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REVIEW ARTICLE

ROLE OF CIRCULAR RNAS IN TUMORIGENESIS AND THEIR POSSIBLE APPLICATIONS IN CANCER DIAGNOSIS AND TREATMENT

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Abstract

Circular RNAs (circRNAs) play critical roles in the development and progression of diseases, especially in cancer. Thus, expanding our understanding of circRNAs will enrich knowledge of cancer and give new opportunities for cancer therapy. In this review, we discuss the genesis of circular RNAs, their types and epigenetic aspects in tumorigenesis of abnormal cellular proliferation. The possible applications of individual circRNAs as biological markers in laboratory diagnosis of cancer received considerable attention recently.

Introduction:-

Non-coding RNAs(ncRNAs) including long non-coding RNAs (lncRNAs), microRNAs (miRNAs) andcircular RNAs (circRNAs), are considered important epigenetic regulatorsof many physiological processes ***(Gangwar et al., 2018; Guay and Regazzi, 2013; Tang et al., 2017; Zaiou and Bakillah, 2018; Zaiou et al., 2018)***.

Circular Rnas Characteristics:

CircRNAs are covalently closed single-stranded molecules generated from precursor mRNA***(Chen, 2016; Starke et al., 2015)***.CircRNAs are typicallygenerated from one to five exons with length between afew hundred to thousands of nucleotides ***(Guo et al., 2014; Lasda and Parker, 2014)***. They are usually formed by back-splicing ***(Jeck et al., 2013)***.

There are several important properties of circRNAs generated from back-splicing:

1. CircRNAs have a closed ring structure without either 5′–3′ polarity or a polyadenylated tail soit is considered to be resistant to degradation by exonucleases and much more stable than linear RNA***(Suzuki and Tsukahara, 2014).***
2. CircRNAs primarily reside in the cytoplasm, whereas a small number of circRNAsare located in the nucleus ***(Memczak et al., 2013).***
3. Most circRNAs have highly conserved sequences between different species ***(Chen et al., 2016).***
4. CircRNAs exhibit tissue-specific and dynamic developmental stage-expression patterns ***(Salzman et al., 2013)***.
5. CircRNAs play a regulatory role at the level of transcription or post-transcription.

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Functional Mechanisms of Circ RNAs:

Acting as miRNA sponges:

It has been proven that some circRNAsare rich in miRNA response elements (MREs)and may serve as miRNA sponges. MiRNAs are small,noncoding RNAs with approximately 22 nucleotides lengths thatplay an important role in post-transcriptional gene expression hrough binding to specific target sites withinthe mRNA 3′-untranslated region (3′-UTR), leading todecreased mRNA stability and suppression of translation***(Bartel, 2009; Su et al., 2019).*** The best example is CDR1which harbors more than 70 selectively conserved miR-7binding sites ***(Hansen et al., 2013).***

Interacting with RNA binding proteins:

CircRNAsthat harbor binding sites for RNA-binding proteins mayserve as protein sponges. For example, the circRNA originatingfrom the PABPN1 locus (circ-PABPN1) binds to humanantigen R/ELAV-like protein 1 (HuR) and prevents HuRfrom binding to PABPN1 mRNA, subsequentlysuppressingPABPN1 translation ***(Abdelmohsen et al., 2017).***

Regulating transcription or splicing:

Some circRNAs have been demonstrated to regulate gene transcription through combining with RNA polymerase IIcomplex and translating related proteins ***(Chen, 2016)***.

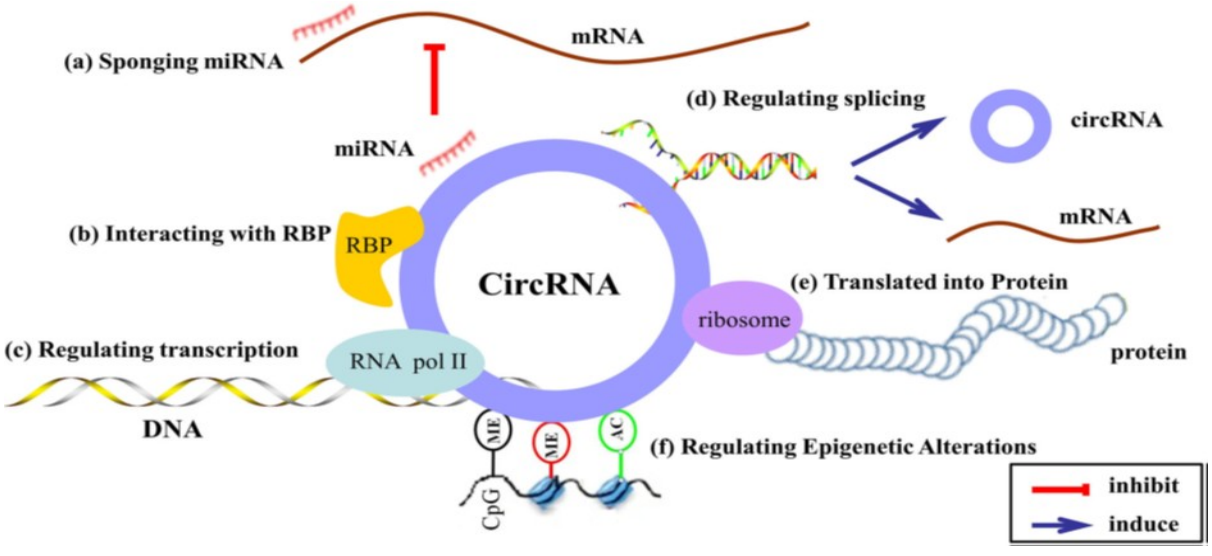
Translating proteins:

CircRNAswere initially defined asa distinct class of endogenous noncoding RNA that could nottranslateproteins, because of lacking 5′-3′ and polyadenylatedtails, as well as internal ribosome entry sites (IRES)***(Liu et al., 2017; Wang and Fang, 2018)***.

Regulating epigenetic alterations:

Aberrant DNA methylation and histone modificationsassociated with epigenetic gene expression are frequentlyfound in cancer ***(Bird, 2002; Lv et al., 2016)***.

Figure 1: The functional mechanisms of CircRNAsa). (a) Acting as miRNA sponge; (b) Binding with RNA binding protein (RBP); (c) Regulating transcription ;(d) regulating splicing; (e) translated into Protein; (f) Regulating epigenetic alterations.***(Su et al., 2019).***



Circrnas And Cancer Regulation:

Effects on cellular proliferation:

Many studies have shown that circRNAs can affectthe process of cell proliferation byregulating the cell cycle***(Zhou et al., 2018)***. One example of circular RNAs involved in cancer proliferation is Circ-ITCH which can be used as asponge for miR-7 and miR-214 to inhibit the proliferation of cancer cells bydownregulating β-Catenin through Wnt/β-Catenin pathway***(Guo et al., 2017)***.

Effects on cell cycle proteins:
CircRNAs can also indirectly affect cellcycle-related proteins or transcription factors by combining miRNAs to regulate theproliferation of tumor tissues. For example, circ-TCF25***(Zhong et al., 2016)***and circ-100290 ***(L. Chen et al., 2017)***canenhance the function of CDK6 by binding to downstream miRNAs(as miR-107 and miR-103a-3p).

Contribution to invasion and metastasis:
During metastasis process, the cellsmay undergo some alterations such as hypernomicproliferation, "epithelial-mesenchymal-transition" (EMT)***(Dwyer et al., 2017)***, and increased expression of matrixmetalloproteinase (MMP). CircRNAs play a role as sponges in the regulation of tumorcell invasion and metastasis.

CircRNA also impacts the invasion and metastasis of tumor cells by suppressingmiRNAs that directly target MMP, thereby up regulating the expression of MMP intissues. For example, in non-small cell lung cancer, circRNA_100876 acts as a spongefor miR-136, which binds to the 3'-UTR of MMP-13 mRNA and suppresses itstranscription***(Yao et al., 2017).***

Influence on angiogenesis:
Angiogenesis is one basic characteristics of tumors and plays a keyrole in tumor progression ***(Hanahan and Weinberg, 2011)*** ."Angiogenic switches" such as transforming growthfactor (TGF), vascular endothelial growth factor (VEGF), and matrixmetalloproteinase (MMPS) are correlated with regulation of angiogenesis. CircRNAcan impact the expression of these factors to regulate angiogenesis of tumors byacting on miRNA. Circ-100876 prevents miR-136 from binding to theMMP-13 3' UTR sequence by sponging miR-136, which enhances the expression of MMP-13 ***(Yao et al., 2017)***.

Impact on apoptosis:
Cancer cells acquired theability to evade apoptosis signals as Bcl-2 and Bax***(Li et al., 2017; Su et al., 2019)***. Silencing of hsa_circ_0007534 inhibitedproliferation while promoting the apoptosis of CRC cells.Moreover, the Bcl-2/Bax ratio was decreased followinghsa_circ_0007534 silencing, which demonstrated thathsa_circ_0007534 inhibits CRC cellproliferation, at leastpartially, by inducing apoptosis ***(R. Zhang et al., 2018)***.

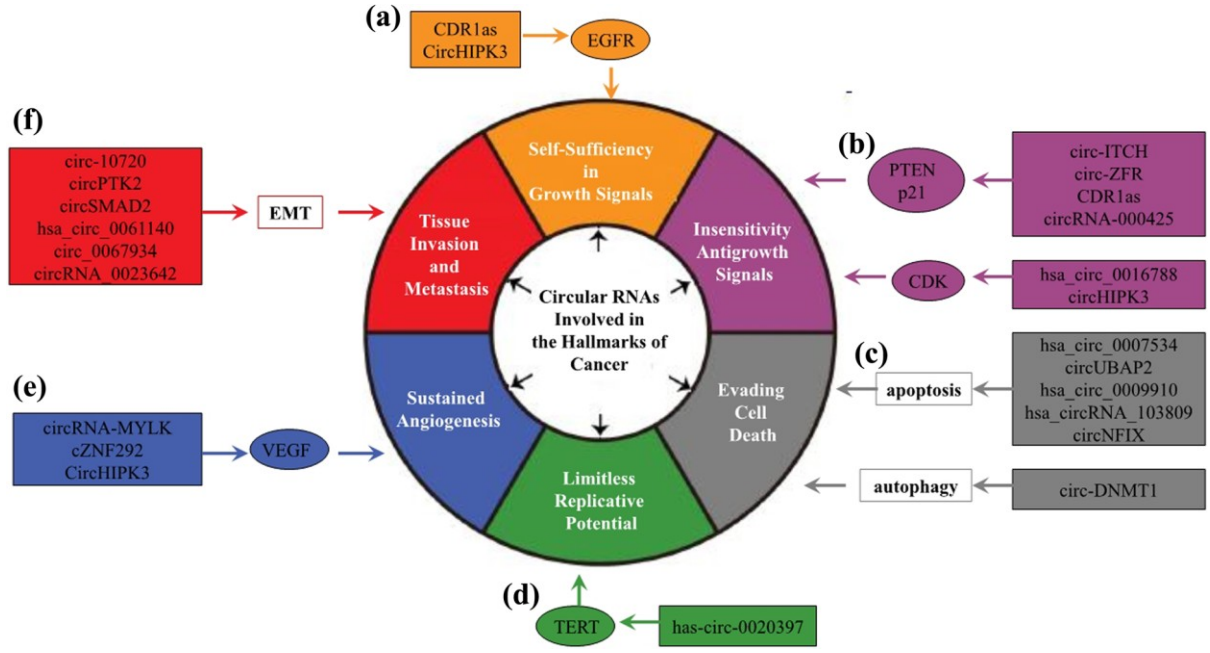


Figure 2:- CircRNAs involved in the hallmarks of cancer***(Su et al., 2019)***.

CDR1as and circHIPK3 active cell proliferative through regulating EGFR; (b) Circ-ITCH et al. promotecancer cells evading antigrowth signals by preventing expression or activation of tumor suppressors, such as PTEN and CDK; Hsa_circ_0007534 et al. promote cancer cells evading cell death via regulating cellular apoptosis or autophagy; Hsa-circ-0020397 limits replicative potential of cancer cell trough regulating TERT; (e) CircRNA-MYLK et al. sustain angiogenesis trough regulating VEGF; (f) Circ-10720 et al. regulate the process of EMT and thus tissue invasion and metastasis of cancer.

Circrnas As Biomarkers In Cancer:-

CircRNAs can be potentially valuable prognosticand diagnostic biomarkers for cancer. Recently,many studies have demonstrated circRNAs may be stablyexpressed and present in relatively high quantities in humanbody fluids, such as saliva, plasma, serum andexosomes,which also makes circRNAs ideal candidates asnoninvasive liquid biopsy biomarkers for cancer ***(Kun-Peng et al., 2018)***.Examples of circ RNAs as biomarkers in cancer are listed below.

Circ-ZEB:-

Circ-ZEB1.33 is overexpressed in humanHCC tissues and serum of HCC patients, its levels in HCC tissue and serum were correlatedwith different TNM stages and overall survival inHCC patients, suggesting circ-ZEB1.33 may serve as avaluable biomarker in HCC prognosis prediction ***(Gong et al., 2018)***

Hsa_circ_0000190:-

Has circ 0000190 is downregulated in gastric cancer (GC) tissues andplasmasamples ***(S. Chen et al., 2017)***. Its expression levels were significantlyassociated with tumor size, distal metastasis,lymphatic metastasis, TNM stage and CA19–9 levels***(S. Chen et al., 2017).***

Hsa_circ_0000745:-

Has circ 0000745 is decreased in GC tissues andplasma samples ***(M. Huang et al., 2017)***. The expression level of hsa_circ_0000745 in GC tissues was correlated with tumordifferentiation,while the expression level in plasma wascorrelated with tumor-node metastasis stage.

Table 1:- CircRNAsfrom biological fluids as biomarkers in cancer(Su et al., 2019).

Source	Cancer type	Circ RNA	Expression	Associated clinical features	Reference
Saliva	OSCC	hsa_circ_0001874	Up	TNM stage, tumor grade	***(Zhao et al., 2018)***
Plasma	GC	Hsa_circ_0000190	down	tumor diameter, lymphatic metastasis, distal metastasis, TNM stage	***(S. Chen et al., 2017)***
	GC	hsa_circ_0000673	down	TNM stage	***(Chang et al., 2018)***
	BC	hsa_circ_0001785	down	histological grade, TNM stage, distant metastasis	***(Yin et al., 2018)***
	HCC	hsa_circ_0001445	down	AFP level	***(X. Zhang et al., 2018)***
Serum	HCC	circ-ZEB1.33	up	TMN stages,	***(Gong et al., 2018)***

Emerging Role Of Circular Rnas In Hepatocellular Carcinoma:-
Hepatocellular carcinoma accountsfor approximately 80% of primary liver cancers and the fifth leading cause of mortality from cancerrelateddiseases worldwide ***(Bray et al., 2018)***.

CircMTO1 is downregulated in HCC tissues,and correlated with the poor survival of patients. Aftersilencing circMTO1, the level of cell proliferation andinvasion is significantly increased, and the percentage ofapoptosis is reduced (***Han et al., 2017)***.

CSMARCA5 isdecreased in HCC tissues, and correlated with aggressivebiological behaviors, such as poorer tumor differentiation, advanced tumor stage, tumor size and the presenceof microvascular invasion ***(Yu et al., 2018)***.

Circ_0067934 ishighly expressed in HCC tissues, when compared withadjacent normal tissues, and promotes cell proliferationand metastasis in vitro and in vivo via the miR-1324/FZD5/Wnt/b-catenin axis ***(Zhu et al., 2018)***.

The expression of CircRNA_100338 iscorrelated with decreased cumulative survival rate, increasedvascular invasion and lung metastasis in HCCpatients (***X.-Y. Huang et al., 2017)***.

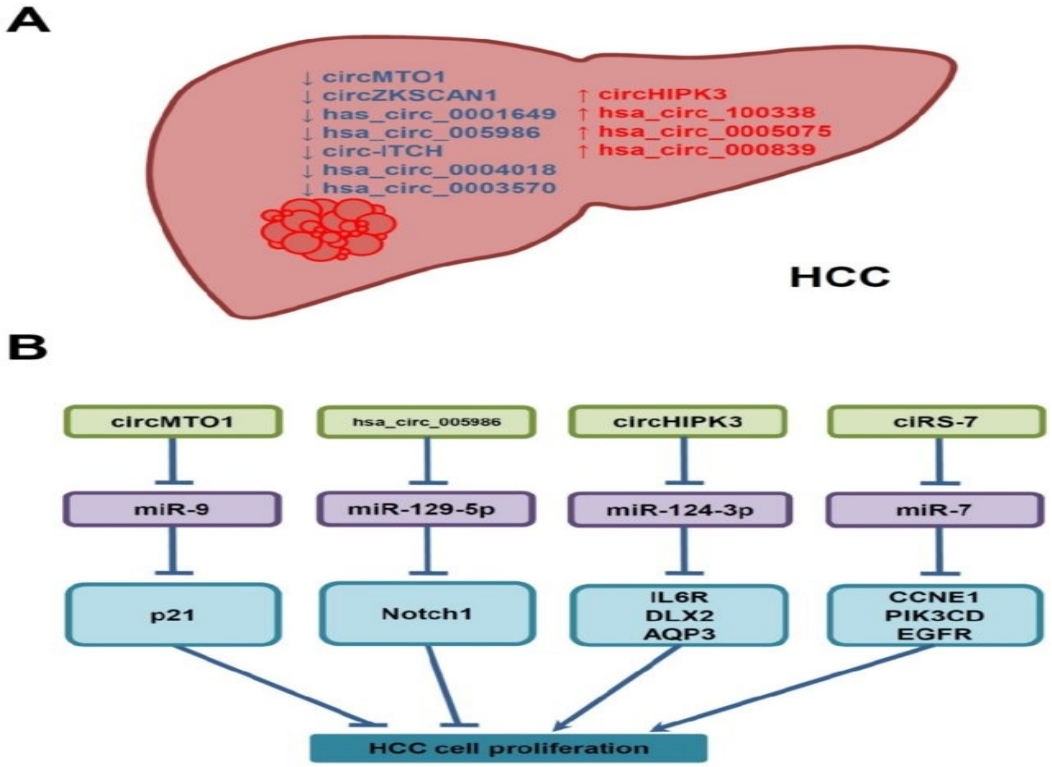


Figure 3:- Deregulated circRNAs in HCC, and their underlying molecular mechanisms in the proliferation of HCC cells. (A) CircRNAs are down- (↓, blue) or up-regulated (↑, red) in HCC. (B) Deregulated circRNAs may interfere with HCC cell proliferation by sponging miRNAs and increasing the expression of miRNA-target genes (Qiu et al., 2018).

Conclusion:-

In the past few years, the regulatory effects of circRNAs have been illustrated on pathophysiologic processes, including tumorigenesis. In this review, we briefly summarized the recent advances regarding circRNAs in the hallmarks and the possibility as biomarkers for cancer.

The stability, universality, and specificity of circRNAs make it to be a potential valuable prognostic and diagnostic biomarker for cancer, and the functions and regulatory roles that circRNAs play in tumor cells make it possible to be a target for the treatment of cancer. However, the study of circRNAs is far from being able to be incorporated into clinical practice, and there are still problems requiring further investigation in this field. For example, further investigation is needed regarding the precise mechanisms, other than those of miRNA sponge activity, of circRNAs underlying the initiation and progression of cancer. Furthermore, more controlled and large-scale clinical studies are required before cancer-specific circRNAs can be recommended for diagnosis and treatment. Understanding circRNA willgenerate new hypotheses regarding cancer pathogenesis. We hope that the appropriate and precise use of circRNAs in clinical applications might eventually create breakthroughs for cancer therapy in the upcoming years.

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