

## Original Paper

# The Emerging Roles of Circular RNAs in Colorectal Cancer

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### Abstract

*Colorectal cancer (CRC) is one of the most common malignant diseases and the fourth common cause for death in the world. Circular RNAs (circRNAs) are a group of non-coding RNAs (ncRNAs), which have a covalent closed loop without 5' and 3' ends. Studies indicated that many circRNAs are differently expressed in CRC cells and tissues. Their different expression levels are significantly correlated with clinicopathological features and overall survival time of CRC patients. Additionally, they regulate CRC cell proliferation, apoptosis, invasion, and migration mainly by acting as competing endogenous RNAs (ceRNAs). In this review, we reviewed CRC-associated circRNAs, described their functions and mechanisms, discussed their potential as diagnostic or prognostic biomarkers and therapeutic targets of CRC.*

### Keywords

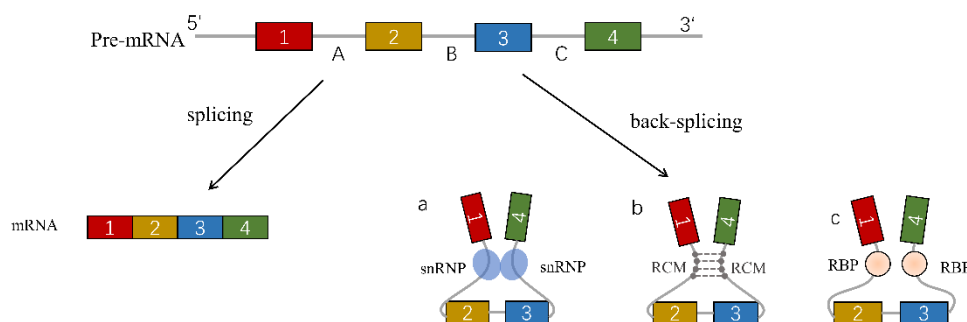
*circRNA, colorectal cancer, competing endogenous RNAs, biomarker*

### 1. Introduction

Colorectal cancer (CRC), usually defined as adenocarcinoma in colon or rectum, is one of the most common malignant diseases and the fourth common cause for death in the world as well (Kolligs, 2016). The occurrence and development of CRC are affected by both genetic and environmental factors (Kolligs, 2016). CRC is often considered as a “lifestyle” disease. Besides, deregulation of oncogenes (*K-ras* and *N-ras*), suppressor genes (*APC*, *DCC*, *TP53*, *SMAD2*, *SMAD4*) and genes related to DNA repair (*MMR* and *MUTYH*) may contribute to the carcinogenesis of CRC (Taborda, Ramirez, & Bernal, 2017).

Recently, high-throughput technology has showed that lots of our genome are transcribed; but many of them cannot be translated to proteins but have functions and are named as non-coding RNAs (ncRNAs) (Lizarbe et al., 2017). NcRNAs can regulate several biological processes like proliferation, differentiation, apoptosis and inflammation. Deregulation of ncRNAs would promote or repress the occurrence and development of tumors including CRC (Lizarbe et al., 2017). There are several ways for the classification of ncRNAs. Based on shape, they can be divided into two groups: linear ncRNAs and circular RNAs (circRNAs). According to their length, linear RNAs can be further divided into two classes: 1) small ncRNAs (sncRNAs), which length is shorter than 200nt. The most attractive type of sncRNAs is microRNAs (miRNAs); 2) long ncRNAs (lncRNAs), which length is up to 200nt. Nowadays, growing studies reveal that miRNAs and lncRNAs are involved in the development, progression, and prognosis of CRC (Shaker et al., 2018; Wang et al., 2015).

CircRNAs have a covalent closed loop without 5' and 3' ends (Tian et al., 2018; Li et al., 2018). According to their component, circRNAs can be divided into three types: exonic circRNAs, intronic circRNAs, and exon-intron circRNAs (Fu et al., 2018). There are three ways of circularization (Figure 1): Spliceosome-dependent circularization, cis-elements driven circularization and protein factors regulation circularization (Liu et al., 2017; Chen, 2016). It has been demonstrated that circRNAs are more stable than linear RNAs, have tissue-specificity, regulate gene expression, and exist in exosomes that can be secreted into body fluids (Salzman et al., 2013; Dou et al., 2016). These features have been renewed interest in cancer research including CRC research.



**Figure 1. CircRNAs are Produced from pre-mRNA by Back-splicing**

There are three ways of circularization:

- Spliceosome-dependent circularization: Two sites of pre-mRNA (one 5' donor site and one 3' acceptor site) with spliceosomal small nuclear ribonucleoproteins (snRNPs) joined together to form a circRNA following the spliceosomal machinery.
- Cis-elements driven circularization: Cis-elements contain reverse complementary sequences (RCM) and repetitive elements like Alu which enriched in introns and promote circularization of exons.
- Protein factors regulation circularization: RNA-binding protein (RBP) including the splicing factor Muscleblind (Mbl) and quaking (QKI) can bind with introns of pre-mRNA and upregulate circRNAs.

Growing studies showed that circRNAs have several functions in biological processes, like regulating gene transcription (Zhang et al., 2013), taking part in selective splicing (Ashwal-Fluss et al., 2014), inhibiting the maturation of RNA (Holdt et al., 2016), participating in translation process (Das, Gorospe, & Panda, 2018), promoting protein-protein interactions (Hinds et al., 1992), and affecting protein localization (Du et al., 2017). But in cancer researches, the relatively more studied topic is that circRNAs act as competing endogenous RNAs (ceRNAs) to affect cancer associated gene expression (Greene et al., 2017; Hsiao et al., 2017; Li et al., 2017).

For CRC, a growing number of circRNAs have been identified deregulated expression in CRC tissues and cell lines. Some circRNAs are involved in CRC progression by regulating cell proliferation, apoptosis, migration, invasion and so on (Table 1). Other circRNAs may be potential diagnostic or prognostic biomarkers of CRC (Table 2).

**Table 1. Deregulated CircRNAs in Colorectal Cancer (CRC) and Their Functions**

No.	CircRNA	Alias	Deregulation	Gene symbol	Genome position	Function	Gene affected*	Ref.
1	hsa_circ_0026782	circITGA7	↓	ITGA7	chr12: 56094682-56094938	proliferation (-); metastasis (-); invasion (-)	↓ miR-370-3p → NF1, ↓ RREB1 → ITGA7 (Ras pathway)	Li et al. (2018)
2	hsa_circ_0026344	—	↓	ACVR1	chr12: 52314542-52317145	proliferation (-); invasion (-); apoptosis (+)	↓ miR-21 and miR-31	Yuan et al. (2018)
3	hsa_circ_0000567	hsa_circ_001983	↓	SETD3	chr14: 99924615-99932150	proliferation (-); migration (-)	—	Wang and Li (2018)
4	hsa_circ_0014717	—	↓	CCCT3	chr1: 156290629-156304709	proliferation (-); colony formation (-); arrest G0/G1 phase (+)	→ P16	Wang et al. (2018)
5	ciRS-7	CDR1as; hsa_circ_0001946	↑	CDR1	chrX: 139865339-139866824	proliferation (+); migration (+); invasion (+); apoptosis (-)	↓ miR-7, →EGFR/RAF1 (EGFR/RAF1/MAPK pathway)	Weng et al. (2017)
6	hsa_circ_0001724	hsa_circ_000984	↑	CDK6	chr7: 92462409-92463134	proliferation (+); migration (+); invasion (+); tumor formation (+)	↓ miR-106b, →CDK6	Xu et al. (2017)
7	hsa_circ_0000069	hsa_circ_001061	↑	STIL	chr1: 47745912-47748131	proliferation (+); migration (+); invasion (+)	—	Guo et al. (2016)
8	—	circ-BANP	↑	BANP	—	proliferation (+)	—	Zhu et al. (2017)
9	—	circCCDC6	↑	CCDC6	—	proliferation (+);	↓ miRNA-33b and	Hsiao

		66		6		migration (+); metastasis (+); proliferation (+); migration (+); invasion (+); apoptosis (-)	miR-93, → MYC	et al. (2017)
10	—	circRNA_ 100290	↑	SLC30 A7	chr1: 101372407– 101379362		↓ miR-516b, →FZD4 →Wnt/b-catenin pathway	Fang et al. (2018)
11	hsa_circ_0020 397	—	↑	DOCK1	chr10: 128768965– 128926028	proliferation (+); viability (+); invasion (+); apoptosis (-)	↓ miR-138, → PD-L1 and TERT	Zhang, Xu and Wang (2017)
12	hsa_circ_0000 284	circHIPK 3; hsa_circ_ 000016	↑	HIPK3	chr11: 33307958– 33309057	proliferation (+); migration (+); invasion (+); apoptosis (-)	cMyb→circHIPK 3, ↓ miR-7, →proto-oncogene s (FAK, IGF1R, EGFR, YY1)	Zeng et al. (2018)
13	hsa_circ_0007 534	—	↑	DDX42	chr17: 61869771– 61877977	proliferation (+); apoptosis (-)	—	Zhang et al. (2018)

\*: ↓, inhibit; →, promote.

**Table 2. CircRNAs as CRC Diagnostic/Prognostic Biomarkers**

No.	CircRNA	Deregu lation	Biomarker	Patients (n)	Detection method	Prognostic/diagn osis value	Refs.
1	hsa_circ_0026782; circITGA7	↓	diagnostic	177	qRT-PCR;	AUC 0.879; sensitivity 0.928; specificity 0.667	Li et al. (2018)
2	hsa_circ_0026344	↓	prognostic	32	qRT-PCR; ISH	—	Yuan et al. (2018)
3	circRNA0003906	↓	diagnostic	122	qRT-PCR	AUC 0.818; sensitivity 0.803; specificity 0.725	Zhuo et al. (2017)
4	Hsa_circ_0001649; hsa_circ_001599	↓	diagnostic	64	qRT-PCR	AUC 0.857; sensitivity 0.828; specificity 0.781	Ji et al. (2018)
5	hsa_circ_0000567	↓	diagnostic	102	qRT-PCR	AUC 0.865; sensitivity 0.828; specificity 0.781	Wang and Li (2018)
6	hsa_circ_0014717	↓	diagnostic prognostic	46	qRT-PCR	AUC 0.683	Wang et al. (2018)
7	hsa_circ_0001451; hsa_circ_001988	↓	diagnostic	31	qRT-PCR	AUC 0.788; sensitivity 0.68;	Wang et al. (2015)

						specificity 0.73	
8	circCCDC66	↑	diagnostic; prognostic	229	qRT-PCR	AUC 0.884	Hsiao et al. (2017)
9	ciRS-7; CDR1as; hsa_circ_0001946	↑	prognostic	362	qRT-PCR	—	Weng et al. (2017)
10	circRNA_100290	↑	prognostic	44	qRT-PCR ; ISH	—	Fang et al. (2018)
11	hsa_circ_0000284; circHIPK3; hsa_circ_000016	↑	prognostic	178	qRT-PCR	—	Zeng et al. (2018)

qRT-PCR, quantitative reverse transcription-polymerase chain reaction; AUC, area under ROC curve; ISH, In situ hybridization;

## 2. Down Regulated CircRNAs in CRC

### CircITGA7

The integrin subunit alpha 7 (ITGA7) pre-mRNA can produce two types of RNA: ITGA7 mRNA and circITGA7. CircITGA7, also known as hsa\_circ\_0026782, is produced from exon 4 of ITGA7 pre-mRNA via back-splicing (Li et al., 2018). ITGA7 is one of the extracellular matrix binding proteins. Its gene has been reported to be a tumor-suppressor gene in prostate cancer, leiomyosarcoma and malignant pleural mesothelioma (Li et al., 2018; Tan et al., 2013; Laszlo et al., 2015). Recently, circITGA7 and ITGA7 were both found to down-regulated in CRC cell lines and tissues (Li et al., 2018). In the meanwhile, lower circITGA7 expression was significantly associated with tumor size, lymph node metastasis, metastasis and TNM stage (Li et al., 2018). Overexpression of circITGA7 can restrain CRC cell proliferation, migration and arrest cells in G0/G1 phase (Li et al., 2018). In sharp contrast, the knockdown of circITGA7 has opposite results. Further study showed that circITGA7 could modulate Ras signaling pathway. Mechanism study showed that circITGA7 bound with miR-370-3p and then increased expression of neurofibromin 1 (NF1), one Ras pathway inhibitor, resulting in suppressing Ras signaling pathway (Li et al., 2018). Besides, circITGA7 can upregulate ITGA7 expression by inhibiting Ras responsive element binding protein 1 (RREB1) in CRC (Li et al., 2018). These data indicated that circITGA7 might be a new target for CRC treatment.

### Circ\_0026344

Nowadays, miR-31 and miR-21 are reported to be oncogenic miRNAs in multiple cancers including in CRC (Saraggi et al., 2018; Zadeh, Ranji, & Motamed, 2015; Pennelli et al., 2015; Cui, Zhang, Ye, Zheng, Song, Deng, ... Guo, 2013; Zhang, Guo, Li, Xiao, Miao, Jiang, & Zhuo, 2010; Guo, Miao, Xiao, Huan, Jiang, Meng, & Wang, 2009; Zheng et al., 2015; Ning et al., 2014; Gao et al., 2014; Zheng, Cui, Sun, Zhou, Yuan, Huo, ... Guo, 2011-2012; Wu et al., 2017). Circ\_0026344, a significantly

down-regulated circRNA in CRC tissues, was correlated with advanced stages, metastasis and poor prognosis in CRC patients (Yuan et al., 2018). Circ\_0026344 was further found acting as a miRNA sponge of miR-31 and miR-21 in CRC (Yuan et al., 2018). Afterwards, overexpression of circ\_0026344 significantly suppressed CRC cell proliferation, enhanced apoptosis while significantly decreased invasion *in vitro* and inhibited CRC growth *in vivo* (Yuan et al., 2018). Interestingly, overexpression of either miR-21 or miR-31 could only reverse part effects of up-regulated circ\_0026344 on proliferation, invasion and apoptosis, but both miR-31 and miR-21 ectopic expression at the same time reversed all the effects on CRC cells (Yuan et al., 2018). These results indicate that circ\_0026344 function by binding both miR-31 and miR-21 in CRC cells. In a word, circ\_0026344 may play a tumor-suppressive role in CRC through sponge both miR-31 and miR-21.

#### CircRNA0003906

CircRNA0003906 is a 14317 nt circRNA. Its gene is located at chr6:29989443–30003760. Zhuo et al. reported that circRNA0003906 was significantly downregulated in several CRC cell lines and CRC tissues (Zhuo et al., 2017). Low expression of circRNA0003906 was associated with poor differentiation and lymphatic node metastasis. The area under ROC curve (AUC) was 0.818, with the specificity and sensitivity of 0.725 and 0.803, respectively (Zhuo et al., 2017). These indicate that circRNA0003906 may be a biomarker in the diagnosis of CRC.

#### Hsa\_circ\_0001649

The circRNA hsa\_circ\_0001649 is generated from Snf2 histone linker phd ring helicase (*SHPRH*) gene. It has been reported that hsa\_circ\_0001649 is a potential novel prognosis biomarker and therapeutic target in hepatocellular carcinoma (HCC), pancreatic ductal adenocarcinoma, cholangiocarcinoma and glioma (Xu et al., 2018; Wang et al., 2018; Qin et al., 2016; Jiang et al., 2018). For CRC, qRT-PCR results showed that hsa\_circ\_0001649 expression was significantly down in CRC tissues and cell lines (Ji et al., 2018). What's more, Ji et al. detected the expression of hsa\_circ\_0001649 in serum from before and after surgery CRC patients, and found that hsa\_circ\_0001649 levels after surgery were increased (Ji et al., 2018). Besides, the AUC was 0.857 with specificity 0.781 and sensitivity 0.828 (Ji et al., 2018). These results showed that hsa\_circ\_0001649 could be a new biomarker for CRC.

#### Hsa\_circ\_0000567

Hsa\_circ\_0000567, a circRNA with 683 nt, is generated from exon 2-6 of SET domain-containing 3 (*SETD3*) pre-mRNA. Firstly, RNase R and actinomycin D treatments proved that hsa\_circ\_0000567 was more stable than linear mRNA (Wang & Li, 2018). Then, hsa\_circ\_0000567 was verified to down-regulate in CRC tissues, and was negatively correlated with tumor size, distant metastasis, lymph node metastasis and TNM stage (Wang & Li, 2018). The consistent results from *in vitro* experiments demonstrated that hsa\_circ\_0000567 played a suppressor role in CRC (Wang & Li, 2018). Furthermore, loss-of-function assay showed that hsa\_circ\_0000567 suppressed proliferation and migration of CRC cells (Wang & Li, 2018). The AUC was 0.865, with specificity 0.764, sensitivity 0.833, and Youden's index 0.598 (Wang & Li, 2018). Since that some circRNAs stably exist in plasma or exosomes (Li et al.,

(2015), Wang et al. observed whether hsa\_circ\_0000567 in blood exosome from CRC patients can be detected. They found that hsa\_circ\_0000567 levels in blood exosome from CRC patients were lower than those from normal subjects (Wang & Li, 2018). These findings indicated that circulating hsa\_circ\_0000567 may be used more conveniently for CRC screening.

#### Hsa\_circ\_0014717

Hsa\_circ\_0014717 of 516 nt in length is transcribed from chr1:156290629-156304709. Its lower expression was closely associated with distant metastasis, TNM stage and poor prognosis (Wang et al., 2018). CCK-8 assay, colony formation assay and flow cytometry were demonstrated that overexpression of hsa\_circ\_0014717 could weaken cell viability and colony formation number as well as arrest cell at G0/G1 phase (Wang et al., 2018). The phenomenon of slower tumor growth and lower tumor weight in nude mice indicated that hsa\_circ\_0014717 might be a tumor suppressor in CRC (Wang et al., 2018). Further study showed that hsa\_circ\_0014717 could enhance the expression of P16 (Wang et al., 2018). Previous studies showed that P16 is one of cyclin-dependent protein kinase inhibitors (Witkiewicz et al., 2011; Rayess, Wang, & Srivatsan, 2012). The rescue assay was performed to reveal that knockdown of P16 could reverse the inhibition function of hsa\_circ\_0014717 in CRC cells (Wang et al., 2018). These data suggested that hsa\_circ\_0014717 may be a prognosis marker for CRC diagnosis and a promising target for CRC therapy.

### 3. Up Regulated CircRNAs in CRC

#### Circular RNA CCDC66

CircRNACCDC66 (circRNACCDC\_66\_10\_8) was found upregulated in CRC cell lines, the pre-cancerous polyp tissues and CRC tissues (Hsiao et al., 2017). The high expression of circRNACCDC66, but not linear transcript of CCDC66, was correlated with poor prognosis of CRC (Hsiao et al., 2017). The AUC of circRNACCDC66 was 0.8843, indicating that circRNACCDC66 is a good biomarker for CRC prognosis and diagnosis (Hsiao et al., 2017). Further study demonstrated that knockdown of circRNACCDC66 by siRNA successfully reduced CRC cell proliferation, migration and invasion (Hsiao et al., 2017). Besides, knockdown circRNACCDC66 inhibited tumor growth and cancer invasion in xenograft and orthotopic mouse models, respectively. Gene set enrichment analysis (GSEA) showed that predicted circRNACCDC66-regulated genes were enriched in tumor transcriptome (Hsiao et al., 2017). The levels of the targeted genes such as enhancer of zeste 2 polycomb repressive complex 2 subunit (*EZH2*), DNA methyltransferase 3 beta (*DNMT3B*), Yes associated protein 1 (*YAPI*) and MYC proto-oncogene (*Myc*), were significantly downregulated by circCCDC66 knockdown or upregulated by overexpression of circCCDC66 (Hsiao et al., 2017). In addition, luciferase assays further demonstrated that circRNACCDC66 may protect oncogenic target genes by sponging a number of miRNAs in CRC (Hsiao et al., 2017). These findings indicated that circRNACCDC66 may be a useful biomarker for CRC diagnosis and prognosis and may emphasize a novel oncogene function of circRNAs in CRC.

### CiRS-7

CiRS-7 (also named as cerebellar degeneration-related protein 1 antisense RNA, hsa\_circ\_0001946 or Cdr1as) is a super star in the circRNA field. It is a ceRNA by acting as a miRNA sponge with 73 binding sites of miR-7 to efficiently influence miR-7 activities in many human diseases (Weng et al., 2017; Hansen et al., 2013; Zhang et al., 2017). To date, ciRS-7 has been declared to express extensively in astrocytoma, neuroblastomas, gastric cancer, esophageal squamous cell carcinoma, liver cancer, and lung carcinomas (Weng et al., 2017; Su et al., 2018; Sang et al., 2018; Pan, 2018; Xu et al., 2017; Li et al., 2017). Thus, it is an important regulator in cancers. Recently, Weng et al. discovered that upregulated ciRS-7 in CRC tissues was significantly correlated with some clinicopathological features such as tumor size, lymph node metastasis, distant metastasis, and TNM stages (Weng et al., 2017). Besides, the higher expression of ciRS-7 was associated with poor overall survival. *In vitro* experiments showed that ciRS-7 overexpression notably reduced miR-7 function in CRC cells, which was consistent with *in vivo* experiments (Weng et al., 2017). Further study found that ciRS-7 weakened the inhibitory effect of miR-7 on Raf-1 proto-oncogene (*RAF1*) and epidermal growth factor receptor (*EGFR*) (Weng et al., 2017). In addition, the related expression between ciRS-7, miR-7 and EGFR/RAF1 in CRC tissues indicated that ciRS-7 may play a crucial role in EGFR/RAF1 pathways via sponging miR-7 (Weng et al., 2017). These results showed that ciRS-7 may be served as a therapeutic target in CRC.

### Hsa\_circ\_000984

Hsa\_circ\_000984 is a circRNA transcribed from chromosomal region 7q (92462409-92463134), encoded by the cyclin-dependent kinase 6 (*CDK6*) gene. CDK6, involving in progression of cell cycle, takes part in the development and prognosis of multiple cancers (Tadesse et al., 2015). Xu et al. found that hsa\_circ\_000984 was highly expressed in CRC tissues and cell lines, correlated with advanced TNM stage in clinicopathological features analysis (Xu et al., 2017). CCK-8 assay and colony formation assay showed that knockdown of hsa\_circ\_000984 could obviously reduce cell growth rate and the numbers of colony formation mainly due to the disorder of cell cycle (Xu et al., 2017). Meanwhile, knockdown of hsa\_circ\_000984 could abate the invasion and migration both in CRC cell lines and CRC xenograft model (Xu et al., 2017). As for molecular mechanisms, further studies demonstrated that hsa\_circ\_000984 can be served as a miRNA sponge of miR-106b to regulate downstream target gene *CDK6* (Xu et al., 2017). These suggested that hsa\_circ\_000984 may play a significant role in CRC progression and therapeutic strategy.

### Hsa\_circ\_0000069

Hsa\_circ\_0000069 is an up-regulated circRNA selected from a circRNA microarray and later validated in both CRC cell lines and tissues (Guo et al., 2016). The expression of hsa\_circ\_0000069 was related to CRC patients' TNM stages (Guo et al., 2016). In CRC cells, knockdown of hsa\_circ\_0000069 distinctly inhibited cell proliferation, invasion and migration (Guo et al., 2016). Furthermore, flow cytometry analysis revealed that knockdown of hsa\_circ\_0000069 arrested cells at G0/G1 phase (Guo



et al., 2016). These suggested that hsa\_circ\_0000069 may be involved in CRC progression, and may be a therapeutic target for CRC treatment.

#### Circ-BANP

Circ-BANP, a circular RNA screened by a circRNA microarray of CRC with remarkably upregulated expression, later was validated by both RT-PCR and fluorescence in situ hybridization in CRC tissues and cell lines (Zhu et al., 2017). It was also reported to promote lung cancer growth, migration and invasion via miR-503/LARP1 signaling recently (Han et al., 2018). However, there is no correlation between circ-BANP expression and clinicopathologic features of patients with CRC (Zhu et al., 2017). Simultaneously, Zhu et al. used siRNA to explore the biological function of circ-BANP and found that knockdown of circ-BANP could suppress the CRC cell proliferation, colony formation and expression of p-Akt (Zhu et al., 2017). These suggested that circ-BANP could implicate in the proliferation of CRC cells by PI3K/Akt pathway.

#### CircRNA\_100290

Another up-regulated circRNA in CRC tissues and cell lines is circRNA\_100290 that was also validated by qRT-PCR and ISH (Fang et al., 2018). Further analysis showed that higher level of circRNA\_100290 expression was correlated with metastasis leading to poor prognosis in CRC (Fang et al., 2018). Knockdown circRNA\_100290 could inhibit proliferation, migration and invasion but increase the cellular apoptosis of CRC cells (Fang et al., 2018). Furthermore, through bioinformatics analysis, qRT-PCR, luciferase reporter assay, circRNA\_100290 was found direct interact with miR-516b to target frizzled class receptor 4 (FZD4) and then active cyclin D1 (CCND1), cyclin D1 (CCND2), MYC, SRY-box 4 (SOX4) and transcription factor 7 (TCF7) (target genes of Wnt/b-catenin pathway) (Fang et al., 2018). Then, rescue assays verified the role of circRNA\_100290/miR-516b/FZD4/Wnt/b-catenin pathway in CRC (Fang et al., 2018). These data revealed that circRNA\_100290 may be a potential biomarker and a hopeful treatment target for CRC.

#### Has\_circ\_0020397

Hsa\_circ\_0020397, generated from dedicator of cytokinesis 1 (DOCK1), was observed upregulated in CRC cell lines (Zhang, Xu, & Wang, 2017). It was predicted to have several binding sites with miR-138 by using common bioinformatic algorithms (miRanda and RNAhybrid 2.2) (Zhang, Xu, & Wang, 2017). After that, qRT-PCR analysis and dual-luciferase reporter assay got the consistent conclusion as the prediction (Zhang, Xu, & Wang, 2017). Further study showed that hsa\_circ\_0020397 could repress the function of miR-138, leading to increasing the expression of miR-138 target gene telomerase reverse transcriptase (*TERT*) and programmed death-ligand 1 (*PD-L1*) (Zhang, Xu, & Wang, 2017). Previous studies showed that TERT activated telomerase, which maintain telomere length, offer cancer cells unlimited replication capacity (Liu, Yuan, & Xu, 2016). Besides, overexpression of hsa\_circ\_0020397 could promote CRC cells viability, invasion but inhibit apoptosis by increasing the expression of miR-138 target genes (*TERT* and *PD-L1*) (Zhang, Xu, & Wang, 2017). These suggested that hsa\_circ\_0020397 may serve as an oncogene in CRC initiation and progression.

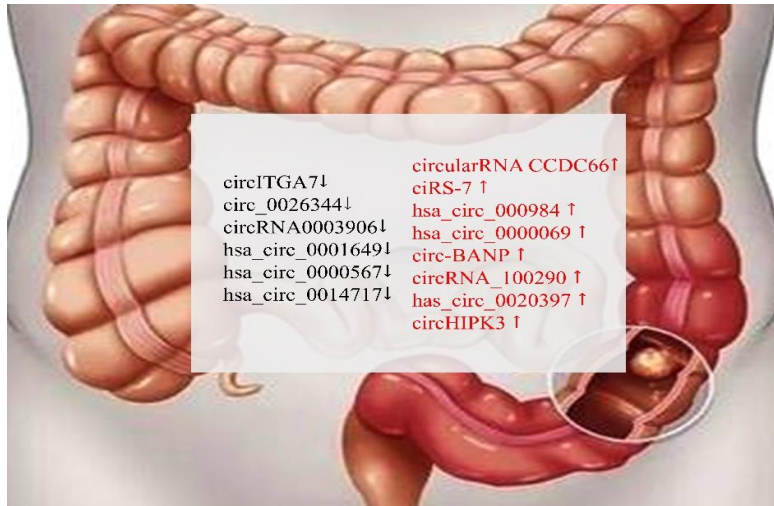
### CircHIPK3

CircHIPK3 (hsa\_circ\_0000284), generated from the homeodomain interacting protein kinase 3 (*HIPK3*) gene Exon2, plays as oncogene or anti-tumor roles in various cancers. CircHIPK3 may be an oncogene in glioma tissues, nasopharyngeal carcinoma, epithelial ovarian cancer, hepatocellular carcinoma, and gastric cancer (Jin et al., 2018; Liu et al., 2018; Ke et al., 2018; Cheng et al., 2018; Chen et al., 2018). However, circHIPK3 was down-regulated in osteosarcoma (OS) tissues, and overexpression of circHIPK3 could restrain OS cell proliferation, invasion and migration (Xiao-Long, Kun-Peng, & Chun-Lin, 2018). Besides, Li et al. showed that circHIPK3 may sponge miR-558 to suppress the expression of heparanase (HPSE) resulted in inhibiting bladder cancer growth and metastasis (Li et al., 2017).

CircHIPK3 was found to up-regulate in CRC tissues and cell lines (Zeng et al., 2018). Its expression levels were significantly correlated with tumor size, lymph metastasis, distant metastasis, and TNM stage (Zeng et al., 2018). Cox multivariate survival analysis showed that high circHIPK3 expression was an independent prognostic factor for CRC patients (Zeng et al., 2018). Further *in vivo* study showed that circHIPK3 knockdown significantly inhibited the number of colony, cell proliferation but promoted the number of apoptotic cells to retard the CRC progression (Zeng et al., 2018). CircNet database, qRT-PCR, luciferase reporter assays combined with FISH demonstrated that circHIPK3 may sponge miR-7 in CRC (Zeng et al., 2018). More recently, miR-7, a well-known tumor suppressor, was reported to interact with ciRS-7 which has 73 binding sites to regulate CRC progression (Weng et al., 2017). CircHIPK3 overexpression significantly reversed suppression of aggressive phenotypes induced by miR-7 in CRC cells and promotion the expression of miR-7 target oncogene (YY1 transcription factor (*YY1*), EGFR, protein tyrosine kinase 2 (*FAK*) and insulin like growth factor 1 receptor (*IGF1R*)) via acting as a miRNA sponge (Zeng et al., 2018). Moreover, xenograft mouse models and metastasis models demonstrated that silencing of circHIPK3 with overexpression of miR-7 may play an additive inhibitory effect on growth and metastasis in CRC (Zeng et al., 2018). These results suggested that circHIPK3/miR-7 axis may be a treatment target for CRC.

#### 4. Conclusion and Future Perspectives

Growing reports showed that some circRNAs are differently expressed in CRC cells and tissues (Figure 2). Their different expression levels are significantly correlated with clinicopathological features and overall survival time of CRC patients. Some of them can take part in CRC progression through regulating CRC cell proliferation, migration, invasion, and apoptosis mainly by acting as miRNA sponge. Because circRNAs are more stable, abundant, tissue-specific, exist in exosomes, they are potential diagnostic or prognostic biomarkers and therapeutic targets for CRC.



**Figure 2. Deregulated CircRNAs in Colorectal Cancer (CRC). Some CircRNAs are Differently Expressed in CRC**

(Blank, down-regulated; red, up-regulated.)

Compared with proteins, mRNAs, miRNAs and lncRNAs, there are a lot of unknown space of CRC-related circRNAs that we should study. So far, among these reported CRC-associated circRNAs, only a few functional circRNAs have been explored. Scientists studied circRNAs mainly through RNA sequencing or microarray, focused on validating the function of miRNAs sponge. Many questions about CRC-associated circRNAs' biogenesis, degradation, function, and molecular mechanisms are waiting to be answered.

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