International Journal of
Science Inventions Today
www.ijsit.com
ISSN 2319-5436
Review Article

# CIRCULAR RNA IN COLORECTAL CANCER: NEW ADVANCES

Dibesh Shrestha, WenLiang Li*, Jian Huang, Zhu Zhu, RuiZe Zhou and Quan Yang

*Department of Oncology, First Affiliated Hospital of Kunming Medical University, No. 295, XiChang road, Kunming City, Yunnan Province, PRC*

## ABSTRACT

A newly revived area of research in the field of RNA is the circular RNA (circRNA). Although only very recently identified to be a pertinent player in the regulation of cellular processes, it has become a hotspot for research regarding many disease processes. Due to their unique structural properties they have caught the attention of scientists as potential biomarkers of disease. Moreover, as they have been found to have global influence on cellular processes, they are also thought to have potential as novel therapeutic targets. Here we review the salient features of circular RNAs and provide an update on the studies which explore the relationship between circular RNAs and colorectal cancer.

**Keywords**: circRNA, colorectal cancer, cancer biomarker, long non-coding RNA, cancer therapy

## INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide (1) accounting for morbidity, mortality and economic burden in developing and developed countries alike. Although recognized as a great health problem and continuous efforts being made in improving the screening, diagnostic and therapeutic practices all over the world, the incidence of CRC is still increasing, especially in the younger age group (2). Therefore, it is of utmost importance that novel methods be sought to improve the diagnosis and treatment of this condition.

Long non-coding RNAs (lncRNAs) are a class of transcripts which are known to influence every step of the gene expression pathway (3, 4). Circular RNAs (circRNAs) are a class of regulatory non-coding RNAs which were first discovered in the 1970s (5, 6). Since then, although this species of RNA were identified in several other studies (7-11), they were mostly dismissed as "junk" and labeled transcription errors, products of aberrant RNA splicing and so remained understudied for decades. However, with advances in high throughput RNA sequencing technologies, researchers have revealed circRNAs are infact abundant, stable and naturally occurring in eukaryotic cells (12) and are associated with many disease processes in humans. In the present day, circRNAs have reemerged as a hotspot for research in the field of RNA.

The phenomenon of circularization of RNA in CRC was first identified by Renkonen et al a decade ago, but the authors were unaware of its biological and clinical significance. However, even then, they hinted towards possible use of these "unusual" products in diagnosis and screening of CRC (13). With accumulating evidence in the present day, their prediction may soon be a reality in clinical practice.

We aim to summarize the recent works that explore the relationship between circRNA and CRC in this review after a brief appraisal of the general features of circRNA.

### Structure of circRNAs:

CircRNAs have a unique structural difference from other lncRNAs in that they do not possess 5' caps and 3' polyadenylated tails. This difference accounts for the inability detecting these species of RNA by traditional molecular methods that relied on a polyadenylated free RNA end or poly(A) enrichment of samples (14). Infact their 3' and 5' ends are covalently linked to form the characteristic circular structure, via the so-called "back-splicing" mechanism in which a splice donor is joined to an upstream splice acceptor (12). Several mechanisms for the biogenesis of circRNAs have been postulated: *intron-pairing driven circularization* (12), *RBP pairing driven circularization* (15, 16), *lariat-driven circularization (exon skipping)* (12) and *co- or post-transcriptional biogenesis* (16).

### General properties of circRNA:

**Abundance:** circRNAs are synthesized from >14% of transcribed human fibroblasts and they can be considerably more abundant than associated linear transcripts (12, 17). **Resistance:** circRNAs seem to be resistant to RNase R activity, an exonuclease that degrades linear RNA molecules (17). This property is utilized by the new generation molecular methods to isolate circRNAs in experimental models (14). **Stability:** circRNAs

are very stable in cells with a half life of about 48 hours (12). **Specificity:** numerous circRNAs have been identified that are cell-type and developmental stage specific (17, 18).

Because of these special properties, circRNAs could be considered as ideal potential candidates of clinically applicable biomarkers. Moreover, the correlation between the expression of exosomal proteins, microRNAs (miRNAs) and circRNAs could increase the sensitivity and specificity of diagnostic tests. Studies have also concluded that there are distinct circRNAs expressed in different clinical conditions (19). Additionally, circRNAs were reproducibly and easily detected in standard clinical blood samples and in human saliva (20, 21), and were also found to be transported to exosomes where they were found to be stable and in high quantities (22, 23), highlighting that circRNAs are indeed promising candidates of biomarkers for human diseases.

circRNAs are generally classified as (i) sense or exonic – comprising of exons of a linear transcript, (ii) intronic – containing intron of a linear transcript, (iii) antisense – overlapping exon(s) of the opposite strand, (iv) intragenic – transcribed from the same gene locus of the linear transcript but cannot be classified as exonic or intronic, (v) exonic intronic – containing both exons and retained introns and (vi) intergenic – located between 2 genes (24). Exonic circRNAs tend to be located in the cytoplasm (25) while intronic RNAs (ciRNAs) and exonic intronic (EIciRNAs) tend to be located in the nucleus (26).

## Proposed functions of circRNAs:

**miRNA sponge:** miRNAs are a class of small non-coding RNAs that have been shown to be indispensable to the cellular life processes. Some circRNAs have been found to function as "sponges" of miRNAs, i.e. they sequester these miRNAs and affect their downstream functions (protein translation, gene expression etc.) (18). A very well studied example is the ciRS-7/CDR1as which contains >70 binding sites for miR-7 (12), which in turn is speculated to be central in many disease processes such as glioblastoma, tongue squamous cell carcinoma, neurodegenerative disorders etc. (27-30). miR-7 was also found to be instrumental in up- and down-regulation of various oncogenic factors in cancer related signaling such as EGFR (30), Pak1 (31), IRS-1 (32), mTOR (33), IGF1R (34).

SrycircRNA, a circRNA found in mouse testis, is another sponge of miRNA which was found to have 16 putative binding sites for miR-138 (18).

Very recently, hsa_circ_0005986 was identified as a sponge of miR-129-5p and its downregulation accelerated cell proliferation by promoting the G0/G1 to S phase transition in hepatocellular carcinoma (35). Some other RNAs that have been identified as miRNA sponges include circITCH (36, 37), circFoxo3 (38), circHRCR (39), circHIPK3 (40, 41), hsa_circ_001569 (42), circABCB10 (43).

The sponging activity was supported by a study in which circRNAs were found to be devoid of single nucleotide polymorphisms in miRNA binding sites (44). However, since very few mammalian circRNAs are found to have more than 10 miRNA binding sites, it is debatable whether miRNA sponge activity is general of all circRNA (14).

**Transcription regulation:** circRNA circMbl is generated from the locus of splicing factor MBL. circMbl renders the pre-mRNA non-productive, thus significantly affecting production of MBL protein (16). ciRNAs and EIciRNAs have little enrichment of miRNA binding sites and the knockdown of these circular RNAs was found to lead to reduced expression of their parent genes (17, 26). Furthermore, researchers have also found that the interaction of EIciRNAs with U1snRNP and RNA polymerase II could enhance the transcription of their parental genes (45). Similarly, circITCH and its interaction with miR-7, miR-17 and miR-214 could increase the levels of ITCH itself (36, 37).

**Protein translation:** researchers have found that circRNAs could translate proteins in vitro (46) and in vivo (47) if they possess internal ribosome entry sites (IRES) or prokaryotic ribosome binding sites. Till date, at least one naturally occurring circular RNA – HDV, a satellite virus of hepatitis B virus – is known to encode a single protein in eukaryotic cells (7). However, there have been no experimental evidence showing that naturally occurring endogenous circRNAs can code for proteins.

## DISCUSSION

### Circular RNAs in Colorectal Cancer:

In the recent years a few studies that explore the relationship between circular RNA and CRC have been concluded. Below we summarize the pertinent findings of these studies. Table 1 provides a summary of the different circular RNAs that have been identified in colorectal cancer.

| circRNA | Chromos-ome | Gene | Regulation | miRNA associated | Functional target | Reference |
|---|---|---|---|---|---|---|
| hsa_circ_001988 | chr4 | FBXW7 | Down | - | - | (48) |
| circCCDC66 | chr3 | CCDC66 | Up | miR-33b, miR-93 | MYC | (49) |
| ciRS-7 | chrX | CDR1 | Up | miR-7 | EGFR/RAF1/MAPK | (34) |
| Cir-ITCH | | | Down | miR-7, miR-20a | Wnt-β catenin | (37) |
| hsa_circ_0000069 | chr1 | STIL | Up | | | (50) |
| circRNA_001569 | chr16 | ABCC1 | Up | miR-145 | E2F5/BAG4/FMNL2 | (42) |
| circ-BANP | chr16 | BANP | Up | - | PI3k/Akt | (51) |

| | | | | | | |
|---|---|---|---|---|---|---|
| hsa_circRNA_103809 | chr5 | ZFR | Down | - | - | (21) |
| hsa_circ_RNA104700 | chr8 | PTK2 | Down | - | - | (21) |
| hsa_circ_0001649 | chr6 | SHPRH | Down | - | - | (52) |
| hsa_circ_0014717 | chr1 | CCT3 | Down | - | p16/CDK4/RB | (53) |
| hsa_circ_0020397 | chr10 | DOCK1 | Up | miR-138 | TERT/PDL-1 | (54) |
| hsa_circ_0071589 | chr4 | FAT1 | Up | miR-600 | EZH2/p53 | (55) |
| hsa_circ_000984 | chr7 | CDK6 | Up | miR-106b | CDK | (56) |
| circRNA_100290 | chr1 | SLC30A7 | Up | miR-516b | FZD4/Wnt-β catenin | (57) |
| circRNA_0003906 | chr6 | - | Down | - | - | (58) |
| circ_HIPK3 (hsa_circ_0000284) | chr11 | HIPK3 | Up | miR-7 | FAK/IGF1R/EGFR/YY1 | (59) |
| ciRS-7 | chrX | CDR1 | Up | miR-7 | EGFR/RAF1/MAPK | (60) |
| circ_0026344 | chr12 | ACVRL1 | Down | miR-21 miR-31 | - | (61) |
| hsa_circ_0055625 | chr2 | DUSP2 | Up | miR-106b | ITGB8 | (62) |
| hsa_circ_0136666 | chr8 | PRKDC | Up | miR-136 | SH2B1 | (63) |
| hsa_circRNA_103809 | chr5 | ZFR | Down | miR-532-3p | FOXO4 | (64) |
| circDDX17 | chr22 | DDX17 | Down | miR-21-5p | - | (65) |
| hsa_circ_0006508 | chr17 | VMP1 | Up | - | - | (65) |
| hsa_circ_0007534 | chr17 | DDX42 | Up | - | - | (66) |

**Table 1:** Circular RNAs identified in colorectal cancers

## Circular RNAs as diagnostic markers in CRC:

As mentioned previously, circRNAs can be reproducibly and relatively easily be detected in clinical blood samples. Similarly, expression level of circRNA in exosomes was found to be more abundant than in the CRC cells themselves (22). In addition to being an evidence for the potential use of circRNA as an early diagnostic marker in CRC, this could also signify that circRNAs could have as yet undiscovered extracellular or endocrine effects. Some other circRNAs studied have been proposed as potential diagnostic markers. Hsa_circ_001988 is a downregulated circRNA whose sensitivity and specificity on ROC analysis was 0.68 and 0.73 respectively with area under the curve (AUC) of 0.788 (95% CI = 0.68-0.90, P<0.001) (48). Additionally, its decreased expression was significantly correlated with perineural invasion and differentiation, but the terms of this relationship have not been specified by the authors. circCCDC66 is an upregulated circRNA, whose AUC was 88% making it a good diagnostic biomarker (49). Similarly, it was reported that the AUC of hsa_circRNA_103809 and hsa_circRNA_104700 were 0.699 (p<0.0001) and 0.616 (p<0.0001) respectively (21). circRNA0003906 was also identified to have an AUC of 0.818 (95% CI = 0.749-0.888) (58). The sensitivity and specificity of hsa_circ_0001649 were 0.828 and 0.781, respectively, and AUC was 0.857 (52). Similarly, AUC for ROC of hsa_circ_0014717 was 0.683 (p=0.001) (53).

Taken collectively, circRNAs have the promise of being a novel diagnostic biomarker for CRC which, at least, can be used in addition to the existing diagnostic methods to perhaps increase the diagnostic yield. More research looking into this potential role is required.

## Circular RNAs as prognostic markers CRC:

Circular RNA expression has been shown to be associated with clinicopathological parameters in CRC in many studies thereby serving as prognostic indicators. circCCDC66, for example, was found to promote cell proliferation, migration and metastasis. It was overexpressed in CRC cells and higher expression levels were associated with poorer prognosis (49). ciRS-7 expression was found to be correlated with advanced tumor stage, tumor depth and metastasis. It was also found to be an independent risk factor for overall survival (34, 60). Similarly, decreased expression of hsa_circ_0014717, circ_0026344 was found to be associated with significantly shorter overall survival in CRC patients (53, 61). On the other hand, higher expression levels of hsa_circ_0071589, circHIPK3, hsa_circ_0136666, were associated with poorer prognosis in CRC patients (55, 59, 63).

## Circular RNAs as markers for directing therapy and monitoring therapeutic efficacy in CRC:

The therapy of colorectal cancer is based upon the TNM classification and the stage of the disease. Such classification has helped clinicians to carefully categorize patients and select appropriate patients for the most appropriate therapy greatly improving the quality of care and outcomes. In recent times circular RNAs are also emerging as potential markers for directing therapy. Expression of circular RNAs in CRC tissues, whether increased or decreased, have been shown to correlate with the stage of disease. Overexpression of ciRS-7

correlates with advanced tumor stage, tumor depth and metastasis (34). Similarly, overexpression of hsa_circ_0000069, circ_001569, hsa_circ_0055625, hsa_circ_0071589, hsa_circ_0007534, hsa_circ_000984 have been shown to correlate with tumor characteristics like pathological grade of differentiation, tumor depth, nodal and distal metastases (42, 50, 56, 62, 66). Meanwhile, the downregulated circular RNAs having significant association with tumor characteristics are hsa_circRNA_103809, hsa_circRNA_104700, hsa_circ_0001649, hsa_circ_0014717, circRNA0003906, circ_0026344 and circDDX17 (21, 52, 53, 57, 61, 65). That said, not all of the mentioned circular RNAs show the same association with clinicopathologic characteristics and each circular RNA could have its own unique role either singly or in combination. Perhaps future research could shed light on this issue. Moreover, in one study circular RNA was proposed to have the potential to serve as a marker for monitoring therapeutic efficacy in CRC. Hsa_circ_0001649 was found to be downregulated not only in CRC tissue samples but also in corresponding serum samples (52). After surgical removal of tumors, the serum levels were significantly elevated compared to pre-operative levels. This could signify that circular RNAs could have a role in post-therapeutic surveillance of CRC in adjunct to methods currently applied in clinical practice.

## Circular RNAs as markers of resistance to therapy in CRC:

Chemoradiotherapy, as an adjunctive or singular modality, plays a crucial role in the management of CRC patients along with surgical resection of tumor. However, it is well established that not all patients with CRC need chemoradiotherapy and among those who do require some form of adjunctive therapy, not all patients will benefit from it. Much attention has been attracted by this clinical problem. Currently, genetic testing in combination with pathological characterization of tumor tissues help guide clinicians in identifying and selecting appropriate patients for chemoradiotherapy. Circular RNAs could also serve this purpose. Hsa_circ_0007031 was found to interact with micro RNAs (discussed below) to promote chemoradiotherapy resistance in CRC (67).

## Interaction between circular RNAs and micro RNAs in CRC:

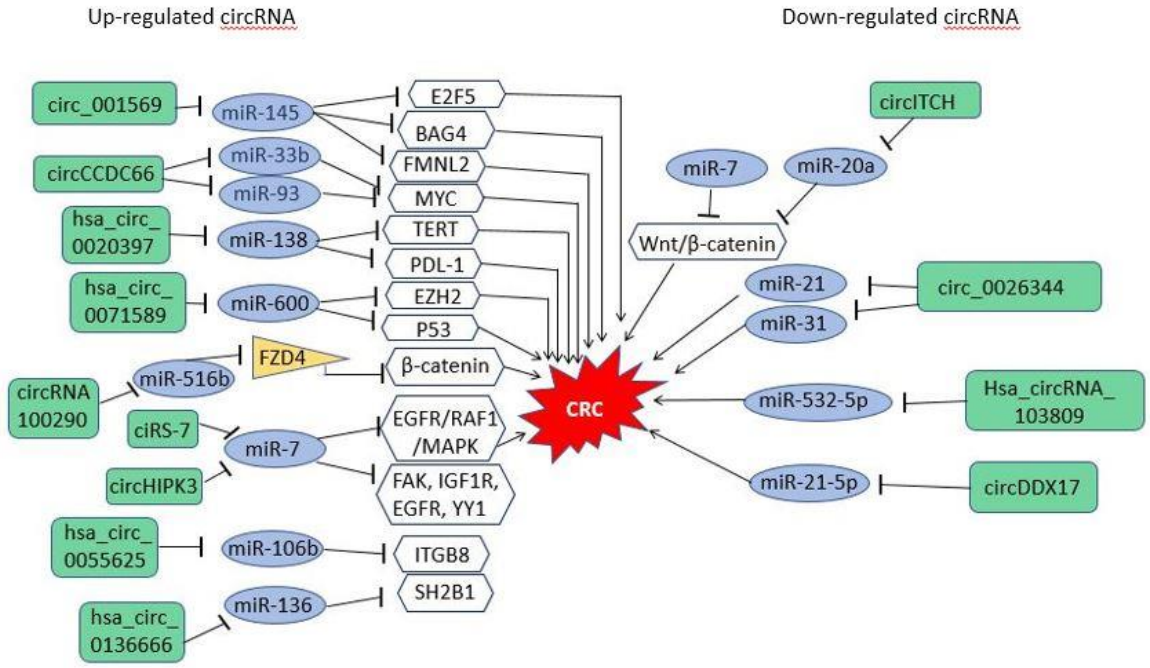


**Figure 1:** Illustration of the interaction between circular RNAs and micro RNAs in the pathogenesis of colorectal cancer

Circular RNAs are known to interact with micro RNAs and thus have varying effects by regulating the activities of downstream targets. Figure 1 provides an illustration of the interactions between circular RNAs and micro RNAs discovered in colorectal cancer so far. Here we briefly summarize these ineractions.

Hsa_circ_0020397 was found to inhibit the function of miR-138 thus, upregulating the targets of miR-138, TERT and PDL1, which are involved in regulating cancer cell growth; TERT stabilizes telomere length thereby immortalizing cancer cells, whereas PDL1 inhibits T-cell activity and cancer cell apoptosis (54). Hsa_circ_0071589 could bind to miR-600 and inhibit its tumor suppressive effects through EZH2 suppression (55). Hsa_circ_000984 acted as a sponge of miR-106b and upregulated its downstream target CDK6, which is a well-known contributing factor in a variety of cancers by regulation of cell cycle (56).

Wnt/β-catenin signaling pathway is abnormally activated in CRC and FZD4 serves as a receptor in this signaling pathway. circRNA_100290 was shown to promote FZD4 expression by inhibiting miR-516b (57). circHIPK3 was found to sponge miR-7, a well-known tumor suppressor, and contribute to tumor progression by increasing the expression of miR-7 targets – FAK, IGF1R, EGFR and YY1 (59). ciRS-7 also acts as a sponge of miR-7. Weng et al found that ciRS-7 could suppress miR-7 activity and thereby activate the EGFR/RAF1/MAPK pathway, an important pathway in carcinogenesis (60). Similar function of ciRS-7 was also reported in another study (34). circCCDC66 was found to be able to bind to miR-33b and miR-93 to promote the oncogenic activity of the downstream target MYC (49). circ_0026344 also could bind to multiple miRNAs. It was shown to bind miR-21 and miR-31 to inhibit carcinogenesis in CRC; thus, its downregulation could promote CRC (61).

circ_001569 was shown to combine with miR-145, and up-regulate miR-145 targets E2F5, BAG4 and FMNL2 to exert its tumor promoting function in CRC cells (42). Similarly, hsa_circ_0055625 was found to inhibit the tumor suppressive role of miR-106b by binding to it, hence, increasing ITGB8 mRNA levels and promoting tumor proliferation, migration and invasion (62). miR-136 is a tumor suppressor and SH2B1, and oncogene, is a downstream target of miR-136. Hsa_circ_0136666 was found to function as a ceRNA of miR-136 to facilitate expression of SH2B1 in CRC cells (63). In another study, it was speculated that hsa_circ_103809 competitively binds to miR-532-5p which in turn regulates FOXO4 activity of cell cycle progression and inhibition of apoptosis (64).

## Circular RNAs as regulators of cell cycle in CRC:

Hsa_circ_0014717 was shown to be able to upregulate the expression of the tumor suppressor p16 and induce cell cycle arrest (53). It is known that p16 inhibits aberrant cell cycle progression by binding to CDK4 or CDK6, thus blocking the formation of cyclin D/CDK4 or 6 complexes which leads to cell cycle arrest at G1/S phase by inhibition of phosphorylation of RB. Hsa_circ_000984 was found to promote cell proliferation, migration and invasion by sponging miR-106b and upregulating its downstream target CDK-6, which is a well-known contributing factor in a variety of cancers by its regulation of cell cycle (56).

## Analysis of function of circular RNAs in CRC:

A couple of studies have investigated the function of circular RNAs in CRC by utilizing the Gene Ontology (GO) analysis and the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis (68, 69). Zeng et al identified differentially expressed circular RNAs in CRC tissues from patients with pulmonary metastasis compared with those without. They reported that the genes producing upregulated circular RNAs were involved in DNA repair and those producing downregulated circular RNAs were enriched in signal transduction especially Wnt and NF-κβ signaling (70). These are al major mechanisms identified in CRC pathogenesis. Li et al reported that the host genes of differentially expressed circRNAs were associated with important CRC-related pathways. The most enriched GO terms in their study were cell communication, autophagosome and GTP binding (65). They also uncovered that circDDX17, the most significantly dysregulated circRNA in their study, could potentially bind to hsa_miR_21_5p, which was involved in KEGG pathway for COLORECTAL CANCER and MicroRNAs in cancer. In the same study, the authors found that another circRNA hsa_circ_0006508 was significantly upregulated in CRC tissues. It was derived from the newly discovered autophagy and CRC related gene VMP1. Thus, it could be speculated that hsa_circ_0006508 might be involved in autophagy in CRC by regulating the host gene VMP1 (65).

In the study conducted by Chen et al the most enriched GO terms were cell cycle, mitotic cell cycle and cell cycle process (71). Furthermore, the authors concluded that a new circular RNA hsa_circ_0126897_CBC1 could be involved in the regulation of cell proliferation, migration and invasion of CRC based on studies done on its host gene SLC4A4 (71).

One more circular RNA was identified by Zhang et al – hsa_circ_0007534 (66). It was upregulated in CRC tissues and cell lines and its expression was correlated with higher tumor stage and lymph node metastasis. No miRNA interaction was identified in this study.

## CONCLUSION

Studies involving circRNAs, although being undertaken at a staggering rate, are still in their infancy. The above discussion clearly supports the idea that this newly re-discovered non-coding RNA seems to have global influence in carcinogenesis. However, studies that provide incontrovertible evidence of their central role in cancer pathways are still lacking. Even fewer studies have related colorectal cancer with circRNAs. Studies are heterogeneous in terms of their findings. Some circular RNAs that may be pro-carcinogenic in one type of cancer may be anti-carcinogenic in another type of cancer. The explanation for this phenomenon is still unclear. Till date, only two circular RNAs – ciRS-7 and hsa_circRNA_103809 – have been reproducibly shown to play a role in CRC. It is still unclear what impact these sorts of findings have on clinical practice. In general, global circRNA downregulation seems to be a general rule in colorectal cancer tissues. Pivotal roles have been proposed for both upregulated as well as downregulated circRNAs, however, precise mechanisms of how these circRNAs, downregulated or upregulated, are involved in cancer pathways need to be discovered. Several studies have elaborated the circular RNA/micro RNA/downstream targets pathway in pathogenesis of cancers. These pathways must be reproducible and such endeavors must be undertaken in the future. Similarly, mechanisms of circRNA transport from nucleus to cytoplasm and their clearance are yet to be uncovered.

Although initial studies have shown promising results with regards to the usefulness of circRNAs as diagnostic and prognostic biomarkers and potential therapeutic targets, more rigorous, large scale studies are required to identify the most suitable circRNAs for translation of their utility to clinical practice. Research for future therapies of some diseases have already begun to look into the potential role of circularization, i.e. to either decrease circularization of functional transcripts or to sequester exons contributing to dysfunctional transcripts (14, 72). Future researches will predictably be directed towards the possibilities of translation of proteins from engineered or naturally occurring circRNAs and develop methods to identify clinical diagnostic and prognostic biomarkers in easily accessible compartments of the body such as peripheral blood, saliva etc.

## REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel R, Torre L, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018 68(6):394-424
2. Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A, et al. Colorectal cancer statistics, 2017. *CA Cancer J Clin* 2017 67(3):177-93
3. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. *Mol Cell*. 2011;43(6):904-14

4. Yoon JH, Abdelmohsen K, Gorospe M. Posttranscriptional gene regulation by long noncoding RNA. *J Mol Biol* 2013 425(19):3723-30.
5. Sanger HL, Klotz G, Riesner D, Gross HJ, Kleinschmidt AK. Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. *Proc Natl Acad Sci U S A* 1976 73(11):3852-6.
6. Hsu MT, Coca-Prados M. Electron microscopic evidence for the circular form of RNA in the cytoplasm of eukaryotic cells. *Nature* 1979 280(5720):339-40.
7. Kos A, Dijkema R, Arnberg AC, van der Meide PH, Schellekens H. The hepatitis delta (delta) virus possesses a circular RNA. *Nature* 1986 323(6088):558-60.
8. Nigro JM, Cho KR, Fearon ER, Kern SE, Ruppert JM, Oliner JD, et al. Scrambled exons. *Cell* 1991 64(3):607-13
9. Capel B, Swain A, Nicolis S, Hacker A, Walter M, Koopman P, Goodfellow P, Lovell-Badge R Circular transcripts of the testis-determining gene Sry in adult mouse testis. *Cell* 1993 73(5):1019-30.
10. Zaphiropoulos PG. Circular RNAs from transcripts of the rat cytochrome P450 2C24 gene: correlation with exon skipping. *Proc Natl Acad Sci U S A* 1996 93(13):6536-41.
11. Pasman Z, Been MD, Garcia-Blanco MA. Exon circularization in mammalian nuclear extracts. *RNA* 1996 2(6):603-10
12. Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, et al. Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA* 2012 19(2):141-57.
13. Renkonen E. Novel splicing associations of hereditary colon cancer related DNA mismatch repair gene mutations. *Journal of Medical Genetics* 2004 41(7):e95-e.
14. Jeck WR, Sharpless NE. Detecting and characterizing circular RNAs. *Nat Biotechnol* 2014 32(5):453-61.
15. Conn SJ, Pillman KA, Toubia J, Conn VM, Salmanidis M, Phillips CA, et al. The RNA binding protein quaking regulates formation of circRNAs. *Cell* 2015 160(6):1125-34.
16. Ashwal-Fluss R, Meyer M, Pamudurti NR, Ivanov A, Bartok O, Hanan M, et al. circRNA biogenesis competes with pre-mRNA splicing. *Mol Cell* 2014 56(1):55-66.
17. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 2013 495(7441):333-8.
18. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, et al. Natural RNA circles function as efficient microRNA sponges. *Nature* 2013 495(7441):384-8.
19. Abu N, Jamal R. Circular RNAs as Promising Biomarkers: A Mini-Review. *Front Physiol* 2016 7:355.
20. Bahn JH, Zhang Q, Li F, Chan TM, Lin X, Kim Y, et al. The landscape of microRNA, Piwi-interacting RNA, and circular RNA in human saliva. *Clin Chem* 2015 61(1):221-30.
21. Pfeffer S, Memczak S, Papavasileiou P, Peters O, Rajewsky N. Identification and Characterization of Circular RNAs As a New Class of Putative Biomarkers in Human Blood. *Plos One* 2015 10(10).

22. Dou YC, Cha DJ, Franklin JL, Higginbotham JN, Jeppesen DK, Weaver AM, et al. Circular RNAs are downregulated in KRAS mutant colon cancer cells and can be transferred to exosomes. *Sci Rep* 2016 6:37982
23. Li Y, Zheng Q, Bao C, Li S, Guo W, Zhao J, et al. Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis. *Cell Res* 2015 25(8):981-4.
24. Qu S, Zhong Y, Shang R, Zhang X, Song W, Kjems J, et al. The emerging landscape of circular RNA in life processes. *RNA Biol* 2017 14(8):992-9.
25. Qu S, Yang X, Li X, Wang J, Gao Y, Shang R, et al. Circular RNA: A new star of noncoding RNAs. *Cancer Lett* 2015 365(2):141-8.
26. Zhang Y, Zhang XO, Chen T, Xiang JF, Yin QF, Xing YH, et al. Circular intronic long noncoding RNAs. *Mol Cell* 2013 51(6):792-806.
27. Junn E, Lee KW, Jeong BS, Chan TW, Im JY, Mouradian MM. Repression of alpha-synuclein expression and toxicity by microRNA-7. *Proc Natl Acad Sci U S A* 2009 106(31):13052-7.
28. Pogribny IP, Filkowski JN, Tryndyak VP, Golubov A, Shpyleva SI, Kovalchuk O. Alterations of microRNAs and their targets are associated with acquired resistance of MCF-7 breast cancer cells to cisplatin. *International journal of cancer* 2010 127(8):1785-94.
29. Jiang L, Liu X, Chen Z, Jin Y, Heidbreder CE, Kolokythas A, et al. MicroRNA-7 targets IGF1R (insulin-like growth factor 1 receptor) in tongue squamous cell carcinoma cells. *The Biochemical journal* 2010 432(1):199-205.
30. Kefas B, Godlewski J, Comeau L, Li Y, Abounader R, Hawkinson M, et al. microRNA-7 Inhibits the Epidermal Growth Factor Receptor and the Akt Pathway and Is Down-regulated in Glioblastoma. *Cancer Research* 2008 68(10):3566-72.
31. Reddy SD, Ohshiro K, Rayala SK, Kumar R. MicroRNA-7, a homeobox D10 target, inhibits p21-activated kinase 1 and regulates its functions. *Cancer Research* 2008 68(20):8195-200.
32. Webster RJ, Giles KM, Price KJ, Zhang PM, Mattick JS, Leedman PJ. Regulation of epidermal growth factor receptor signaling in human cancer cells by microRNA-7. *J Biol Chem* 2009 284(9):5731-41.
33. Giles KM, Brown RA, Epis MR, Kalinowski FC, Leedman PJ. miRNA-7-5p inhibits melanoma cell migration and invasion. *Biochem Biophys Res Commun* 2013 430(2):706-10.
34. Tang W, Ji M, He G, Yang L, Niu Z, Jian M, et al. Silencing CDR1as inhibits colorectal cancer progression through regulating microRNA-7. *Onco Targets Ther* 2017 10:2045-56.
35. Fu L, Chen Q, Yao T, Li T, Ying S, Hu Y, et al. Hsa_circ_0005986 inhibits carcinogenesis by acting as a miR-129-5p sponge and is used as a novel biomarker for hepatocellular carcinoma. *Oncotarget* 2017 8(27):43878-43888.
36. Li F, Zhang L, Li W, Deng J, Zheng J, An M, et al. Circular RNA ITCH has inhibitory effect on ESCC by suppressing the Wnt/β-catenin pathway. *Oncotarget* 2015 6(8):6001-13.

37. Huang G, Zhu H, Shi Y, Wu W, Cai H, Chen X. cir-ITCH plays an inhibitory role in colorectal cancer by regulating the Wnt/β-catenin pathway. *PLoS One* 2015 10(6).
38. Yang W, Du WW, Li X, Yee AJ, Yang BB. Foxo3 activity promoted by non-coding effects of circular RNA and Foxo3 pseudogene in the inhibition of tumor growth and angiogenesis. *Oncogene* 2016 35(30):3919-31.
39. Wang K, Long B, Liu F, Wang JX, Liu CY, Zhao B, et al. A circular RNA protects the heart from pathological hypertrophy and heart failure by targeting miR-223. *Eur Heart J* 2016 37(33):2602-11.
40. Li Y, Zheng F, Xiao X, Xie F, Tao D, Huang C, et al. CircHIPK3 sponges miR-558 to suppress heparanase expression in bladder cancer cells. *EMBO Rep* 2017 18:1646-1659.
41. Zheng Q, Bao C, Guo W, Li S, Chen J, Chen B, et al. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. *Nat Commun* 2016 7:11215.
42. Xie H, Ren X, Xin S, Lan X, Lu G, Lin Y, et al. Emerging roles of circRNA_001569 targeting miR-145 in the proliferation and invasion of colorectal cancer. *Oncotarget* 2016 7(18):26680-91.
43. Liang HF, Zhang XZ, Liu BG, Jia GT, Li WL. Circular RNA circ-ABCB10 promotes breast cancer proliferation and progression through sponging miR-1271. *Am J Cancer Res* 2017 7(7):1566-1576.
44. Thomas LF, Saetrom P. Circular RNAs are depleted of polymorphisms at microRNA binding sites. *Bioinformatics* 2014 30(16):2243-6.
45. Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, et al. Exon-intron circular RNAs regulate transcription in the nucleus. *Nat Struct Mol Biol* 2015 22(3):256-64.
46. Chen C, Sarnow P. Initiation of protein synthesis by the eukaryotic translational apparatus on circular RNAs. *Science* 1995 268(5209):415-7.
47. Perriman R, Ares M. Circular mRNA can direct translation of extremely long repeating-sequence proteins in vivo. *RNA* 1998 4(9):1047-54.
48. Wang X, Zhang Y, Huang L, Zhang J, Pan F, Li B, et al. Decreased expression of hsa_circ_001988 in colorectal cancer and its clinical significances. *Int J Clin Exp Pathol* 2015 8(12):16020-5.
49. Hsiao KY, Lin YC, Gupta SK, Chang N, Yen L, Sun HS, et al. Noncoding Effects of Circular RNA CCDC66 Promote Colon Cancer Growth and Metastasis. *Cancer Res* 2017 77(9):2339-2350.
50. Guo JN, Li J, Zhu CL, Feng WT, Shao JX, Wan L, et al. Comprehensive profile of differentially expressed circular RNAs reveals that hsa_circ_0000069 is upregulated and promotes cell proliferation, migration, and invasion in colorectal cancer. *Onco Targets Ther* 2016 9:7451-7458.
51. Zhu M, Xu Y, Chen Y, Yan F. Circular BANP, an upregulated circular RNA that modulates cell proliferation in colorectal cancer. *Biomed Pharmacother* 2017 88:138-44.
52. Ji W, Qiu C, Wang M, Mao N, Wu S, Dai Y. Hsa_circ_0001649: A circular RNA and potential novel biomarker for colorectal cancer. *Biochem Biophys Res Commun* 2018 497(1):122-6.
53. Wang F, Wang J, Cao X, Xu L, Chen L. Hsa_circ_0014717 is downregulated in colorectal cancer and inhibits tumor growth by promoting p16 expression. *Biomed Pharmacother* 2018 98:775-82.

54. Zhang XL, Xu LL, Wang F. Hsa_circ_0020397 regulates colorectal cancer cell viability, apoptosis and invasion by promoting the expression of the miR-138 targets TERT and PD-L1. *Cell Biol Int* 2017 41(9):1056-64.
55. Yong W, Zhuoqi X, Baocheng W, Dongsheng Z, Chuan Z, Yueming S. Hsa_circ_0071589 promotes carcinogenesis via the miR-600/EZH2 axis in colorectal cancer. *Biomed Pharmacother* 2018 102:1188-94.
56. Xu XW, Zheng BA, Hu ZM, Qian ZY, Huang CJ, Liu XQ, et al. Circular RNA hsa_circ_000984 promotes colon cancer growth and metastasis by sponging miR_106b. *Oncotarget* 2017 8(53):91674-91683.
57. Fang G, Ye BL, Hu BR, Ruan XJ, Shi YX. CircRNA_100290 promotes colorectal cancer progression through miR-516b-induced downregulation of FZD4 expression and Wnt/beta-catenin signaling. *Biochem Biophys Res Commun* 2018 504(1):184-9.
58. Zhuo F, Lin H, Chen Z, Huang Z, Hu J. The expression profile and clinical significance of circRNA0003906 in colorectal cancer. *Onco Targets Ther* 2017 10:5187-93.
59. Zeng K, Chen X, Xu M, Liu X, Hu X, Xu T, et al. CircHIPK3 promotes colorectal cancer growth and metastasis by sponging miR-7. *Cell Death Dis* 2018 9(4):417.
60. Weng W, Wei Q, Toden S, Yoshida K, Nagasaka T, Fujiwara T, et al. Circular RNA ciRS-7-A Promising Prognostic Biomarker and a Potential Therapeutic Target in Colorectal Cancer. *Clin Cancer Res* 2017 23(14):3918-28.
61. Yuan Y, Liu W, Zhang Y, Zhang Y, Sun S. CircRNA circ_0026344 as a prognostic biomarker suppresses colorectal cancer progression via microRNA-21 and microRNA-31. *Biochem Biophys Res Commun* 2018 503(2):870-5.
62. Zhang J, Liu H, Zhao P, Zhou H, Mao T. Has_circ_0055625 from circRNA profile increases colon cancer cell growth by sponging miR-106b-5p. *J Cell Biochem* 2019 120(3):3027-37.
63. Jin C, Wang A, Liu L, Wang G, Li G. Hsa_circ_0136666 promotes the proliferation and invasion of colorectal cancer through miR-136/SH2B1 axis. *J Cell Physiol* 2019 234(5):7247-56.
64. Bian L, Zhi X, Ma L, Zhang J, Chen P, Sun S, et al. Hsa_circRNA_103809 regulated the cell proliferation and migration in colorectal cancer via miR-532-3p / FOXO4 axis. *Biochem Biophys Res Commun* 2018 505(2):346-52.
65. Li XN, Wang ZJ, Ye CX, Zhao BC, Li ZL, Yang Y. RNA sequencing reveals the expression profiles of circRNA and indicates that circDDX17 acts as a tumor suppressor in colorectal cancer. *J Exp Clin Cancer Res* 2018 37(1):325.
66. Zhang R, Xu J, Zhao J, Wang X. Silencing of hsa_circ_0007534 suppresses proliferation and induces apoptosis in colorectal cancer cells. *Eur Rev Med Pharmacol Sci* 2018 22:118-126.
67. Xiong W, Ai Y-Q, Li Y-F, Ye Q, Chen Z-T, Qin J-Y, et al. Microarray Analysis of Circular RNA Expression Profile Associated with 5-Fluorouracil-Based Chemoradiation Resistance in Colorectal Cancer Cells. *BioMed Research International* 2017 2017:1-8.

68. The Gene Ontology Consortium. et al. The Gene Ontology Project in 2008. *Nucleic Acids Res* 2008 36:D440-D444.
69. Kanehisa M, Goto S. KEGG: Kyoto Encylopedia of Genes and Genomes. *Nucleic Acids Res* 2000 28(1):27-30.
70. Zeng Y, Xu Y, Shu R, Sun L, Tian Y, Shi C, et al. Altered expression profiles of circular RNA in colorectal cancer tissues from patients with lung metastasis. *International Journal of Molecular Medicine* 2017 40:1818-1828.
71. Chen S, Zhang L, Su Y, Zhang X. Screening potential biomarkers for colorectal cancer based on circular RNA chips. *Oncol Rep* 2018 39(6):2499-512.
72. Spitali P, Aartsma-Rus A. Splice modulating therapies for human disease. *Cell* 2012 148(6):1085-8.