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# REGULATORY ROLE OF CIRCULAR RNAS IN CELLULAR SIGNALING PATHWAYS

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## ABSTRACT

Circular RNAs (circRNAs) have become a unique category of endogenous non-coding RNAs with essential roles in cell signaling networks. The purpose of the study is to provide a systematic analysis of circRNA involvement in the regulation of important signaling pathways and how they might be relevant to disease pathogenesis. An in-depth literature and database-driven analysis was performed to assess circRNA biogenesis, molecular pathways, and interactions that are pathway specific. It was discovered that CircRNAs are able to regulate signaling cascades in a manner that is mainly mediated by microRNA (miRNA) sponging, RNA-binding proteins interaction, and transcriptional and post-transcriptional regulation. It is worth noting that circRNAs have a great regulatory impact on key pathways such as PI3K/AKT, Wnt/ -catenin, MAPK/ERK, NF-kB and TGF- beta, and influence the processes of cellular proliferation, apoptosis, and differentiation. CircRNA expression dysregulation was found to be always related to the development of different diseases, especially cancer, cardiovascular diseases, and neurodegeneration. Moreover, circRNA mRNA miRNA circRNA networks provide new integrative analysis to support the potential of circRNAs as diagnostic biomarkers and therapeutic targets. Although there has been progress in high-throughput sequencing and bioinformatics tools, there are still issues of functional validation and clinical translation. On the whole, this paper is a systematic review of the role of circRNA in signaling regulation and highlights their recent prominence in the field of molecular biology and exact medicine.

**KEYWORDS:** Circular RNA (circRNA), Cellular Signaling Pathways, miRNA Sponging, RNA-Binding Proteins, PI3K/AKT Pathway, Wnt/β-Catenin Signaling, MAPK/ERK Pathway, NF-κB Signaling, TGF-β Pathway, Gene Regulation, Non-Coding RNA, Disease Biomarkers.

## 1. INTRODUCTION

Cellular signaling pathways coordinate the key biological functions, cell proliferation, cell differentiation, cell apoptosis, and cell metabolism. Accurate control of these pathways is essential to cellular homeostasis and their dysregulation to the pathogenesis and pathophysiology of the development of complex diseases, including cancer, cardiovascular diseases, and neurodegenerative diseases. Non-coding RNA (ncRNA) has come to prominence in recent years as a key regulator of gene expression and intracellular signaling pathways, extending the knowledge of molecular control mechanisms beyond the protein-coding genes. (2014)). Circular RNAs (circRNAs) have been of great interest among ncRNAs with respect to their distinctive covalently closed-loop design feature, that is, they do not contain 5 end-caps or 3 end-poly (A) tails and therefore, are highly stable and cannot be degraded by exonuclease. CircRNAs were originally referred to as byproducts of splicing, but have recently been known to be functional regulatory molecules of transcriptional, and post-transcriptional gene regulation. Their capacity to group as microRNA (miRNA) sponges, their interaction with the RNA-binding proteins and their capability to regulate signaling pathways underlines their importance in cell physiology. (2020)). Although there is increasing evidence of circRNA roles in major signaling pathways, including PI3K/AKT, Wnt/ -catenin, MAPK/ERK, NF- or TGF- pathways, the current research is mostly limited and focused on one pathway or a disease setting. There is a dearth of a comprehensive, and integrative view of circRNA-mediated regulatory networks in a variety of signaling pathways. Moreover, there are still difficulties in correlating circRNA expression patterns and functional results and clinical use (Cargnello, M., & Roux, P. P. (2011)).

Thus, the current article seeks to offer a well-organized and methodical review of circRNA biogenesis, molecular processes, and how it regulates key cellular signaling pathways. Furthermore, the paper discusses the consequences of circRNA dysregulation in disease development and their potential to be diagnostic biomarkers and therapeutic targets.

## 2. RELATED WORK

In recent studies, circular RNAs (circRNAs) have been analyzed using experimental, computational, and integrative systems biology methods to examine the different regulatory functions of these structures in cellular signaling networks. CircRNA identification and quantification High-throughput sequencing technologies, especially RNA sequencing (RNA-seq) have been extensively used in circRNA identification and quantification, and thousands of circRNAs have been identified in diverse tissues and disease states. (2015)). These are usually accompanied with quantitative real-time PCR (qRT-PCR) and Northern blotting validation, which makes the circRNA detection reliable. Simultaneously, bioinformatics-based models have been created to anticipate circRNA interplays with microRNAs (miRNAs) and messenger RNAs (mRNAs). Interaction networks of circRNA–miRNA-mRNA can be built using databases and tools like circBase, CircInteractome, and StarBase which rely on sequence complementarity and binding energy models (Hansen, T. B., Jensen, T. I., Clausen, B. H., Bramsen, J. B., Finsen, B., Damgaard, C. K., and Kjems, (2013)). Such computational frameworks are often applicable to the competing endogenous RNA (ceRNA) hypothesis describing circRNAs as miRNA sponges that control gene expression on the post-transcriptional level. (2020)). Moreover, network-based and systems biology models have also been utilized to map circRNA-implicated major signaling pathways, including PI3K/AKT, Wnt/ beta-catenin, and MAPK/ERK pathways. These models combine multi-omics data to detect important regulatory nodes and pathways interactions (Jeck, W. R., Sorrentino, J. A., Wang, K., Slevin, M. K., Burd, C. E., Liu, J., Marzluff, W. F., and Sharpless, N. E.). (2013)). Recent works have also covered machine learning methods to predict circRNA functions and disease associations to improve the scale of circRNA studies (Jiang, N., Dai, Q., Su, X., Fu, J., Feng, X., and Peng, J. (2020)).

Although these developments have been made, there are still a number of limitations. First, not all predictions made by computations are sufficiently validated experimentally, which results in possible false-positive interactions. Second, current models do not typically examine cross-talk between two or more signaling pathways as circRNA functions and their effects are typically analyzed individually. Third, the majority of the research is disease-specific, which restricts the applicability of results to biological systems, in general. Moreover, neither standardized pipelines to detect and functionally annotate circRNAs are available yet, thereby impacting reproducibility and comparability among studies. Thus, integrative approaches that integrate experimental validation with sound computational modeling are eminently required to dissect circRNA-mediated regulatory networks within a variety of signaling pathways in a systematic manner. Overcoming these obstacles will advance the circRNA functionalities and fast track its translations to clinical use.

## 3. Biogenesis and Characteristics of Circular RNAs

Circular RNAs (circRNAs) are a special type of endogenous non-coding RNAs that are produced by a non-canonical splicing called back-splicing. Through this process, a downstream splice donor site is covalently attached to an upstream splice acceptor site thus creating a closed-loop RNA structure. CircRNAs have no 5 5 -caps or 3 -poly(A) ends, unlike linear RNAs, which serves to give them extraordinary stability and catabolismal resistance. Complementary intronic sequences, RNA-binding proteins, and spliceosomal machinery control the formation of circRNAs, which suggests that circRNA biogenesis is a tightly regulated process in cells, as shown in Fig. 1.

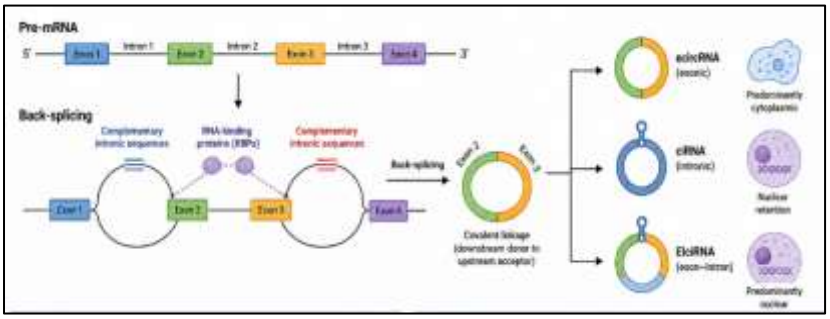


**Fig. 1. Biogenesis of Circular RNAs via Back-Splicing and Formation of circRNA Subtypes**

### 3.1 Types of Circular RNAs

There are three major types of CircRNAs according to their origin and structure. Exonic circRNAs (ecircRNAs) only consist of exonic sequences and are mostly found in the cytoplasm, where they participate in post-transcriptional

control. Intronic circRNAs (ciRNAs) are expressed on intronic regions and are normally maintained in the nucleus where they can control the transcription of genes. Exonintron circRNAs (EIciRNAs) comprise exonic and intronic sequences and are as well predominantly nuclear and are also involved in regulating transcriptional activity, interacting with RNA polymerase II and other transcriptional regulators.

### 3.2 Key Features of Circular RNAs

The CircRNAs have a number of unique characteristics that highlight their biological significance. To begin with, they contain covalently closed circular structures, which offer them great stability, and they are therefore more resistant to degradation as compared to linear RNAs. Second, circRNAs are resistant to degradation by RNase, thus they are able to stand up in diverse cellular contexts. Thirdly, they show tissue and stage-specific expression patterns, indicating that they play a role in specific cellular roles. Lastly, circRNAs have been shown to be evolutionarily conserved across species, meaning that they have critical regulatory functions in cell physiology.

## 4. Mechanisms of circRNA-Mediated Regulation

Circular RNAs (circRNAs) can control gene expression via several molecular processes that take place at transcriptional, post-transcriptional, and translational degrees. These processes specify the functional purposes of the circRNAs regardless of particular signaling conditions as shown in Fig. 2.

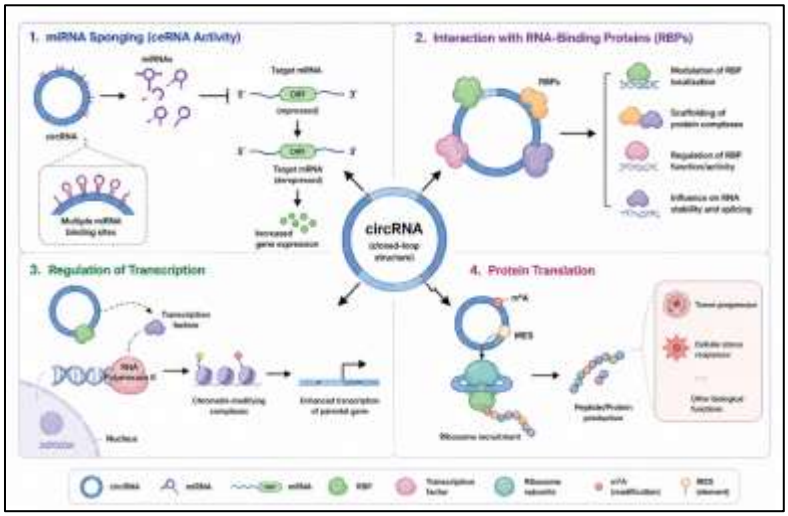


**Fig. 2. Mechanisms of circRNA-Mediated Gene Regulation**

### 4.1 miRNA Sponging

Another of the most widely researched activities of circRNAs is the use of circRNAs as microRNA (miRNA) sponges. In this process, circRNAs become competing endogenous RNAs (ceRNAs), where they are able to bind to certain miRNAs in its complementary sequences. This competition does not allow miRNAs to bind on to their target messenger RNAs (mRNAs), thus alleviating translational repression or degradation of target genes. (2014)). To illustrate, some circRNAs have more than one binding site to a single miRNA and hence they can be used to regulate whole gene sets. The mechanism is vital in the regulation of cell proliferation, apoptosis and differentiation signaling pathways (Lee, S., Rauch, J., and Kolch, W. (2020)).

### 4.2 Interaction with RNA-Binding Proteins (RBPs)

Direct association with RNA-binding proteins (RBPs) is another way that CircRNAs control cellular functions. The interactions have the capability of affecting protein activity, localization, and stability. CircRNAs can be used as protein scaffolds, regulating protein-protein interactions or forming RNA-protein complexes. In other instances, circRNAs trap RBPs, and stop their interaction with their standard targets, which can modify downstream signaling events (Legnini, I., Di Timoteo, G., Rossi, F., Morlando, M., Briganti, F., Sthandier, O., Fatica, A., Santini, T., Andronache, A., & Wade, M.). (2017)). This process is especially critical in regulating the transcriptional control and signal transduction pathways.

### 4.3 Regulation of Transcription

Some circRNAs are situated in the nucleus and are involved in controlling gene expression. These circRNA have the opportunity to be recognized by transcription factor, RNA polymerase II, or chromosome-modifying complexes and effect gene expression at transcriptional level. Exon intron circRNAs (EIciRNAs) and intronic circRNAs (ciRNAs) have been reported to positively affect the transcription of their parent genes through the regulatory complex in the

nucleus. (2019)). This process underscores how circRNAs can be used to modulate gene expression and cellular homeostasis.

### 4.4 Protein Translation

Even though circRNAs have mostly been categorized as non-coding RNAs, recent studies show that certain circRNAs have the capacity to encode functional peptides or proteins. This is supported by the existence of an internal ribosome entry site (IRES) or N6-methyladenosine (m6A) modifications that have the capabilities of cap-independent translation. (2012)). CircRNA-derived peptides have been observed to play a role in many biological processes, such as tumour development and cellular stress responses. This ability to be translated widen the functional diversity of circRNAs and disputes the conventional category non-coding RNAs.

## 5. circRNAs in Major Cellular Signaling Pathways

Based on the molecular pathways in Section 4, circRNAs have regulatory implications on various cellular signaling pathways. Such pathway specific interactions demonstrate the functional role of circRNAs in a variety of biological and pathological processes.

### 5.1 PI3K/AKT Signaling Pathway

The phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling is a key mediator of cell survival, growth, metabolism and apoptosis. One mechanism of control over this pathway by miRNA sponging is used by CircRNAs to control the expression of PI3K, AKT, and downstream effectors. As an example, circHIPK3 has been identified to sponge several miRNAs, such as miR-124, thus triggering the PI3K/AKT pathway and increasing cell proliferation in cancer. (2020)). Likewise, this pathway is regulated by circRNA CDR1as (ciRS-7), which indirectly targets miR-7, which has been shown to silence oncogenic signaling elements. PI3K/AKT signaling through circRNAs leads to tumor growth, survival, and apoptotic resistance (Salzman, J., Gawad, C., Wang, P. L., Lacayo, N., and Brown, P. O.). (2012)).

### 5.2 Wnt/β-Catenin Signaling Pathway

The Wnt/b-catenin pathway plays a fundamental role in cell fate determination, embryonic development and tissue homeostasis. CircRNAs can modify this pathway by modulating the stability of β-catenin and transcription. Indicatively, circRNA ciRS-7 serves as a miR-7 sponge which results in the amplification of Wnt pathway effectors and amplification of 2-catenin signaling (Shen, B., Wang, Z., Li, Z., Song, H., and Ding, X.). (2019)). Also, circITCH has been found to suppress tumor progression by blocking Wnt/ B -catenin signaling via sponging oncogenic miRNAs (Vlahopoulos, S. A., Cen, O., Hengen, N., Agan, J., Moschovi, M., Critselis, E., Adamaki, M., Bacopoulou, F., Copland, J (2015)). CircRNAs are important in the process of epithelial-mesenchymal transition (EMT) and cancer metastasis through these interactions.

### 5.3 MAPK/ERK Signaling Pathway

The mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway converts extracellular signals to control cell growth, differentiation, and cell responses to stress. CircRNAs regulate this pathway through miRNA interaction with regulatory proteins of kinase signal pathways. As an example, circRNA circRNA-MYLK enhances the activation of the MAPK/ERK pathway by sponging miR-29a, resulting in higher proliferation and angiogenesis in cancer cells (Wang, L., Tong, X., Zhou, Z., Wang, S., Lei, Z., Zhang, T., Liu, Z., Zeng, Y., Li, C (2018)). Likewise, circHIPK3 is involved in the regulation of ERK signaling via its association with several miRNAs (Yang, Y., Fan, X., Mao, M., Song, X., Wu, P., Zhang, Y., Jin, Y., Yang, Y., Chen, L. L., and Wang, Y.). (2017)). These communications underscore the potential of circRNAs in regulating cell activities in response to external stimuli and stress responses.

### 5.4 NF-κB Signaling Pathway

The nuclear factor kappa B (NF-), or nuclear factor, is a major immune response, inflammatory, and cell survival regulator. CircRNAs play a role in the regulation of NF-kB signaling through the regulation of upstream stimulators and downstream molecules of inflammation. As an illustration, circRNA circ_0000096, sponging miR-34a, has been found to trigger NF-κB signaling, and thus inflammatory responses and tumor development (Zhang, X. O., Wang, H. B., Zhang, Y., Lu, X., Chen, L. L., and Yang, L.). (2014)). Further, circHIPK3 has been shown to induce the NF-KB pathway via its regulatory action on the expression of inflammatory genes (Zeng, Y., Du, W. W., Wu, Y., Yang, Z., Awan, F. M., Li, X., Yang, W., Zhang, C., Yang, Q., and Yee, A.). (2017)). NF-KB signaling via circRNA is implicated in chronic inflammation and cancer progression.

### 5.5 TGF-β Signaling Pathway

Transforming growth factor-beta (TGF-B) signaling pathway is important in cell differentiation, apoptosis and tissue remodelling. CircRNAs control this pathway through regulation of activity and transcriptional responses of SMAD proteins. A case in point is circRNA circPTK2, which up-regulates the TGF-2-induced epithelial-mesenchymal transition (EMT) through the coordinated functions of miR-429 and SMAD signaling (Zhong, Z., Huang, M., Lv, M., He, Y., Duan, C., Zhang, L., and Chen, J.). (2017)). Equally, circRNA circ 0000064 helps promote fibrosis and cancer development by boosting TGF-B pathway activity (Zhou, R., Wu, Y., Wang, W., Su, W., Liu, Y., Wang, Y., Fan, C., Li,

X., Li, G., and Li, Y.). (2018)). The results indicate that circRNAs are important regulators of TGF-B-induced cellular functions. Fig. 3 shows the integrated role of circRNAs in the regulation of major signaling pathways and how they are associated with disease outcomes.

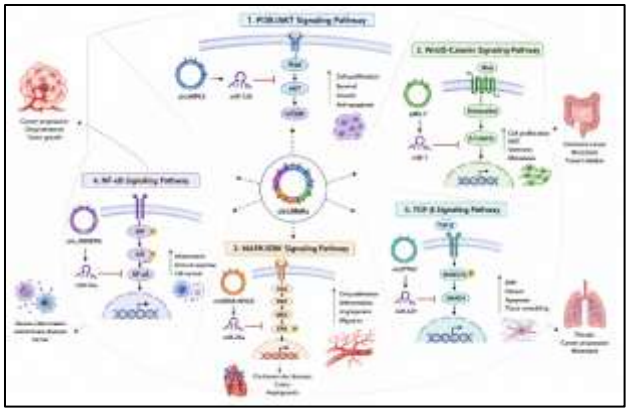

**Fig. 3. circRNA-Mediated Regulation of Major Cellular Signaling Pathways and Associated Diseases**

**6. Disease Association of circRNA-Regulated Signaling**

Circular RNA (circRNA) dysregulation has gained importance as a significant determinant of the pathogenesis of a number of diseases in humans. CircRNAs can regulate the primary cellular signaling pathways affecting essential biological events, including cell growth, cell death, inflammation, and cell differentiation. Abnormal circRNA expression perturbs these regulatory circuits leading to disease development and exacerbation in various physiological systems. (2018)).

**6.1 Cancer**

CircRNAs are also important in tumorigenesis to modulate oncogenic and tumor suppressor signaling pathways. CircRNAs are capable of modulating multiple pathways simultaneously including PI3K/AKT, Wnt/b-catenin and MAPK/ERK through various pathways like miRNA sponging and protein interaction. As an example, circHIPK3 facilitates the proliferation of cancerous cells due to miR-124 sponging and triggering downstream oncogenic signaling pathways Ashwal-Fluss, R., Meyer, M., Pamudurti, N. R., Ivanov, A., Bartok, O., Hanan, M., Evantal, N., Memczak, S., Rajewsky, (2014)). Likewise, ciRS-7 (CDR1as) promotes tumor growth by suppressing miR-7, and thus controls the activation of the oncogenes related to cell proliferation and survival (Artemaki, P. I., Scorilas, A., and Kontos, C. K.). (2020)). Moreover, circITCH was found to suppress Wnt/ -catenin signaling, which demonstrates the dual functions of circRNAs in cancer biology (Cargnello, M., & Roux, P. p. (2011)). These data reveal that circRNAs play a critical role in controlling tumor growth, metastasis, and resistance to therapy.

**6.2 Cardiovascular Diseases**

CircRNAs in cardiovascular diseases play a role in regulating cardiac hypertrophy, fibrosis, and vascular remodeling. They affect signaling pathways including MAPK/ERK and TGF-B that are essential in cardiac functions and cardiac structures. CircRNA HRCR (heart-related circRNA) is an example that has been demonstrated to serve as a miR-223 sponge and protect against cardiac hypertrophy by regulating downstream signaling pathways (Conn, S. J., Pillman, K. A., Toubia, J., Conn, V. M., Salmanidis, M., Phillips, C. A., Roslan, (2015)). Likewise, circFOXO3 engages in interactions with proteins that are involved in regulating cellular migration and stress responses, which add to cardiac aging and dysfunction (Hansen, T. B., Jensen, T. I., Clausen, B. H., Bramsen, J. B., Finsen, B., Damgaard, C. K., and Kjems, J.). (2013)). Abnormal signaling caused by the dysregulation of these circRNAs causes pathological cardiac remodeling and vascular impairment.

**6.3 Neurological Disorders**

CircRNAs are very abundant in the central nervous system and are vital in development of neurons and synaptic plasticity as well as in the protection of the brain. Changes in circRNA have been associated with neurodegenerative diseases, e.g. Alzheimer disease and Parkinson disease. An example is that ciRS-7 (CDR1as) can regulate neuronal signaling via the regulation of miR-7, which participates in the regulation of synaptic-related and neurodegeneration-related genes. (2020)). Moreover, the abnormal circRNAs have the potential to influence other pathways, including oxidative stress and inflammation, resulting in neuronal damage. The implication of circRNAs in the regulation of the neural network and signaling in synapses indicates that circRNAs can be used as biomarkers and therapeutic targets in neurological diseases. (2013)).

In order to give a concise view of circRNA-mediated regulation of various signaling pathways and disease states, Table 1 gives a summary of principal circRNAs, their interaction with targets, the signaling pathways, and their biological activities.

**Table 1. Summary of circRNA-Mediated Regulation in Signaling Pathways and Associated Diseases**

| circRNA | Target miRNA / Interaction | Signaling Pathway | Biological Effect | Disease Association |
|---|---|---|---|---|
| circHIPK3 | miR-124 | PI3K/AKT, MAPK/ERK | Promotes cell proliferation and survival | Cancer |
| ciRS-7 (CDR1as) | miR-7 | PI3K/AKT, Wnt/β-catenin | Enhances oncogene expression and tumor growth | Cancer, Neurological disorders |
| circITCH | miR-7, miR-214 | Wnt/β-catenin | Suppresses tumor progression | Cancer |
| circRNA-MYLK | miR-29a | MAPK/ERK | Promotes angiogenesis and cell proliferation | Cancer |
| circ_0000096 | miR-34a | NF-κB | Activates inflammatory signaling | Cancer, Inflammatory diseases |
| circPTK2 | miR-429 | TGF-β/SMAD | Regulates EMT and fibrosis | Cancer, Fibrosis |
| HRCR | miR-223 | MAPK/ERK | Inhibits cardiac hypertrophy | Cardiovascular diseases |
| circFOXO3 | Protein interaction (CDK2, p21) | Stress-response pathways | Induces cardiac senescence | Cardiovascular diseases |
| circZNF609 | Protein translation | PI3K/AKT | Regulates cell cycle and proliferation | Cancer |
| circDLGAP4 | miR-143 | MAPK signaling | Protects against neuronal damage | Neurological disorders |

**7. circRNA Regulatory Network**

Circular RNAs (circRNAs) are regulators of complex gene expression networks whose interaction with microRNAs (miRNAs), messenger RNAs (mRNAs), and signaling proteins. The interactions create circRNA-miRNA-mRNA regulatory axes, which are vital in regulating the cellular signaling cascades and disease development.

Table 1 summarizes the results of circRNAs that regulate various signaling pathways by regulating a set of miRNA-circRNA interactions. As an example, circHIPK3 and ciRS-7 (CDR1as) are circRNAs that serve as miRNA sponges, and in turn, can regulate downstream events like PI3K/AKT and Wnt/ -catenin. These circRNAs alleviate repression of target genes regulated by miR-124 and miR-7, which play a role in cell proliferation, survival, and differentiation. In a similar fashion, circRNAs like circITCH and circPTK2 control important pathways, such as Wnt /-catenin and TGF- B, respectively, by altering miRNA functions and transcriptional responses. These interactions put on the limelight the capacity of circRNAs to modulate several signaling cascades at a given time making them be shown as prime regulators of cell networks.

Notably, the circRNA regulatory network is very interlinked, with single circRNAs frequently targeting a number of miRNAs and affecting over one signaling pathway. This multi-target regulatory ability helps their implication in various diseases, such as cancer, cardiovascular disorders, and neurological disorders.

On the whole, circRNA-miRNA-pathway network represents systems-level insights into the role of circRNAs in the coordination of complex biological functions and highlights their promise as biomarkers and therapeutic targets.

**8. Experimental and Computational Approaches**

Circular RNAs (circRNAs) identification and functional characterization are phenomena that demand a combination of experimental and computational methods. These techniques allow the correct identification, confirmation, and computational analysis of circRNA-mediated regulatory networks. Recent innovations in high-throughput sequencing and bioinformatics tools have contributed greatly to the knowledge of circRNA biology and their contribution in cellular signaling pathways.

**8.1 Experimental Techniques**

CircRNAs and their interaction with the functions are detected and validated by implementing a variety of experimental methods.

One of the most popular approaches to circRNA discovery is RNA sequencing (RNA-seq). Back-splicing junctions, which are typical of circRNAs, can be detected by high-throughput RNA-seq, making it possible to genome-wide profile circRNA expression in various tissues and conditions.

To confirm the level of circRNA expression (identified in a sequencing study) quantitative real-time PCR (qRT-PCR) is typically employed. The specific primers that amplify circular RNA junctions guarantee the correct identification of circRNAs and identify the difference between them and linear transcripts.

CircRNA- miRNA interactions are investigated through luciferase reporter assays. Changes in the activity of the luciferase are a measure of circRNA binding to a target miRNA in this technique, which is used to offer functional proof of miRNA sponging processes.

RNA immunoprecipitation (RIP) is used to investigate circRNA-RNA-binding protein (RBP) interactions. This approach makes it possible to identify protein partners of circRNAs, thereby demonstrating their involvement in the post-transcriptional regulation and signaling pathways.

**8.2 Bioinformatics Tools**

Computational tools and databases prove to be important in circRNA studies as they allow predicting and analyzing circRNA interactions and regulatory networks.

circBase is another popular database which contains in-depth information on circRNA sequences, genomic location, and annotation. It is one of the main sources of known circRNAs.

CircInteractome allows predicting circRNA-miRNA and circRNA-RNA-binding protein interactions. The tool is especially helpful in building circRNA-miRNA-protein interaction networks.

StarBase combines big-data CLIP-seq and degradome sequencing data to infer RNA-RNA and RNA-protein interactions. It gives insights about circRNA-mediated regulation, and assists in identifying functional targets.

These computational methods are used to supplement experimental methods allowing analysis on a large scale and generation of hypotheses. Experimental validation coupled with bioinformatics predictions is necessary to correctly describe the functions of circRNAs and their roles in complicated signaling pathways.

## CONCLUSION

Circular RNAs (circRNAs) have become key regulators of cellular signaling pathways, orchestrating complex gene expression networks by means of miRNA sponging, RNA-binding protein interaction, transcriptional regulation, and translational potential. This paper gives a broad account of circRNA biogenesis, functional mechanisms and how they are regulated by most of the primary signaling pathways such as PI3K/AKT, Wnt/ 2 -catenin, MAPK/ERK, NF- kappa B and TGF- 2. The combination of pathway-related and disease-related studies indicates the essential role of circRNAs in cellular homeostasis and disease development like cancer, cardiovascular diseases, and neuro-related disorders. Nevertheless, despite the achievements, there are still numerous issues in the study of circRNA. Detection and quantification of the RNA remains a technically challenging task with overlapping sequences with linear RNAs. Additionally, a large number of circRNA interactions are not experimentally validated, and circRNA-regulating networks are too complicated to be broadly interpreted in functional terms. No standardized databases and analytical pipelines also complicate the issues of reproducibility and cross-study comparisons. The investigations of the future must aim at creating precise high-throughput validation methods, enhanced computational models and standard bioinformatics systems. Besides, the combination of multi-omics techniques, such as transcriptomics, proteomics, and epigenomics, will give a better understanding of circRNA regulatory networks. Notably, to achieve the translational potential of circRNAs in diagnostic biomarkers and therapeutic targets, clinical validation is essential. Overall, circRNAs are an exciting future of molecular biology, and they can greatly reshape our perspective on gene regulation and allow us to use new approaches to diagnosing and treating diseases.

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