**Role of Circular-RNAs in Cancer Diagnosis**

**Authors**

Naveen Bandi

MVSc Student

Divison of Veterinary Pathology

SKUAST- Jammu, India

[naveenbandi0067@gmail.com](mailto:naveenbandi0067@gmail.com)

Dr. Shafiqur Rahman

Assistant Professor

Divison of Veterinary Pathology

SKUAST- Jammu, India

[srahmanskuastj@gmail.com](mailto:srahmanskuastj@gmail.com)

Riya Abrol

Phd Scholar

Divison of Veterinary Pathology

SKUAST-Jammu, India

[rriyaabrol478@gmail.com](mailto:rriyaabrol478@gmail.com)

Harnoor Kaur

MVSc Student

Divison of Veterinary Pathology

SKUAST- Jammu, India

[noor.soodan@gmail.com](mailto:noor.soodan@gmail.com)

**Abstract**

Circular RNA (circRNA) is a novel member of the noncoding RNA with distinct properties and diverse cellular functions, which is being explored steadily. The number of endogenous circRNAs linked to cancer is constantly expanding, yet it is still unknown how most of them function. CircRNAs are generally extremely stable molecules, and some have been demonstrated to work as effective microRNA sponges with the ability to regulate gene expression. Numerous circRNAs have tissue-specific expression patterns and are highly conserved, yet they frequently do not correlate well with host gene expression. In this article, we go over what is currently known about circRNAs and how they might be used in oncology as therapeutic targets as well as diagnostic and prognostic biomarkers. Our discussion of circRNA cancer research's prospects and current concerns is concluded with a discussion of how to make it easier to translate fundamental circRNA research into therapeutic applications.

**Key words**: Circular RNA, stable molecules, microRNA sponges, cancer, diagnosis.

1. **INTRODUCTION**

Circular RNAs (circRNAs) are a class of endogenous single-stranded closed circular RNA molecules that are created when hundreds of genes in eukaryotes undergo a covalently closed continuous loop by backsplicing or skipping events in their precursor mRNA. The lack of 3' poly(A) tails and 5' end caps is a defining characteristic of circRNAs. Circular RNAs are more durable than their corresponding linear RNA isoforms and resistant to exonuclease RNase R because they lack accessible ends [21, 28]. The widespread expression of circRNAs across species has been discovered using high-throughput sequencing and bioinformatics techniques. They serve as effective microRNAs and protein sponges, and they also play significant roles in regulating transcription and splicing. Their great stability, abundance, and evolutionary conservation among species indicate their unique features and numerous physiological functions. The majority of circRNAs also exhibit abnormal expression in pathological circumstances and in a tissue-specific way, such as during the onset and development of cancer. They serve as effective microRNAs and protein sponges, and they also play significant roles in regulating transcription and splicing. Their great stability, abundance, and evolutionary conservation among species indicate to their unique features and numerous physiological functions. The majority of circRNAs also exhibit abnormal expression in pathological circumstances and in a tissue-specific way, such as during the onset and development of cancer. In this article, we focus on the traits, roles, and mechanisms of action of circRNAs in cancer. We also give a brief account of current developments in the field of circRNA and discuss potential uses of circRNAs as novel therapeutic targets and cancer biomarkers in the future.

1. **Biogenesis of circRNAs**

circRNAs are derived from pre-mRNAs, and hosted by protein-coding genes, indicating that RNA polymerase II (RNA pol II) is responsible for their transcription, The normal spliceosomal machinery may also be necessary for modulating circRNA biogenesis. The normal spliceosomal machinery may also be necessary for modulating circRNA biogenesis, canonical spliceosomal machinery may also be necessary for modulating circRNA biogenesis. There are two current models explaining the formation of circRNAs the exon skipping or lariat intermediate model, and the direct back-splicing model [10]. In the exon skipping or lariat intermediate model, canonical splicing occurs first, generating a linear RNA with skipped exons. By using backsplicing to determine the creation of circRNA, the lengthy intron lariat containing these skipped exons is created. In the direct back-splicing model, processing starts with back-splicing determining a circRNA and an exon–intron (s)–exon intermediate, which can be processed in a linear RNA with skipped exons[10, 21, 28]. The biogenesis of circRNAs is also regulated by splicing factors and RNA-binding proteins [2]. The splicing factor muscleblind (MBL) can be involved in circRNA biogenesis. MBL promotes the circularization of the circular RNA circMBL, binding to the introns flanking the circRNA generated from the second exon of its RNA [2]. Interestingly, the circRNA also contains binding sites for its parental gene MBL [2]. This is an illustration of how a splicing factor can control how many circRNAs are produced. The MBL example also shows that circRNAs are produced cotranscriptionally and compete with normal pre-mRNA splicing. CircRNA biogenesis's mechanisms of action, however, are not entirely understood. CircRNAs can come from introns, intergenic regions, antisense RNAs, 3' UTRs, 5' UTRs, and the exons of coding areas [11]. Exonic circRNAs (ecircRNAs), which make up more than 80% of all known circRNAs, are among them and are the principal source of circRNAs in human cells. Although exon skipping, a type of alternative splicing, is thought to be a key regulator of circRNA creation, the precise method by which they are created is yet unknown. Additionally, circRNAs may differ from canonical splicing of linear RNAs in that a single gene locus may result in a variety of circRNAs through alternate gene locus back-splice site selection. [11]. High-throughput sequencing has so far identified three additional circRNA types: circular intronic RNAs (ciRNAs), which only contain introns; exon-intron cirRNAs (EIciRNAs), which contain both introns and exons; and tRNA intronic circRNAs (tricRNAs), which can splice into stable circRNA via pre-tRNA splicing.

1. **Characteristics of circRNAs**

CircRNAs are a class of stable circular RNA molecules that are evolutionarily well-preserved in mammalian cells. More than a million circRNAs exist in human tissues as detected by high-throughput sequencing [29]. CircRNAs are predominantly found in the cytoplasm, whereas a small number of circRNAs are located in the nucleus. The evolution of circRNAs in different species appears to be relatively conserved [27]. CircRNAs have a low overall abundance, but certain of them express themselves far more strongly than linear RNAs do. Circular RNA expression levels and functions are independent of linear RNA isoforms. Therefore, information about a disease that cannot be determined by conventional RNA analysis may be present in circRNA expression. Although the efficiency of circRNA formation is very low, the half-life of circRNA is long due to its resistance to RNA exonucleases, enabling circRNAs to maintain stable levels in the body under normal conditions. Yet RNA interference can be utilized to reduce the production of circular RNA since endonucleases can cut open circular RNA.

1. **The Biological Roles of circRNAs**
2. **circRNAs Can Function as miRNA Sponges:** It is recognized that miRNAs play a major role in several biological and pathological processes, including cancer. By directly base-pairing to target places inside mRNAs, they can affect how genes are expressed. [3,4,5]. Since circRNAs are primarily found in the cytoplasm, the majority of circRNAs may operate as competitive endogenous RNAs and modulators of miRNA activity by vying for these locations. Li et al. [24] reported that cirRNA itchy E3 ubiquitin protein ligase (cir-ITCH) enhanced the amount of ITCH and inhibited tumor growth by acting as a miRNA sponge. According to Chen et al. [8]. By functioning as a sponge for members of the miR-125 family, circPVT1 may encourage cell growth. Importantly, some research has suggested that ciRS-7 has conceptually altered the mechanistic understanding of miRNA networks by acting as a designated miR-7 inhibitor or sponge by decreasing miR-7 activity and raising amounts of miR-7-targeted transcripts. The expression of ciRS-7 and miR-7 in the mouse brain showed substantial overlap, according to in situ profiling experiments, indicating that the bulk of the miR-7 expressed in the brain was bound to ciRS-7. Therefore, the high association between ciRS-7 and miR-7 can add to the cellular pool of RNA-induced silencing complex components that are available. As a result, miRNA regulation and miRNA activity may generally be less prominent in tissues that express ciRS-7/miR-7. The majority of circRNAs, however, might be used for purposes beyond regulating miRNAs.
3. **circRNAs function in Haematological Malignancies:** Deep sequencing of RNA from biological materials has been used often in RNA-seq methodologies for transcriptome investigations to look into and categorize changes in the expression and structure of transcriptomes. Numerous circRNAs with tissue- and developmental stage-specific expression have been discovered by RNA-seq, and circRNAs are also widely expressed in the hematopoietic compartment.
4. **circRNAs from Translocations Have Oncogenic Function:** According to Guarnerio et al. [13] circRNAs can be produced by transcription of fusion genes produced by chromosomal translocations [20]. They discovered that circRNAs derived from several tumor-associated translocations, such as promyelocytic leukemia-retinoic acid receptor-alpha (PML-RARA) in promyelocytic leukemia and mixed lineage leukemia (MLL)-AF9 in acute myeloid leukemia (AML), and termed these fusion-circRNAs (f-circRNAs). It's significant that they discovered that f-circRNAs (f-circPR and f-circM9) may support cell survival and aid in cellular transformation and resistance to therapy, indicating that these f-circRNAs are physiologically active and exert pro-proliferative and pro-oncogenic actions. Guarnerio et al. [13]. In THP1 cells, suppression of f-circRNAs produced from MLL-AF9 induced apoptosis and elevated p27 and p21 expression, indicating that f-circRNAs may also be important for cell viability [1].
5. **circRNAs Role in Malignant Tumors:** We take into account current information in the context of cancer hallmarks to think about the potential functions of circRNAs in different facets of tumor biology. [4,5,6].
6. **circRNAs Role in the Hallmarks of Cancer Sustaining Proliferative Signaling:** CircRNAs may show a substantial role in controlling the growth of cancer and long-term proliferative signaling. One of the most effective experimental examples is circ-FOXO3, which has lower expression in tumors and may have an impact on the expression of FOXO3, p53, and PUMA.7 Endogenous circ-FOXO3 inhibition can have the opposite result, whereas ectopic circFOXO3 suppressed tumor progression and extended mouse lifespan. Less blood vessel growth may have been a factor in the smaller tumors that cells expressing circ-FOXO3, FOXO3, and FOXO3P produced compared to control cells. Alternately, the creation of the circ-FOXO3-p21-CDK2 (cyclin-dependent kinase 2) ternary complex may halt CDK2's function and hence halt the continuation of the cell cycle.50 To identify a proto-oncogenic circRNA (circ-PRKCI), Qiu et al. [26] combined bioinformatics analyses of altered circRNAs with focal copy-number variations in lung adenocarcinoma (LAC). Circ-PRKCI was one of the most common genomic aberrations in multiple cancers and may promote proliferation and tumorigenesis of LAC. hsa\_circ\_0014717, which is reduced in colon cancer cells, and able to inhibit carcinogenesis by promoting the expression of p16.
7. **Evasion of Growth Suppressors and/or Impairment of Differentiation Signals:** The majority of tumor suppressor genes encode proteins that can slow tumor growth; nevertheless, the absence of one or more of these "brakes" may hasten the development of certain cancersCircRNAs can help tumor suppressors in addition to these other ways by limiting the growth of cancer cells. Hepatocellular carcinoma (HCC) proliferation, migration, and invasion were all markedly reduced when circC3P1 was overexpressed. CircC3P1 may also stimulate the production of PCK1 via sponging miR-4641 in HCC cells.53 Inhibiting the activity of the genes circZKSCAN1 and zinc finger with KRAB and SCAN domain 1 (ZKSCAN1) may promote tumor development and cell division [33]. They also showed that ZKSCAN1 circRNA contributed to various cancer-related signaling pathways while ZKSCAN1 mRNA predominantly controlled cellular metabolism, highlighting the crucial function of ZKSCAN1 mRNA and circRNA in HCC cells. By functioning as an oncogenic miR-9 sponge to increase the expression of p21, research demonstrated that circMTO1 can slow the evolution of HCC, suggesting that it might be a good candidate as a target for HCC therapy.
8. **Enabling Replicative Immortality:** Compared to normal cells, tumor cells are known to have a substantially higher replication capacity. CircRNAs enriched in the nucleus can then connect with the opposing strand of its genomic DNA through base pairing during this process. DNA replication is defined as the process of creating two identical copies from one original DNA molecule.11 As a result, they can create a DNA-RNA triple helix that interferes with DNA replication.11 But there are currently no reliable results for this theory and hallmark.
9. **circRNAs role in tumor-promoting Inflammation:**There is a clear link between inflammation and cancer, according to numerous research [4] In many cancer cells, the crucial functions of non-coding RNAs (ncRNAs), such as miRNAs,17 lncRNAs,9, and circRNAs, were subsequently shown. Bahn et al.60 performed a gene ontology analysis of the genes overlapping suspected circRNAs in human chronic fatigue syndrome and discovered 422 circRNAs in human saliva by bioinformatics studies. These salivary circRNAs are thought to be involved in inflammatory reactions and intercellular signaling since they were discovered to be substantially enriched in a variety of closely associated categories, including chemotaxis, the development of T cell polarity, and integrin-mediated signaling pathway [36]. Alternately, the proteolytic activation of inflammatory cytokines like IL-18 and IL-1b by caspase-1 may help to create an inflammatory milieu [19]. Additionally, compared to non-tumor tissues, osteosarcoma (OS) tissues express caspase-1 at a higher level [19]. According to Jin et al.62's findings taken together, the role of caspase1/miR-214/circ-0016347 in inflammation-related pathways in the emergence of OS is potentially important for effective treatment.
10. **Activation of Invasion and Metastasis:** CircRNAs from humans have been found to aid in the invasion and spread of tumors. Metastatic tumor cells preferentially express several well-studied circRNAs. Numerous circRNAs that were selectively increased in cancer cells were studied by Hsiao et al. [47] using matched tumor colorectal and healthy tissue samples. In particular, they found that circCCDC66 controlled the migration, invasion, and anchorage-independent development of a variety of pathogenic processes in colorectal cancer cells. In mouse studies, circCCDC66 silencing prevented tumor growth and cancer invasionAccording to a study by Xu et al. [31], hsa\_circ\_000984 can function as a competing endogenous RNA (ceRNA) by competitively binding miR-106b, upregulating the expression of cyclin-dependent kinase 6 (CDK6), and promoting a malignant phenotype in tumor cells.
11. **Induction of Angiogenesis**: Numerous research groups have explained the impact of hypoxia on endothelial cells and assessed the expression of circRNA because hypoxia is thought to be a crucial trigger for angiogenesis. Numerous circRNAs were shown to be strongly altered by hypoxia by Boeckel et al [6]. Among these, they discovered that circRNA cZNF292 has a role in the control of endothelial cell growth and displayed proangiogenic properties in vitro. Li et al.[ 25] also mentioned how loss-of-function studies on hsa\_circ\_0003575 silencing might encourage the proliferative and angiogenesis abilities of human umbilical vein endothelial cells. Upregulating circRNA-MYLK may affect the signaling pathways for vascular endothelial growth factor A (VEGFA) and VEGF receptor 2 (VEGFR2), which, according to Zhong et al. [42], may enhance angiogenesis, metastasis, and growth in breast cancer models.
12. **Use of CircRNAs as cancer biomarkers**

CircRNAs are excellent options for use as biomarkers due to their universality, conservatism, tissue/cell specificity, and stability in expression patterns and features [48, 49]. CircRNAs may be used as disease biomarkers because they have been found in human blood, saliva, and stomach fluids [50, 51]. In comparison to linear RNA, Memczak et al. found that circRNA was present at a considerably greater level in blood [50]. These circRNAs could also be reliably and simply found in blood samples. In the blood, a large number of circRNAs are expressed at high levels, but the corresponding linear RNAs are present in average or low abundances. As a result, blood circRNA might offer disease-relevant data that can't be obtained from conventional RNA analysis. CircRNAs have been demonstrated to be at least two times more abundant in exosomes than in producing cells. By using bioinformatics analysis, Bahn and colleagues discovered 422 circRNAs in human cell-free saliva and showed that these circRNAs are involved in intercellular signaling and inflammatory reactions [51]. Furthermore, human gastric juice contains circRNAs. Shao et al. show that storage at 4 °C for 8 hours or freeze-thawing for 8 cycles had no impact on the levels of hsa\_circ\_0014717 expression in gastric juice .Numerous studies have recently examined the clinical utility of circRNAs in cancer and have demonstrated that some circRNAs not only perform better than the corresponding mRNA in terms of stability and diagnostic value but also reflect the characteristics of tumorigenesis at various stages, which has great potential for cancer diagnosis

1. **CircRNAs and cancers**

CircRNAs have just recently been studied about cancer. CircRNAs complete effect on cancer is still unknown. Here, we go over recent developments and the relationship between circRNA expression and clinical traits as well as their diagnostic and predictive abilities ( file : Table 1, 2, 3,4).

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| **Table 1** | | | | | |
| **Cancer** | **Circular RNA** | **expression** | **Function** | **Mechanism** | **Ref** |
| **Hepatocellular Carcinoma (HCC)** | circC3P1 | Down | tumor suppressor | stimulates phosphoenolpyruvate carboxykinase 1 expression through sponging of miR-4641 in HCC cells & significantly suppresses the proliferation of HCC cells | 37 |
| hsa\_circ\_0067531 | Down | - |  | 34 |
| hsa\_circ\_0004018 | Down | - | correlates with serum alpha-fetoprotein (AFP) level, tumor diameter, and differentiation | 12 |
| circRNA\_100338 | Up | - | functions as an endogenous sponge for miR-141-3p  in HCG and high expression of circRNA\_100338 is closely associated  with metastasis progression in HCC patients | 17 |
| circ\_000839 | Up | - | inversely correlates with miR-200b |  |
| circMTO1 | Down | tumor suppressor | suppresses HCC progression by acting as the sponge of oncogenic miR-9 to enhance p21 expression and serves as a prognostic factor for poor survival of patient | 46 |
| circZKSCAN1 | Down | tumor suppressor | mediates several cancer-related signaling pathways and inhibits cell proliferation, migration, and invasion | 33 |
| ciRS-7 | Down | - | High expression of ciRS-7 is significantly correlated with hepatic microvascular invasion, and AFP level, and thus partly related to the deterioration of HC | 30 |

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| **Table 2** | | | | | |
| **Lung Adenocarcinoma (LAC)** | **Circular RNA** | **Expression** | **Function** | **Mechanism** | **Ref.** |
| circRNA\_102231 | up | Oncogene | associated with advanced tumor, metastases (TNM), stage, lymph node metastasis, and poor overall survival of lung cancer patients and Induces lung cancer cell proliferation and invasion ability in vitro | 41 |
| circPRKCI | Up | Oncogene | functions as a sponge for both miR-545 and miR-589 and abrogates their suppression of the pro-tumorigenic transcription factor E2F7 51 promotes proliferation and tumorigenesis of LAC | 26 |
| hsa\_circ\_0013958 | Up | Oncogene | promotes cell proliferation and invasion, and suppresses cell apoptosis of LAC and functions as a sponge of miR-134, thus upregulating oncogenic cyclin D1 | 40 |

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| Table 3 | | | | | |
| Bladder Cancer | Circular RNA | Expression | Function | Mechanism | Ref. |
|  | circRNA-MYLK | Down | Oncogene | function as ceRNA for miR-29a, which can contribute to EMT and the development of bladder cancer by activating the VEGFA/VEGFR2 pathway | 38 |
|  | circHIPK3 | Down | tumor suppressor | can abundantly sponge up miR-558 to suppress the expression of heparinase and may suppress angiogenesis and migration of bladder  cancer cells | 23 |
|  | circTCF25 | Up | Oncogene | can downregulate miR-103-3p and miR-107, increase CDK6 expression, and promote proliferation in vitro and in vivo | 39 |
|  | circ-ITCH | Down | tumor suppressor | acts as tumor suppressor by a novel circ-ITCH/miR-17, miR-224/p21, and phosphatase and tensin homolog axis | 32 |

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| **Table 4** | | | | | |
| **Gastric Cancer (GC)** | **Circular RNA** | **Expression** | **Function** | **Mechanism** | **Ref.** |
| hsa\_circ\_0000520 | Down | Oncogene | negatively associated with the TNM stage in GC plasma | 28 |
| hsa\_circ\_0047905 | Up | Oncogene | acts as a tumor promoter in the pathogenesis of GC | 21 |
| hsa\_circ\_0000745 | Down | - | associated with tumor differentiation and the expression level in plasma correlates with the TNM stage | 16 |

**VII. Conclusions**

To sum up, first off, circRNAs can act in human tumors as either tumor promoters or tumor suppressors, much like lncRNAs can. It would be excellent to target carcinogenic circRNAs in a way that does not obstruct the expression of linear mRNA. Second, the majority of circRNA biomarkers that are now in use are not sensitive or specific enough to be used in clinical settings, even though circRNAs may be future cancer biomarkers. For further validation, more research with bigger sample sizes and long-term follow-up clinical data is required. Third, circRNA sponges—which have more MREs than traditional linear miRNA sponges, which only have one—might be durable and efficient miRNA inhibitors. Consequently, there is mounting evidence that circRNAs play a significant role in carcinogenesis, but circRNA research is still in its infancy. The early evidence suggests that circRNA-based diagnostic and therapeutic approaches may play significant roles in the management of cancer.

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