Review Article

Circular RNAs: a novel tool in the development of digestive system biomarker

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Abstract: As a class of endogenous non-coding RNA expressed by regulatory genes, circular RNAs (circRNAs) have attracted widespread attention due to their unique closed-loop structure. CircRNAs can not only exist stably in different tissues and organs, but also widely exist in blood, exocrine, lymph, saliva and other body fluids. They are involved in the regulation of embryonic development, aging and many pathophysiological processes related to various diseases. Recently, the diagnosis and treatment of digestive diseases have made great advances due to the rapid development of fundamental research. However, most diseases are still lack of accurate early diagnosis and miss the best treatment opportunity, the concomitant complications seriously endanger human health and even lead to death. With the circRNAs becoming a hot research spot, the roles of circRNAs in digestive diseases have also been confirmed and recognized. This review mainly introduces the biological characteristics of circRNAs and their association with digestive diseases, expecting to contribute to the future research on digestive diseases and the exploration of circRNAs function.

Keywords: Circular RNAs (circRNAs); digestive disease; diagnosis

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Introduction

As an important member of the non-coding RNA family, circular RNAs (circRNAs), which were formed by special splicing mechanism with closed circular structure, widely exist in various cells to regulate gene expression (1). Since the first observation of circRNAs in the plant-infected viroid by electron microscopy in 1976, circRNAs were once considered to be a class of low-abundance RNA molecules formed by incorrect splicing of exon transcripts (2). In 2012, a great deal of circRNAs was found to exist in archaea and their biological functions were confirmed, making the research of circRNAs in full swing (3). With the widespread application of RNA sequencing (RNA-seq) technology and the rapid development of biophysical techniques, a substantial amount of circRNAs in a variety of organisms are exposed to the research field, and it has been confirmed that the transcription of many human exons can be non-linearly reversed spliced or genetically rearranged to form circRNAs, which account for a considerable proportion of all spliced transcripts (4,5). Benefit from its tissue specificity, disease specificity, time series specificity and high stability, circRNAs have obvious advantages as a biomarker for clinical diseases, and naturally become a hot research molecule in the field of life sciences and medicine. In the interest of investigating the potential of circRNAs as a biomarker, it is necessary to clarify its production mechanism and structural characteristics firstly.

Research method of circRNAs

The place of circRNAs formation is nucleus, and the mature circRNAs are transported to the cytoplasm to exert their regulatory effects. Aggregates of exon-linked complexes during the process of splicing may participate in the regulation of this nuclear mechanism (6,7). The identification and quantitative analysis of circRNAs are the cornerstone and premise of all researches. Although the in-depth studies of circRNAs are still in its infancy, there are many relatively mature experimental techniques, prediction methods, and circRNAs databases are worthy of promotion in the follow-up study. Detection techniques for circRNAs expression in common use include: the circRNAs chip analysis technology, which can be used to analyze differentially expressed circRNAs in tissue samples for preliminary screening work; the quantitative real-time PCR analysis, which extracts tissue/cell total RNA, performs reverse transcription by random primer, and designs circRNAs-specific primers to expand and quantify the template, this technique is widely used in the detection of circRNAs expression; the RNA-fluorescence in situ hybridization (RNA-FISH), which is a technique that hybridizes a fluorescently labeled specific probe to a specific circRNA, by observing the fluorescent signals, it can not only determine the expression of circRNAs, but even the localization analysis of circRNAs can be achieved at the subcellular levels; the northern blot, which is used to degrade the majority of the linear RNA in total RNA while retain circRNAs by ribonuclease R (RNase R), specific for radiolabeled circRNAs after agarose gel electrophoresis probe hybridization, quantitative or qualitative analysis of specific circRNAs by hybridization of DNA and RNA molecules; the biotin-coupled circRNA capture, which also detects circRNAs by biotin-labeled probes, is more sensitive than northern technology and is more suitable for the detection of low-abundance circRNAs; the high-throughput sequencing, also known as "the next-generation" sequencing technology, can perform a variety of bioinformatics analysis work including circRNAs identification, expression calculation, annotation analysis, distribution statistics, conservative analysis of circRNAs and analysis of interacting miRNA, which is useful for deep excavation of circRNAs functions and mechanisms (8-13).

With the rapid development of research techniques and experimental methods, the types and quantities of circRNAs are presenting an obvious increasing trend. The rational organization and utilization of existing research results are the driving force for subsequent research. As a perfect and complementary research tool for circRNAs, the establishment of more and more circRNAs databases has greatly facilitated the work of researchers and accelerated the deep mining of circRNAs to a certain extent. The commonly used circRNAs online databases currently include: circBase, which integrates published circRNAs data, mainly including circRNAs information from these six species: human, mouse, Caenorhabditis elegans (C. elegans), drosophila melanogaster, spearfish and coelacanth; circRNABase, which builds the networks of interactions between miRNAs and circRNAs as well as circRNAs and RNA-binding proteins (RBPs); Circ2Traits, potentially associated with human disease or traits, is used primarily to predict the interaction between miRNAs and human protein-coding genes, long non-coding genes, and circRNAs; circNet, using RNA-seq sequencing for new circRNAs prediction, genome annotation and calculation of circRNAs expression; deepBase v2.0, which collects more than 150,000 circRNAs genes (human, mouse, fruit fly, nematode, etc.), and constructs the most comprehensive expression map of circRNAs (14-18).

Functional mechanisms and properties of circRNAs

CircRNAs are mainly found in the cytoplasm and are also identified in different organisms (3). At present, 1,903 circRNAs have been found in mice, 724 circRNAs have been found in nematodes, and 1,950 circRNAs have been found in human leukocytes. CircRNAs exist in various types of extracellular fluids such as saliva, blood and urine. Its expression level is more than 10 times higher than that of the corresponding linear mRNAs. Thus, it can be seen that circRNAs are widely and abundantly present in nature (4,19). Different from the single formation mode of other endogenous RNAs, the production mechanisms of circRNAs are diverse, among which the two main mechanisms are lariat-driven circularization and intron-pairing-driven circularization (20). The former promotes the covalently bonding of exon 3'-end splicing donor and the 5'-end splicing acceptor to form a closed-loop structure mainly by “missing splicing” and “exon hopping” and then excision of the intron. While the latter used the group I and group II introns to urge the covalently bonding of RNA precursor to form a closed-loop structure and then resected intron (8,21,22). Briefly speaking, circRNAs, which are widely distributed in eukaryotes, facilitate the

formation of circRNAs in the form of reverse head-to-tail connections in the exon sequence of genes mainly rely on a reversed alternative splicing. Because of these special formation mechanisms, circRNAs have the following properties: (I) the closed loop structure is more stable than the linear structure, thus circRNAs are not easily degraded by the accounting exonuclease and can be stably present in the cytoplasm; (II) highly conserved in different species; (III) they may have rapid evolutionary changes that can be specifically expressed in different tissues and at different developmental stages (4,19,23,24). Based on the development of molecular biology methods, high-throughput sequencing, bioinformatics and the establishment of various circRNAs databases, functions of circRNAs have been gradually unearthed. MiRNAs are a class of RNAs with a length of about 21nt, which can directly bind to mRNA targets through complementary base pairing, thereby inhibiting mRNA translation (25,26). Evidence has shown that circRNAs can function as miRNAs sponges by binding miRNAs to miRNA-target miRNAs networks through their own miRNA response element (MRE) (27); they have obvious regulatory effects on alternative splicing and transcription; they can be combined with RBPs or ribonucleoprotein complexes to exert biological functions; part of them can have the function of protein translation through m6A methylation (23,28-31). There are a large number of circRNAs in exosomes, 90% of which are composed of exons. They are highly stable and are not easily cleaved by exonucleases. Their functions are related to miRNAs and are considered to have the possibility of new markers for cancer detection. A great quantity of circRNAs have been proved to be higher and more stable in blood than in tissues, confirming that circRNAs in circulating blood can be a potential marker for the future disease diagnosis (32). Numerous properties and functions endow circRNAs with great potential as disease markers, which are being explored and excavated.

CircRNAs in digestive diseases

CircRNAs and liver disease

As the largest substantial organ and digestive gland in the human abdominal cavity, liver is the central station of the body's metabolism, which plays an extremely important and complex physiological functions. Due to the large volume, brittle texture and poor tolerance to hypoxia of the liver, various pathogenic factors including biological factors such as hepatitis virus, bacteria and parasites, physical and chemical factors such as drugs, industrial reagents, ethanol, external force factors such as impact, extrusion and genetic immune factors directly or indirectly stimulate to induce different degrees of damage to liver cells, and eventually develop into chronic liver diseases such as liver fibrosis, liver cirrhosis and even liver cancer (33,34). Epidemiological survey results show that there are about 240 million patients with chronic hepatitis B (CHB) and 160 million patients with chronic hepatitis C worldwide. About 25% of the general population suffer from nonalcoholic fatty liver disease, about 4.5% to 9.5% have cirrhosis, and about 770,000 people die of cirrhosis every year (35-37). Therefore, liver damage is the common pathological basis of various liver diseases. The global liver disease has the characteristics of high incidence and wide influence, which seriously endangers human health and has a restrictive effect on social and economic development. Effective early prediction and prevention methods have become an important issue of common concern to the medical community all over the world (38,39). CircRNAs have been linked to liver disease.

CircRNAs and viral hepatitis

Viral hepatitis is a type of high-infectious disease caused by a variety of different hepatitis viruses, mainly characterized by liver damage, with widespread epidemics and serious contagiousness. Up to now, hepatitis viruses that have been identified to have a clear pathogenicity including hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis E virus (HEV), in which HBV and HCV are the most common. The infection rate of HBV in China is nearly 60%, there are about 125 million people carrying HBV, of which the number of HBV patients is as high as 30 million; at the same time, the global infection rate of HCV is about 3%, with an estimated 170 million virus-infected patients and about 4 million new cases each year. Viral hepatitis is the chief culprit of many chronic liver diseases. It can develop into liver fibrosis and cirrhosis, which is a serious hazard to human health. Therefore, it is one of the major public health problems in the world (40-44).

In a recent study, a total of 24,708 circRNAs was detected in 3 normal human liver tissues and 6 CHB patients' liver tissues. Among them, 22,843 kinds of circRNAs were found in normal human liver, and 19,821 kinds of circRNAs were found in CHB patients' liver tissues. Simultaneously, compared with normal people, the expression of partial

circRNAs in liver tissue of CHB patients was abnormal: 72 circRNAs expression increased while 95 circRNAs expression decreased, among which hsa_circ_0005389, hsa_circ_0000038 and hsa_circ_0000650 attracted the attention of researchers, and thus established the following four possible regulatory pathways: hsa_circ_0000650-miR-210-5p-HBV, hsa_circ_0005389-miR-4505/miR-6752-5p/miR-5787-IRF7, hsa_circ_0000650-miR-6873-3p-TGFβ2 and hsa_circ_0000038-miR-370/miR-939-HBV (45). Early diagnosis and treatment of HCV are also the focus of viral hepatitis research. HCV is a hepatocyte-specific virus, previous studies have shown that this hepatocyte characteristic of HCV mainly depends on miR-122 in human body. It is well known that HCV benefits from the binding sites of two miR-122s present at the 5' end of the viral genome, and miR-122 can promote the replication together with translation of HCV viral and enhance viral activity, therefore, miR-122 has always been one of the main targets for the treatment of HCV. With further research, artificially designed circRNA sequences containing miR-122 binding sites were used to synthesize artificial circRNA to adsorb miR-122 in hepatitis C cells, thereby blocking the translation of HCV proteins and opening the door to the connection of circRNAs and disease treatment (46).

CircRNAs and liver fibrosis

Liver fibrosis is a common response of the liver to chronic liver injury caused by different etiologies. It is characterized by excessive production and deposition of various components of the extracellular matrix（ECM）of the liver, resulting in imbalance of fibrosis and degradation leading to liver fibrosis. Further development will lead to liver lobule reconstruction, pseudolobuli and nodules formation, which will eventually form cirrhosis. Liver fibrosis is a dynamic and reversible process, so early diagnosis and prevention are of vital importance (47). Activation and proliferation of hepatic stellate cells（HSC）are still considered to be the central event in the occurrence and development of liver fibrosis. When quiescent HSC are activated, they will transform into myofibroblasts, synthesize and secrete ECM together with metalloproteinase inhibitors, which decreases the activity of proteases such as interstitial collagenase, reduced ECM degradation, and finally resulted in the deposition of ECM. Therefore, degrading fibrosis can be achieved by effectively inhibiting HSC activation or promoting its apoptosis (48-52). Fourteen circRNAs were detected to have increased expression in HSC activation models both *in vivo* and *in vitro*, while 55 circRNAs were detected to be reduced in expression, among which mmu_circ_33594, mmu_circ_35216 and mmu_circ_34116 may be closely related to the activation of HSC. Assisted by bioinformatics analysis software and literature review, the "mmu_circ_34116/miR-22-3P/BMP7" signal axis was found to be involved in the activation process of HSC, and mmu_circ_34116 may have a protective effect on HSC activation (53). As a major complication of the radiation therapy for hepatocellular carcinoma (HCC), radiation-induced liver fibrosis (RILF) imposes a heavy burden on the treatment and prognosis. Irradiation can induce an abnormal increase of transforming growth factor β (TGF-β) and stimulate the transition of HSC from resting state to activated state. The abnormal expressions of circRNAs in HSC after irradiation were suspected to be closely related to hepatocyte transcription, proliferation and cycle progression. The hsa_circ_0071410/miR-9-5p regulatory pathway has also been confirmed to be involved in the process of RILF: down-regulated hsa_circ_0071410 could promote miR-9-5p expression, thereby protecting radiation-induced HSC activation (54). Numerous circRNAs have abnormal expression and are involved in the progression of liver cancer. For example, hsa_circ_0001649, hsa_circ_0005986, hsa_circ_0004018, circ-ITCH showed low expression in liver cancer, while hsa_circ_0005075, hsa_circ_100338, hsa_circ_101368, hsa_circ_000302, hsa_circ_0015756, hsa_circ_00000791, hsa_circ_103847 were highly expressed in liver cancer. They are all entrusted with the hope of clearing the mechanism of liver cancer (55-59). As the pre-stage and necessary way for the development of liver cancer, liver fibrosis may have a certain relationship with these circRNAs involved in the regulation of liver cancer progression, and need to be further explored.

CircRNAs and non-alcoholic fatty liver disease (NAFLD)

NAFLD is a clinical pathological syndrome unrelated to alcohol. The hepatic lobule is the main lesion of NAFLD, with hepatic steatosis and fat accumulation as the main pathological features. The global incidence of NAFLD is increasing year by year. The initial stage of the disease has no obvious clinical symptoms in general, however, without prevention and treatment, it could progress to nonalcoholic steatohepatitis (NASH) with the accumulation of liver inflammation and fibrosis, and even causes serious liver dysfunction, cirrhosis, HCC and other serious consequences, which brings tremendous harm to human

being (60). The identification of the pathogenesis of NASH and the molecular mechanism from NAFLD to NASH are of great significance for the prevention and treatment of diseases. Multitudinous miRNAs have been reported to be closely related to hepatic steatosis, which can target the expression level of lipid metabolism-related genes to interfere with the transport, synthesis and oxidation of intracellular triglycerides, thereby regulating the degree of hepatocyte steatosis. In consideration of circRNAs itself carries a large number of miRNA binding sites, it can exert a powerful function of adsorbing miRNAs to inhibit the function of miRNAs. With the deepening of research on circRNAs, the network of circRNAs-miRNAs-NASH is enriched. 69 up-regulated and 63 down-regulated circRNAs as well as 2,760 up-regulated and 2,465 down-regulated mRNAs were identified by chip detection technology in the mouse NASH model established on the methionine choline deficiency (MCD) diet. Combined with qPCR and bioinformatics prediction software, four NASH-related circRNA-miRNA-mRNA pathways were constructed: circRNA_002581-miR-122-Slcla5, circRNA_002581-miR-122-Plp2, circRNA_002581-miR-122-Cpebl, and circRNA_007585-miR-326-UCP2 (61). The expression of circScd1 was significantly decreased in liver tissue of NAFLD mice induced by high-fat diet. Overexpression of circScd1 could have protective effect on this model while inhibition of circScd1 could promote the development of fatty liver. The activation of JAK2/STAT5 signaling pathway may be closely associated with this regulation (62). The regulatory network of circRNA_0046366/circRNA_0046367-miR-34a-PPARα in hepatic steatosis was also confirmed at the cellular level: the expressions of circRNA_0046366 and circRNA_0046367 were significantly lower in hepatocellular steatosis, and were negatively correlated with triglyceride content and lipid peroxidation level. Its mechanism may be related to the competitive binding of miR-34a, which in turn relieves the inhibition of PPARα expression in hepatocytes (63-65). It is reasonable to believe that circRNAs may provide new directions for the diagnosis and molecular mechanisms of NASH.

CircRNAs in liver regeneration (LR)

The liver has a strong ability of regeneration and recovery that other organs cannot match. Under normal circumstances, hepatocytes are mostly in static state and rarely divide, when the liver is injured by virus infection and trauma or partial hepatectomy, the number of hepatocytes is drastically reduced, and various feedback signals stimulate hepatocytes in quiescent phase to exert their powerful ability of proliferation and self-regulation. The hepatocytes of the residual hepatic lobes are transformed from a substantially non-growth state to a rapid growth state by cell proliferation to compensate for the lost, damaged liver tissue and restore the physiological function of the liver. Meanwhile, the organism can accurately sense the size of the regenerative liver and timely stop LR. This whole process is called LR.

By detecting circRNAs at different time points, a total of 2,412 circRNAs were identified, of which 159 circRNAs with altered expression corresponded to 116 linear RNA genes during rat LR. Go a step further, with the help of high-throughput RNA-seq technology, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, the potential regulation mechanism of hepatic metabolic capacity and hepatocyte proliferation during rat LR was initially explored. Key circRNAs in which circ137 and circ2270 regulate the hepatocyte proliferative capacity by targeting the binding of miR-127; circ432, circ2077, circ1366 and circ15 participate in liver energy and substance metabolism networks by regulating MAPK14, KFN1, TNFRSF21 and GOT1, respectively (66). CircRNAs are not only confirmed to play important roles in LR but also the supplement for the research of LR, which will greatly contribute to the further exploration of LR regulation mechanisms.

CircRNAs and intestinal diseases

CircRNAs and Crohn's disease (CD)

CD is a systemic disease characterized by chronic non-specific intestinal inflammatory lesions. The etiology of the disease is not yet clear. It may be related to immune dysfunction and may be complicated by perforation, intestinal obstruction, hemorrhage, localized peritonitis, abscess and tumor lesions. In recent years, the global incidence of CD has continued to increase, but so far there are still no effective cures. The autophagy-associated protein ATG16L1 is closely related to the development of CD and belongs to the CD susceptibility gene. Previous studies have suggested that under physiological conditions, the increased expression of miR-30c, miR-130A, and miR-93 may inhibit ATG16L1 expression in intestinal mucosa of CD patients, which in turn blocks autophagy-dependent intracellular bacterial clearance (67,68). Under pathological conditions, miR-106b has a targeted regulatory effect on ALG16L1 in CD colon tissue (69). On this basis, a significant decrease

in the expression of has_circ_0023397 was detected in the colon tissue of CD patients. At the same time, the expression of miR-106b was increased while the ATG16L1 and autophagy related genes LC3 were decreased. The bioinformatics analysis prediction software showed that there was a targeting regulatory relationship between has_circ_0023397 and miR-106b. Based on these evidences, it can be inferred that hsa_circ_0023397 acts as an adsorption sponge of miR-106b, and its decreased expression leads to increased expression of miR-106b, thereby interfering with autophagy in CD patients, suggesting that hsa_circ_0023397 may be a potential diagnosis marker for CD (70).

CircRNAs and Hirschsprung disease (HSCR)

HSCR, also known as aganglionosis, is due to a disorder of distal motor function that causes stool to stagnate in the proximal colon, leading to enlargement of the intestine and hypertrophy. HSCR is a common developmental malformation, which is accounting for the second most common cause of gastrointestinal tract anomalies. It is a familiar cause of neonatal digestive tract obstruction, often accompanied by intestinal obstruction, enterocolitis, intestinal perforation, peritonitis and systemic complications. The exploration of its pathogenesis will greatly improve the survival rate and quality of life of patients. Among the current exploration on the pathogenesis of HSCR, miRNAs such as miR-218-1, miR-206 and miR-192/215 as well as some abnormally expressed lncRNAs, may exert an inhibitory role in cell proliferation and migration, which is considered to be related to the occurrence and development of HSCR (71-74). Circ-ZNF609 (has_circ_0000615) is an indispensable circRNAs for the development of the central nervous system. The expression of circ-ZNF609 in HSCR is significantly reduced, and it has been confirmed that circ-ZNF609 can block the regulation of AKT3 by miR-150-5p, thus participating in the regulation of cell proliferation and migration (75).

CircRNAs and congenital anorectal malformations (ARM)

The incidence of birth defects in China is about 5.6%. There are about 900,000 new birth defects every year. ARM ranks first in congenital digestive tract malformations. It has a wide variety of phenotypes and about half of ARM children with urinary, reproductive, cardiovascular, bone and other multi-organ diseases, seriously affecting the long-term quality of children (76-79). The Wnt signaling pathway plays a crucial role in the early development of animal embryos, organ formation, tissue regeneration and other physiological processes. Wnt1, Wnt3a, Wnt8, Wnt5a and pathways occupy a central position in all ARM-related signaling pathways and networks. The classical Wnt/β-catenin signaling pathway exerts an irreplaceable role in the normal differentiation of anorectal (80-84). As a newcomer in the current disease marker research, circRNAs have confirmed the correlation with Wnt signaling pathway in osteoblast differentiation and tumor, the relationship between circRNAs and ARM is of great research value. Accordingly, using the Wnt signaling pathway as a bridge to find out the role of circRNAs in the development of ARM, and a comprehensive and detailed network framework is drawn to provide new ideas for the early diagnosis, treatment and genetic mechanism, as well as provide new clues for the study of the pathogenesis of other congenital gastrointestinal malformation.

CircRNAs and ischemia reperfusion (IR) injury

IR injury is one of the common tissues and organ damages, which plays a vital function in the pathological evolution of severe infection, trauma, shock, cardiopulmonary dysfunction and other diseases. In recent years, many studies have reported that intestinal IR can cause local tissue damage in the digestive tract, promote intestinal bacteria and toxins to the systemic circulation, trigger inflammatory cascade reaction, lead to the release of a large number of related mediators and cytokines, and even induce fatal complications—multiple organ dysfunction (MOD). The pathogenesis and prevention measures of the disease have also become the focus of attention (85,86). Ischemic postconditioning (iPoC) is an endogenous protective measure which can significantly reduce the ischemic intestinal tissue from IR injury and is considered as an inhibitor of IR injury.

A total of 9,821 circRNAs and 12,689 mRNA targets were detected by the combination of biochip, real-time PCR, bioinformatics analysis and other technologies in intestinal tissues initially. It was confirmed that the expression levels of circRNA_012412 and circRNA_016863 were abnormally decreased in intestinal IR, but the low expression level was elevated after iPoC treatment. Later four circRNA-miRNA-mRNA pathways were constructed: circRNA_012412-miR-7649-3p-Sertad1, circRNA_012412-miR-3473c-Sertad1, circRNA_012412-miR-6991-3p-Nudcd1 and circRNA_012412-miR-6991-3p-Jam2, which may be involved in regulating the protective mechanism of iPoC on intestinal IR, enriching

the regulatory network of circRNAs with disease (87).

Opportunity and challenge

Although circRNAs has been studied for more than 40 years, it has gradually carried out related large-scale and in-depth researches in the past decade. Among circRNAs, the intron circRNAs does not have the function of molecular sponge because its miRNA binding sites are relatively dispersed, while usually the exon RNA can be used as miRNA molecular sponges (88), The most famous ones include CDR1as with more than 70 miR-7 binding sites and SRY with 16 miR-138 binding sites (27,89). The identity of the miRNA sponges confers their ability to participate in the regulation of life activities. Taking CDR1as as an example, it can negatively regulate the activity of miR-7 and resist the degradation of miR-7. CDR1as is highly co-expressed with its target miR-7 in the developing midbrain, loss of miR-7 function causes a decrease in the volume of the midbrain and an imbalance in the volume of the telencephalon (4,26,90,91). The intron circRNAs located in the nucleus can enhance the transcription of the parental gene by interacting with pol II, thus participating in the regulation of gene expression (92,93). The evolutionary conservation, wide distribution, tissue specificity and expression stability of circRNAs make them have great potential as markers for disease screening and treatment, which is extremely significant for the research and development of human healthy. CircRNAs are inextricably linked to many clinical diseases such as cancer, cardiovascular disease, nervous system disease, digestive system disease, diabetes and so on (94). The CDR1as/miR-7 axis is a very important regulatory axis in disease research. CDR1as has been proved to be indirectly involved in the regulation of liver cancer, atrophic lateral sclerosis, diabetes and other diseases through miR-7 (90,95); the expression level of hsa_circ_002059 is significantly correlated with the tumor stage of gastric cancer, which may be a hopeful biomarker for the diagnosis of gastric cancer (96), cANRIL affects the risk of atherosclerosis by affecting the inhibitory effect of the INK4A/ARF gene by polycomb group (PcG) (97).

With the rapid development of high-throughput sequencing and molecular bioinformatics technology, the researches of circRNAs are getting more and more intensive, the relationship between more circRNAs and disease as well as the potential regulatory networks between them are becoming more sophisticated. The databases of circRNAs information have also gradually developing in quantity and quality, which provides unprecedented convenience for the study of circRNAs. The current number of studies on circRNAs in digestive diseases is on the rise, and it is expected that circRNAs can be used to detect diseases. However, circRNAs still have certain limitations as disease biomarkers. First of all, some abnormally expressed circRNAs in tissues do not have the same expression level in peripheral blood. Therefore, using these circRNAs as biomarkers may lead to invasive detection. On the other hand, the expression level of the circRNAs do not necessarily correlate with the severity of the disease, so it is impossible to judge the severity of disease only based on the expression level of the circRNAs temporarily. Although circRNAs are expected to be a novel biomarker for diseases, it still needs clinical studies for validation. But we have reason to believe that with the deep understanding, circRNAs will be better applied to the diagnosis and treatment of human diseases in the future.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/ncri.2018.11.06). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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