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

**Authors**

Naveen Bandi

MVSc Student

Division of Veterinary Pathology

SKUAST- Jammu, India

naveenbandi0067@gmail.com

Dr. Shafiqur Rahman

Assistant Professor

Division of Veterinary Pathology

SKUAST- Jammu, India

srahmanskuastj@gmail.com

Riya Abrol

Ph.D. Scholar

Division of Veterinary Pathology

SKUAST-Jammu, India

rriyaabrol478@gmail.com

Harnoor Kaur

MVSc Student

Division of Veterinary Pathology

SKUAST- Jammu, India

noor.soodan@gmail.com

**Abstract**

Circular RNA (circRNA) belongs to a class of endogenous single-stranded, non-coding closed circular RNA molecules with distinct properties and diverse cellular functions, which is being explored steadily. CircRNAs are 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. CircRNAs play a role in many physiological functions in cell cycle regulation, angiogenesis in tumors, haematological malignancies and tumor-promoting Inflammation, etc. The number of endogenous circRNAs linked to cancer is constantly expanding, yet it is still unknown how most of them function. In this article, we discuss biogenesis, its role in cancer, and how it might be used in oncology as therapeutic targets as well as diagnostic and prognostic biomarkers.

**Keywords**: Circular RNA, stable molecules, microRNA sponges, cancer biomarkers, 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. They lack 3' poly(A) tails and 5' end caps at ends, resistant to exonuclease RNase R and more durable than their corresponding linear RNA isoforms [21, 28]. The expression of circRNAs between species has been detected using high-throughput sequencing and bioinformatics techniques. They serve as effective microRNAs and protein sponges respectively and 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. 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. During the onset and development of cancer, these circRNAs show abnormal expression in pathological circumstances and tissue-specific ways. They serve as effective microRNAs and protein sponges and play significant roles in regulating transcription, splicing, and numerous physiological functions. They are stable, abundant, and evolutionary conserved among species indicating their unique features. In this chapter we discuss the role, and potential uses of circRNAs as novel therapeutic targets and cancer biomarkers in the future.

1. **Biogenesis of circRNAs**

circRNAs are formed from pre-mRNAs, and biogenesis is regulated by protein-coding genes, RNA polymerase II (RNA pol II), and spliceosomal machinery. 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. 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]. 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 be formed 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. Exon skipping is considered the key regulator of circRNA genesis, the confirmatory 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]. By method 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. **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 the expression of genes. [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 substantial overlap in expression of ciRS-7 and miR-7 in the mouse brain, according to in situ profiling experiments, indicates 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 from Translocations Have Oncogenic Function:** CircRNAs can be created by transcription of fusion genes created by chromosomal translocations, according to Guarnerio et al. [13]. CircRNAs formed from several tumor-associated translocations, such as mixed lineage leukemia (MLL)-AF9 in acute myeloid leukemia (AML) and promyelocytic leukemia-retinoic acid receptor-alpha (PML-RARA) in promyelocytic leukemia, are known as fusion-circRNAs (f-circRNAs). The f-circRNAs (f-circPR and f-circM9) suggest that these f-circRNAs are physiologically active and exert pro-proliferative and pro-oncogenic activities Guarnerio et al. [13]. They may enhance cell survival, aid in cellular transformation, and facilitate resistance to therapy. p27 and p21 expression were raised and apoptosis was induced in THP1 cells when f-circRNAs produced from MLL-AF9 were suppressed, suggesting that f-circRNAs may also be crucial for cell viability [1].
4. **Role of circRNAs 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].
5. **Role of circRNAs in the Hallmarks of Cancer Sustaining Proliferative Signaling:** CircRNAs may play a significant function in regulating long-term proliferative signals and the progression of cancer. Circ-FOXO3, which has decreased expression in tumors and may have an effect on the expression of FOXO3, p53, and PUMA, is one of the most successful experimental instances. While ectopic circ-FOXO3 slowed the growth of tumors and increased mice survival, endogenous circ-FOXO3 suppression can have the opposite effect. The smaller tumors that cells expressing circ-FOXO3, FOXO3, and FOXO3P compared to control cells produced may have been due to less blood vessel development. Alternately, the formation of the ternary complex of circ-FOXO3-p21-CDK2 (cyclin-dependent kinase 2) may inhibit CDK2's activity, which would therefore prevent the cell cycle from continuing. Qiu et al. [26] integrated bioinformatics analysis of changed circRNAs with localized copy-number changes in lung adenocarcinoma (LAC) to find a proto-oncogenic circRNA (circ-PRKCI). One of the most prevalent genetic abnormalities in many malignancies, Circ-PRKCI may encourage LAC proliferation and tumorigenesis. hsa\_circ\_0014717, which is diminished in colon cancer cells and is capable of preventing the development of new tumors by enhancing the expression of p16.
6. **Evasion of Growth Suppressors and/or Impairment of Differentiation Signals:** Although most tumor suppressor genes encode proteins that can limit tumor growth, some malignancies may develop more quickly if one or more of these "brakes" is missing. Along with these other benefits, circRNAs can aid tumor suppressors by preventing the development of cancer cells. When circC3P1 was overexpressed, it significantly inhibited the proliferation, migration, and invasion of hepatocellular carcinomas (HCC). Through sponging miR-4641 in HCC cells, CircC3P1 may also promote PCK1 synthesis. The formation of tumors and the division of cells may be aided by the inhibition of the circZKSCAN1 and zinc finger with KRAB and SCAN domain 1 (ZKSCAN1) gene activities [33]. They also demonstrated the critical role ZKSCAN1 mRNA and circRNA play in HCC cells by demonstrating that ZKSCAN1 circRNA contributed to several cancer-related signaling pathways whereas ZKSCAN1 mRNA mostly governed cellular metabolism. Research has shown that circMTO1 can halt the evolution of HCC by acting as an oncogenic miR-9 sponge to boost the expression of p21, indicating that it might be a potential choice as a target for HCC therapy.
7. **Enabling Replicative Immortality:** It is well known that tumor cells are much more capable of replicating than healthy cells. Then, during this procedure, base pairing takes place, enabling the circRNAs gathered in the nucleus to connect with the opposing strand of its genomic DNA. The process of making two identical copies of a single DNA molecule is known as DNA replication. They can therefore produce a DNA-RNA triple helix that prevents DNA replication. But as of right now, neither this theory nor this hallmark has any trustworthy outcomes.
8. **Role of circRNAs in** **tumor-promoting Inflammation:** Numerous studies have shown a direct connection between inflammation and cancer [4]. Non-coding RNAs (ncRNAs), such as miRNAs, lncRNAs, and circRNAs, were subsequently found to play important roles in a variety of cancer cells. Bioinformatics investigations by Bahn et al. led to the discovery of 422 circRNAs in human saliva after they ran a gene ontology analysis of the genes overlapping putative circRNAs in human chronic fatigue syndrome. Since salivary circRNAs were found to be significantly enriched in several closely related categories, such as chemotaxis, the development of T cell polarity, and integrin-mediated signaling pathways, they are thought to be involved in inflammatory reactions and intercellular signaling [36]. As an alternative, caspase-1's proteolytic activation of inflammatory cytokines including IL-18 and IL-1b may contribute to the development of an inflammatory milieu [19]. Additionally, osteosarcoma (OS) tissues express caspase-1 more strongly than non-tumor tissues [19]. When considered as a whole, the findings of Jin et al. suggest that caspase1/miR-214/circ-0016347 may play a crucial role in the development of OS through pathways connected to inflammation.
9. **Activation of Invasion and Metastasis:** CircRNAs from humans have been discovered to facilitate the invasion and growth of malignancies. Multiple circRNAs are preferentially expressed by metastatic tumor cells. Hsiao et al. [47] used matched tumor colorectal and healthy tissue samples to investigate several circRNAs that were specifically elevated in cancer cells. According to a study by Xu et al., circCCDC66 knockdown decreased tumor growth and cancer invasion in animal trials. By competitively binding miR-106b, increasing the expression of cyclin-dependent kinase 6 (CDK6), and encouraging a malignant phenotype in tumor cells, hsa\_circ\_000984 can serve as a competing endogenous RNA (ceRNA) [31].
10. **Induction of Angiogenesis**: Because hypoxia is considered to be a significant factor in the initiation of angiogenesis, many research teams have examined the effects of hypoxia on endothelial cells and the expression of circRNA. Boeckel et al. [6] demonstrated that several circRNAs are significantly changed by hypoxia. One of these was circRNA cZNF292, which they found to have proangiogenic qualities in vitro and a function in the regulation of endothelial cell growth. Li et al.[25] also discussed how hsa\_circ\_0003575 silencing loss-of-function experiments may promote the proliferation and angiogenesis of human umbilical endothelial cells. According to Zhong et al. [42], upregulating circRNA-MYLK may have an impact on the vascular endothelial growth factor A (VEGFA) and VEGF receptor 2 (VEGFR2) signaling pathways, which could promote angiogenesis, metastasis, and growth in breast cancer models.
11. **Use of CircRNAs as cancer biomarkers**

CircRNAs are excellent choices 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 major amounts found in blood [50]. In the blood, compared with linear RNAs which are present in average or low abundances, circRNAs are expressed at high levels. As a result, blood circRNA might offer disease diagnosis data that can't be diagnosed 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]. Recently many studies observed the clinical use 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-141-3pin HCG and high expression of circRNA\_100338 is closely associatedwith 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 bladdercancer 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**

 CircRNAs have a role in human tumors as either tumor promoters or tumor suppressors, much like lncRNAs. CircRNas are excellent for targeting carcinogenic circRNAs in a way that does not obstruct the expression of linear mRNA. The majority of circRNA biomarkers at present are not sensitive or specific for clinical use, even though circRNAs may act as cancer biomarkers. For this more research should be done with bigger sample sizes and long-term follow-up clinical data is required. CircRNA sponges, which have more MREs than traditional linear miRNA sponges, which only have one might be durable and efficient miRNA inhibitors. circRNAs play a significant role in carcinogenesis, but its 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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