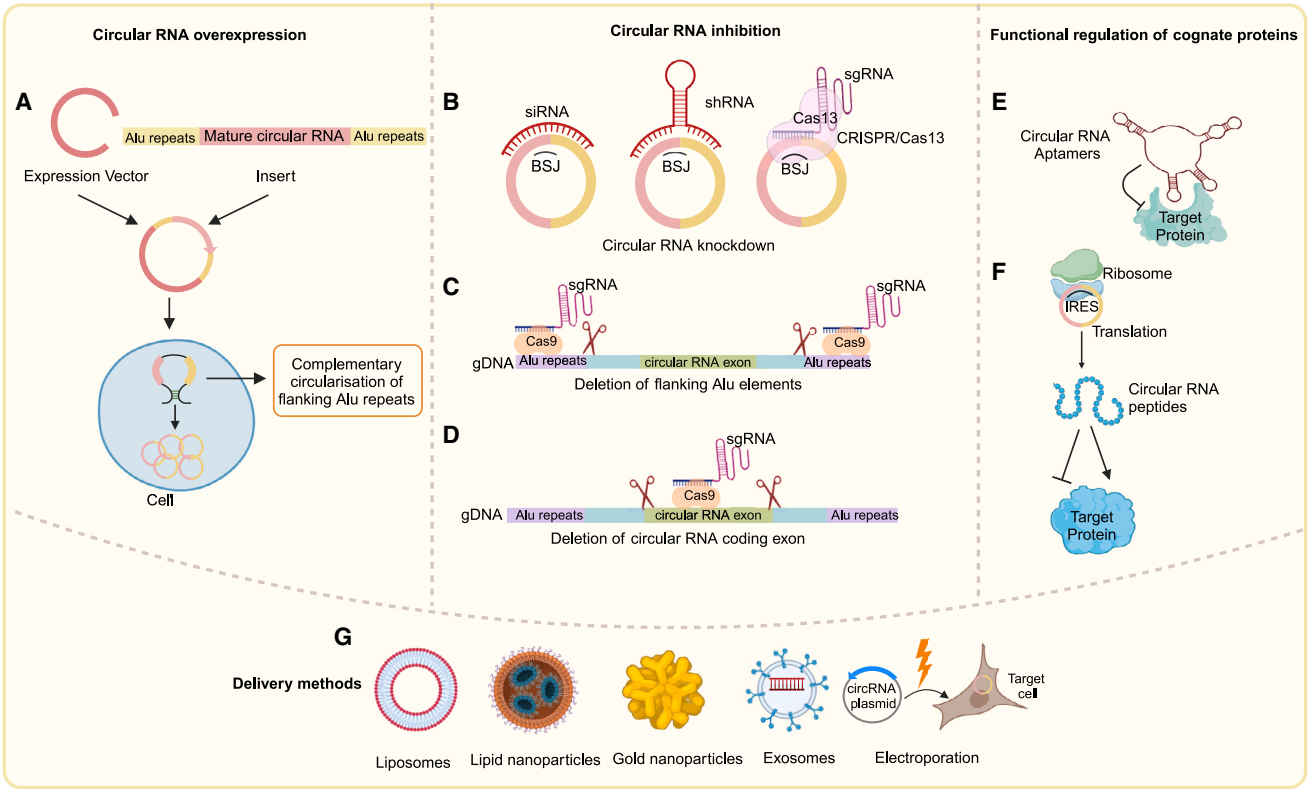

Figure 3. Strategies for modulating circRNA expression

(A) Vectors containing mature circRNA sequences flanked by ALU repeats for stable circRNA expression. (B) SiRNA, short hairpin RNA (shRNA), and CRISPR-Cas13 to specifically degrade circRNAs at the BSDJ. (C) Deletion of intronic regions flanking exons involved in circRNA formation using CRISPR-Cas9. (D) Deletion of exons directly involved in circRNA biogenesis using CRISPR-Cas9. (E) CircRNA aptamers can bind and inhibit specific protein functions. (F) CircRNA-derived peptides can inhibit or enhance protein function by acting as decoys. (G) Liposomes, nanoparticles (lipid and gold), exosomes through electroporation for delivering circRNA expression or inhibitory vectors *in vitro* and *in vivo*.

resistance in a mouse xenograft model, underscoring the potential of circMCTP2 as a modulator of cisplatin resistance in GC.[197] Additionally, Zhang et al.[130] demonstrated that suppression of circNRIP1 restrained tumor growth, whereas circNRIP1 overexpression facilitated xenografted tumor growth *in vivo*, underscoring its potential as a therapeutic target in GC. Furthermore, circCUL2 hindered CRC progression by sequestering miR-208a-3p and modulating PPP6C, influencing cell cycle progression, autophagy, and DNA damage repair. Recent findings by Yang et al. showcase the ability of CircCUL2 to impede tumor progression in mice xenografts, emphasizing its therapeutic potential in CRC treatment.[214] Al-Sudani et al.[215] used systematic evolution of ligands by exponential enrichment to design circular aptamers, notably the modified RNA aptamer AC3, capable of penetrating cancer cells and interacting with SIRT1. This interaction modulated SIRT1 histone deacetylase activity, leading to anticancer effects across diverse cancer cell lines. Furthermore, Li et al.[73] revealed that circARHGAP35, a derivative of the ARHGAP35 gene, exhibits up-regulation in CRC and HCC tissues. They found that while circARHGAP35 protein promotes tumor progression by interacting with TFII-I, its counterpart ARHGAP35 suppresses cancer cell migration and invasion by modulating RhoA activity. This reveals a remarkable opposing biological function of the circRNA-derived protein and its cognate protein.[73] Intriguingly, Li et al.[216] developed a circRNA-LNP platform for therapeutic RNA vaccines, showing greater stability and prolonged protein expression compared with linear RNA *in vitro*, and inducing strong immune responses and significant anti-tumor effects in mouse models. This suggests that circRNA-LNP vaccines could be a viable alternative to traditional mRNA vaccines, with substantial clinical potential.[216]

Several clinical trials are underway investigating the potential of certain circRNAs. For instance, a trial (NCT05771337) examines hsa_circ_0001785 (circ-ELP3) and hsa_circ_100219 (circ-FAF1) in BC serum samples to assess their diagnostic and prognostic utility. Another trial (NCT06042842) focuses on hsa_circ_0004001, a plasma circRNA, as a non-invasive biomarker for early HCC detection. Additionally, a study (NCT04584996) aims to identify differentially expressed circRNAs as potential diagnostic, prognostic, and therapeutic biomarkers for pancreaticobiliary cancer. Furthermore, an active trial (NCT05934045) explores the role of circRNAs as circulating

biomarkers for predicting treatment resistance in ALK-positive anaplastic large-cell lymphoma and their potential as therapeutic targets.

CircRNAs exhibit tissue-specific expression,[217] raising the risk that systemic delivery might affect unintended tissues. Additionally, synthetic circRNAs lack m6A modifications, crucial for immune evasion, which could trigger immune responses.[218] RNA interference methods for circRNA knockdown also face challenges such as rapid degradation, low delivery efficiency, and off-target effects.[219–221] Although CRISPR technology provides a targeted approach, using this approach is complex and less direct than targeting BSJs with RNAi.[222] The CRISPR-Cas13 system offers greater specificity but requires more *in vivo* validation for effective circRNA knockdown.[223] Furthermore, nanoparticle delivery systems like liposomes and gold nanoparticles, face issues such as limited nuclear penetration and lower efficiency compared with siRNA nanoparticles.[224] Gold nanoparticles also show size-dependent toxicity, with smaller particles posing greater risks,[225] necessitating careful property adjustments. Exosomes offer better biocompatibility but are limited by complex manufacturing processes.[226] Future research should focus on improving targeting techniques to fully harness the potential of circRNA-based therapies in clinical settings.

In conclusion, the therapeutic potential of circRNAs in cancer treatment is increasingly evident, with numerous studies demonstrating their ability to regulate all the hallmarks of cancer. Their clinical potential is currently being explored in clinical trials, which may soon establish circRNAs as crucial biomarkers for cancer diagnostics, prognostics, and therapeutic interventions. However, despite these promising advancements, the application of circRNAs in therapy faces significant challenges including effective delivering and targeting these molecules *in vivo*, and the need for a deeper understanding of their interactions within the cellular environment to avoid unintended side effects.

CONCLUSION AND FUTURE PROSPECTS

Understanding the molecular intricacies of cancer is essential for developing effective diagnostic and therapeutic strategies. CircRNAs, with their unique expression patterns and abundance in tumors and bodily fluids, emerging as potential biomarkers for cancer diagnosis and prognosis. These molecules also intersect with personalized medicine, potentially tailoring treatments based on individual circRNA profiles to enhance therapeutic responses and overcome resistance mechanisms. Further insights into how circRNAs modulate gene expression and affect signaling pathways could reveal new therapeutic targets. However, given the sequence similarity between circRNAs and their linear counterparts, the potential for off-target effects on linear mRNAs and other tissues must be carefully evaluated in the design of circRNA-focused strategies. Advanced research methods, including RNA interference with nanoparticle delivery or CRISPR-Cas systems, are being explored to selectively target circRNAs without impacting associated linear mRNAs. Promising strategies for cancer treatment may involve integrating these targeted therapies with conventional treatments, developing synthetic peptides, or creating new drugs targeting circRNA-related pathways. However, transitioning these findings into clinical applications requires comprehensive research, overcoming delivery challenges, and conducting rigorous clinical trials to confirm safety and efficacy.

ACKNOWLEDGMENTS

The authors thank Deepika Antil and other members of the non-coding RNAs lab for their feedback on the manuscript.

AUTHOR CONTRIBUTIONS

I.K. and S.V. reviewed, drafted the manuscript, and created figures. S.K. edited the manuscript. S.N. designed the study, supervised, and contributed to manuscript editing. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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