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REVIEW ARTICLE

The novel roles of circular RNAs in metabolic organs

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Abstract Circular RNAs (circRNAs) with a covalently closed loop structure which was different with linear RNAs, recently re-merged as novel regulator and exerted function in multiple biological processes. Through deep RNA sequencing (RNA-seq) technology coupled with bioinformatic analyses, a number of circRNAs has been identified. Moreover, circRNAs exhibit tissue- and development-specific expression indicating their potential biological significance. Actually, function of circRNAs as miRNA sponge has been well demonstrated in some diseases, besides, circRNAs also could function as RNA binding protein sponge and regulate alternative splicing and gene transcription. Notably, Emerging evidences showed that circRNAs played a pivotal role on the development of diseases including atherosclerotic vascular disease, neurological disorders and liver diseases, and served as diagnostic or predictive biomarkers of some diseases. This review mainly discusses the current advance of circRNAs as regulator involved in many diseases, and highlights circRNAs which have been well elucidated biological and pathogenic mechanism.

Introduction

Different with linear RNAs terminated with 5′ caps and 3′ tails, circular RNAs are characterized by covalently closed loop structures where the 3′ and 5′ RNA ends are joined together.[1,2] This typical circular feature of circRNAs makes them much stable due to the capability of resistance to RNase R digestion.[3,4] Actually, circRNAs were discovered in RNA viruses in 1976, and generally considered to be byproduct of splicing and little biological function in the following years.[5–7] Until recent years, many research groups used high-throughput RNA sequencing technology coupled with bioinformatic analysis to identify a number of circRNAs and demonstrated the function of circRNAs in various organs. In addition, accumulating evidences indicated that the majority of circRNAs are abundant,

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conserved across species and often exhibit cell type or tissue-specific expression, suggesting that circRNAs have potential regulatory roles.[8–10]

Although any loci of genome has the possibility of producing circRNAs, majority of them mainly derived from regions of protein coding genes. Generally, three types of circRNAs can be cataloged including exonic circRNAs(e-circRNA) from exons, intronic circRNAs(ciRNAs) from introns, and retained-intron circRNAs(ElciRNAs) from both.[11–15] Moreover, circRNAs could exert multiple functions depending on the different location. Most ecircRNAs tend to be cytoplasmic and act as miRNAs and RNA binding protein (RBP) sponge, inversely, ElciRNAs and ciRNAs predominantly localize in the nucleus and can regulate alternative splicing and gene transcription. Until now, the function of circRNAs acting as miRNAs sponge was well demonstrated in many studies.[16–18] For example, ciRS-7 (circular RNA sponge for miR-7) contains more than 70 selectively conserved miRNA target sites and strongly suppresses miR-7 activity.[19] Sry, a testis-specific circRNA, serves as a miR-138 sponge due to containing 16 binding sites.[19] Interestingly, a subset of circRNAs with open reading frames (ORFs) has been identified to have the capacity of translation.[20,21] Furthermore, circRNAs also can be modified with N6-methyladenosine (m6A) which promotes efficient initiation of protein translation.[22]

Canonical splicing is responsible for catalyzing pre-mRNA via removing introns and joining exons. However, unlike canonical linear RNAs splicing, back-splicing linked 3′ and 5′ end together to promote circularization.[23,24] Strikingly, the inverted repeat sequences or Alu elements in the introns flanking the exons, bringing the splice sites into close proximity to each other via base-pair, play a vital role for back-splicing.[14,25–27] In addition to cis-elements, trans-factors have been reported to regulate circRNAs biogenesis. For instance, muscleblind (MBL) could bind to its own pre-mRNA and promote circMbl production.[23] Quaking (QKI) positively regulated formation of circRNAs during epithelial to mesenchymal transition, inversely, adenosine deaminase 1 acting on RNA (ADAR1) destabilizes the base-pairing and further suppressed circRNAs biogenesis.[28,29] Therefore, the expression of circRNAs exhibiting cell type or tissue specificity may be due to the combination control of circRNAs biogenesis by cis-elements and trans-factors in corresponding cells and tissues.[30] (See Fig. 1).

Recently, emerging evidences indicated that circRNAs played an important role on the development of metabolic diseases. In this review, we summarized studies concerning the discovery and functions of circRNAs in the metabolic organs including liver, heart, muscle and pancreas, and highlight the circRNAs which have been demonstrated to involve in the development of diseases, such as

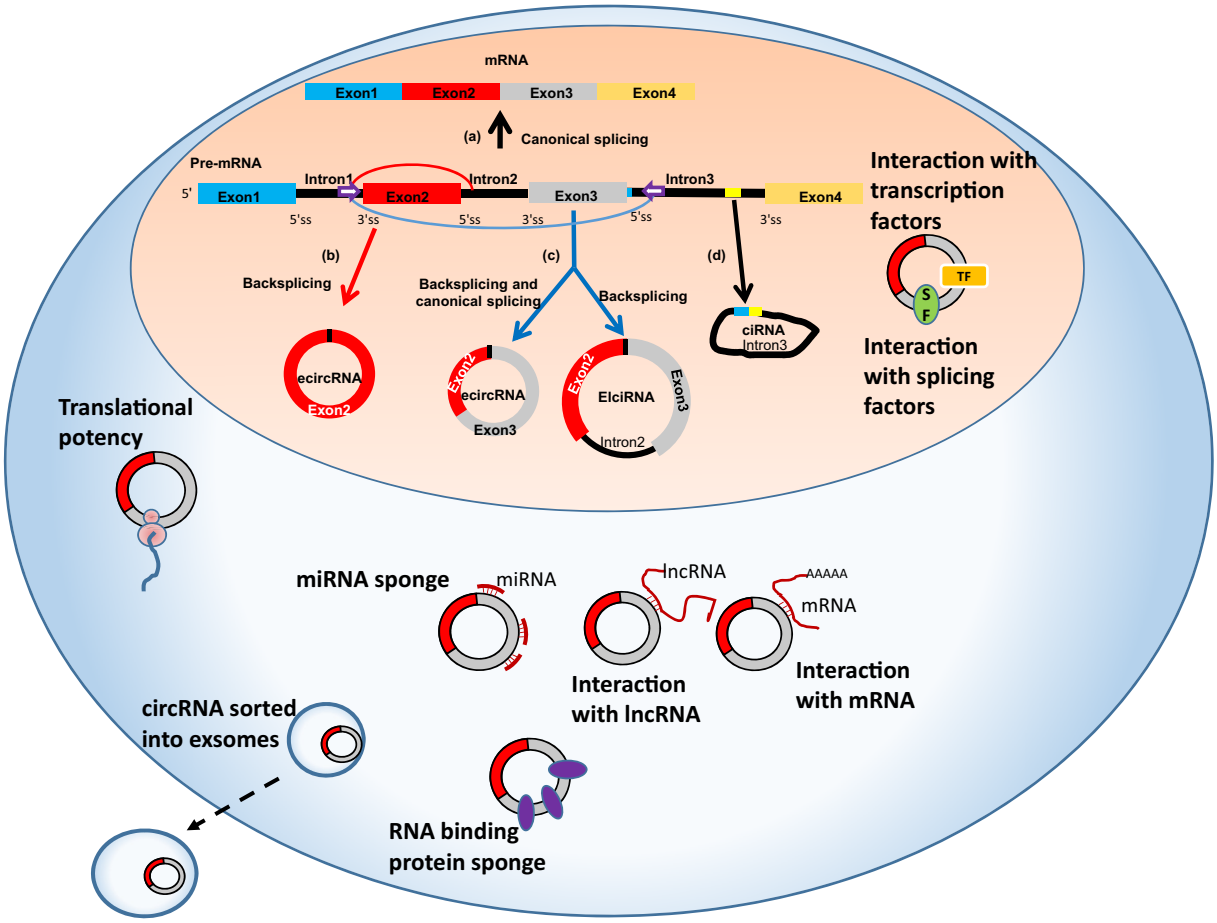


Figure 1 The biogenesis and functions of circRNA.

atherosclerotic vascular disease, neurological disorders, liver diseases as well as the potential links of circRNAs to metabolic diseases, such as type 2 diabetes and NASH.

Function of circRNAs in brain development and diseases

Brain plays a central regulatory role on whole body homeostasis and also can be coordinately response to peripherally secreted hormones such as leptin and insulin to regulate energy homeostasis. To identify the function of circRNAs in brain, several groups used deep RNA profiling to reveal thousands of circRNAs and show that circRNAs were more enriched in brain than other tissues such as heart, liver, lung and testis both in mouse and human. In addition, the expression of circRNAs during brain development, neuronal differentiation and plasticity was dynamic change, suggesting potential regulatory roles.[8,31–33] Remarkably, age-dependent neural accumulation of circRNA in mouse and fly indicated that circRNAs play an pivotal role on disease development of aging central nervous system (CNS).[34,35] Actually, some circRNAs have been demonstrated to involve in neuronal disorders and delineated underlying mechanism. For instance, the role of ciRS-7 on the development of Parkinson's disease (PD) has been well studied as miR-7 sponge.[19,36] In addition, the dysfunction of ciRS-7-miRNA-7-UBE2A circuit contributed to the development of sporadic Alzheimer's disease (AD).[37,38] More importantly, down regulation of CDR1as in mammalian cell culture modified cell migration and over-expression in the zebrafish brain leaded to brain developmental defects.[39] Recently, circ-FBXW7 was identified in Glioma and positively with glioblastoma patient overall survival, strikingly, circ-FBXW7 could encode a novel 21-kDa protein termed FBXW7-185aa that reduced the half-life of c-Myc by antagonizing USP28-induced c-Myc stabilization and served as tumor suppressor.[40]

Regulation of skeletal and cardiac muscle development and function by circRNAs

In order to characterize circRNA in human, mouse and rat hearts, several groups used RNA-Seq analysis to provide extensive catalog of circRNAs and found different expression under postnatal development and cardiac disease, suggesting potential biological functions.[41–43] Actually, some circRNAs have been identified to involve in cardiovascular disease development. For example, heart-related circRNA(HRCR) has been shown to involve in the development of heart diseases via acting as miR-223 sponge. Moreover, the role of miR-223 on inducing cardiac hypertrophy and heart failure, as well as hypertrophy in cardiomyocytes was well studied through targeting apoptosis repressor with CARD domain (ARC).[44–46] In addition, other group found that Cdr1as function as a miR-7a sponge in promoting myocardial infarction (MI) injuries through reducing the activity of miR-7a and upgrading the expression of miR-7a targets like PARP and SP1.[47,48] Circ-Foxo3 was identified high expression in non cancer cells and repressed cell cycle progression by binding to the cell cycle proteins cyclin-dependent kinase 2 (also known as cell division protein kinase 2 or CDK2) and cyclin-dependent kinase inhibitor 1 (or p21), resulting in the formation of a ternary complex. Interestingly, in hearts of aged mice and patients, the expression of circ-Foxo3 was high and positively correlated with extensive senescence. Underlying mechanism was that circ-Foxo3 arrested and relocated ID1, E2F1, FAK, and HIF1a in cytoplasm and blocked their anti-senescent function.[49,50] What's more, circular antisense non-coding RNA in the INK4 locus (circANRIL), forming from the cardiovascular disease risk locus on chromosome 9p21, has been identified to inhibit the development of atherosclerosis. Underlying mechanism is due to binding to PES1 and subsequently impairing exonuclease-mediated pre-rRNA processing and ribosome biogenesis, finally, resulting in the induction of apoptosis and inhibition of proliferation, which are key cell functions in atherosclerosis.[51–54] More recently, circRNA MFACR (mitochondrial fission and apoptosis-related circRNA) has been demonstrated to regulate mitochondrial dynamics and cardiomyocyte apoptosis, and the MFACR/miR-652-3p/MTP18 axis may provide the potential therapeutic target for the treatment of cardiovascular diseases.[55]

Skeletal muscle is insulin sensitive tissue and plays an important role on whole-body energy homeostasis. Not surprisingly, studying the role of circRNAs in muscle is very significant for finding the mechanism of metabolic regulation. An interesting study found that the expression of circRNAs in skeletal muscle of monkey changed with advancing age, indicating that circRNAs may have potential function on age-related muscle disorders.[56] In addition, recent study identified that circRNAs are regulated during murine and human muscle differentiation and whose expression is altered in duchenne muscular dystrophy (DMD) myoblasts. Interestingly, circ-ZNF609 containing an open reading frame can be translated and regulated myoblast proliferation.[20] Another interesting circRNA is circMbl which arises from MBL pre-mRNA. Study demonstrated that MBL could bind to its own pre-mRNA and promoted circMbl production.[23]

Role of circRNAs in liver

Liver is a pivotal metabolic organ which acting as a matabolic regulator for maintaining whole body energy homeostasis. The dysregulation of hepatic metabolism including glucose and lipid metabolism, will contribute to liver diseases such as NAFLD, NASH and HCC. Nonalcoholic steatohepatitis (NASH) is considered as a developed stage of simple steatosis with inflammation infiltration.[57] To identify the function of circRNAs during NASH, circRNA and mRNA profile was done by microarray to construct circRNA-miRNA-mRNA network, exploring the pathogenesis of NASH. Moreover, 69 up and 63 down regulated circRNAs were discovered, but candidates and mechanism need to further study.[58] The capacity of liver regeneration with clinical significance is the most important characterization that distinguishes with other organs. One group identified 159 of 2412 circRNAs remarkably changed during the early stage of rat LR and predicted these changing circRNA may be involved in hepatocyte proliferation, energy metabolism and substance metabolism via networks of circRNA-miRNA.[59] These screening studies showed that circRNAs

may have an important function on biological and pathological condition of liver.

Hepatocellular carcinoma (HCC) is one of the most common of all malignancies in the world. To discover the novel therapeutic target of HCC from circRNAs perspective is very significant. Cdr1as was well demonstrated circRNA and had function on myocardial infarction (MI) injuries and neurodegenerative diseases.[36,48] Recently, two groups have controversial results about its expression in HCC, so further study needs to been done about the expression and role of Cdr1as.[60,61] Screening profile of circRNAs using HCC tissue and para-tumorous tissue was to identify the functional circRNAs involved in HCC. hsa_circ_0004018 and hsa_circ_0005986 expression decreased, but hsa_circ_0005075 and circRNA_000839 expression increased in HCC tissue.[62–65] Moreover, hsa_circ_0005986 has been demonstrated to inhibit carcinogenesis by acting as a miR-129-5p sponge, and circRNA_000839 was identified as downstream of miR-200b which suppresses the invasion and migration of hepatocellular carcinoma.[63,65] What's more, circMTO1 expression was significantly down-regulated in HCC tissue and positively correlated with HCC patient survival. Moreover, circMTO1 suppressed HCC progression by acting as the sponge of oncogenic miR-9 to promote p21 expression and may be a potential target in HCC treatment.[66] Interestingly, circZKSCAN1 decreasing in tumor and host gene ZKSCAN1 both inhibited hepatocellular carcinoma cell growth, migration, and invasion but through different signaling pathways.[67]

Function of circRNA in pancreas

Pancreatic islets exert a crucial role in metabolic homeostasis through the secretion of key endocrine hormones such as insulin and glucagon. Insulin maintains normal level of glucose of serum through promoting intake of peripheral tissues. It was well demonstrated that miR-7 functions as a negative regulator of adult β-cells proliferation via regulating the mTOR Pathway, and ciRS-7 acted as miR-7 sponge. In islet β-cells, overexpression of ciRS-7 inhibits miR-7 function and then in turn improves insulin secretion. Myrip (regulates insulin granule secretion) and Pax6 (enhances insulin transcription) were identified as the potential downstream targets of miR-7.[68,69]

CircRNA, a promising biomarker, especially in exosomes

Biomarker is required for early diagnosis of diseases and then it is useful for improving with effective strategies and treatment approaches. Comparing with miRNAs identified as diagnostic biomarkers of some diseases, circRNAs are more potential promising and novel diagnostic biomarker because of abundant, conserved and stable characteristics.[31,70] Aberrant expression of circRNAs have been observed in many type of cancers. For instance, hsa_circ_0005075, hsa_circ_0001649, hsa_circ_0005986, hsa_circ_0004018, circRNA_000839 and circMTO1 may be novel biomarker of hepatocellular carcinoma.[62–66,71,72] hsa_circ_0013958 and hsa_circ_002059 may be the potential marker of lung adenocarcinoma and gastric carcinoma respectively.[73,74]

Human peripheral whole blood was collected to sequence circRNAs expression for detecting disease relevant circRNAs.[75] To identify diagnostic biomarkers for prediabetes and T2DM, circRNA microarray profiles were did with peripheral blood from control individuals and T2DM patients. Finally, hsa_circ_0054633 was further validated with possibility of being diagnostic biomarker for prediabetes and T2DM.[76] Moreover, hsa_circ_0124644 from peripheral blood can be used as a diagnostic biomarker of coronary artery disease.[77] And hsa_circ_0005836 was discovered from peripheral blood mononuclear cells (PBMCs) and might serve as a novel potential biomarker for TB infection.[78]

Exosomes are nanoscale (30—100 nm) vesicles containing complex cellular signals of RNA, protein and lipids and secreted by most cell types.[79] More recently, adipose tissue macrophages (ATMs) in obese mice secrete miRNA-containing exosomes (Exos), which cause glucose intolerance and insulin resistance when administered to lean mice.[80] This study found that miRNAs in secreted exosomes can be transferred to target tissue or cell to exert function. In addition, RNA-seq analyses revealed that circRNAs were enriched in exosomes compared to the producer cells. And serum exo-circRNA may distinguish patients with cancer from healthy controls, indicating potential possibility of being a circulating biomarker for cancer diagnosis.[81]

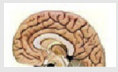
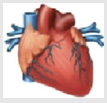
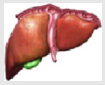
Blood platelets with absence of nucleus are the second most-abundant cell type in peripheral blood, but interesting study showed circRNAs enrichment.[82] Moreover, mRNA sequencing of tumor-educated blood platelets could distinguish cancer patients from healthy population with 96% accuracy.[83] These results suggest that circRNAs blood platelets may be the potential target in the diagnosis and treatment of tumor.

Collectively, most researches cited above lack the clear demonstration of the molecular mechanism, and further insights into the association between circRNAs and cancer would be warranted. Thus, clinical implications of the circRNAs as new clinical diagnostic and prognostic markers need further studies. (See Table 1).

Function of circRNA in other organs and diseases

Immune system is required for maintaining healthy of body. With aging, or immunosenescence, the gradual deterioration of the immune system will contribute to the development of associated diseases. To uncover the role of circRNAs in aging immune cells, circular RNA profiling reveals that circular RNA100783 may be involved in the chronic CD28-associated CD8(+)T cell aging.[84] But more functional circRNAs associated immune need to further explore. Besides the above circRNAs in regulatory and metabolic organs, there are still a lot of circRNAs that were identified to be associated with various cancers including gastric cancer, colorectal cancer, bladder carcinoma and laryngeal cancer. For example, the decreasing of cir-ITCH has been observed in tumor tissue and plays a suppressed role in colorectal cancer (CRC) via regulating the Wnt/β-catenin pathway.[85] CircHIAT1 was

Table 1 CircRNA in metabolic organs.

Metabolic organ	Circular RNA	Association with disease	Physiological function	Study
Brain 	ciRS-7	Parkinson's disease (PD)	miR-7 sponge	19.36
		Alzheimer's disease (AD)	Regulate ciRS-7-miR-7-UBE2A circuit	37
	CDR1as	Brain developmental defect	Unknown	39
	Circ-FBXW7	Glioma	Positively correlate with survival	40
		Suppress tumor development	Encode FBXW7-185aa protein	40
Skeletal muscle 	Circ-ZNF609	Duchenne muscular dystrophy	Encoding protein and regulate myoblast proliferation	20
	Circ-Mbl	Unknown	Promote circ-Mbl Production	23
Cardiac muscle 	HRCR	Heart diseases	miR-223 sponge	46
	Cdr1as	Myocardial infarction (MI)	miR-7a sponge	48
	Circ-Foxo3	Unknown	Suppress cell cycle progression	49
		Correlated with cardiac senescence	Arrested and relocated ID1 E2F1 FAK and HIF1a	50
	Circ-ANRIL	Atherosclerosis	Binding to PES1	51—54
	Circ-MFACR	Cardiomyocyte apoptosis	Regulate MFACR/miR-652-3p/MTP18 axis	55
Liver 	ciRS-7	Hepatocellular carcinoma (HCC)	Target miR-7 in HCC	60.61
	hsa-circ-0004018	Hepatocellular carcinoma (HCC)	Unknown	62
	hsa-circ-0005986	Hepatocellular carcinoma (HCC)	miR-129-5p sponge	63
	hsa-circ-0005075	Hepatocellular carcinoma (HCC)	Unknown	64
	circ-000839	Hepatocellular carcinoma (HCC)	Suppress invasion and migration	65
	Circ-MTO1	Hepatocellular carcinoma (HCC)	Positively correlate with HCC survival and act as miR-9 sponge to promote p21 expression	66
	Circ-ZKSCAN1	Hepatocellular carcinoma (HCC)	Unknown	67
Pancreas 	CiRS-7	Improve insulin secretion	miR-7 sponge	69

suppressed by androgen receptor and enhanced clear cell rental carcinoma cells (ccRCC) migration and invasion by deregulating miR-195-5p/29a-3p/29c-3p expressions.[86] In addition, circPVT1 and circTCF25 were respectively identified in gastric cancer (GC) and Bladder cancer and may become biomarker for the diagnosis.[87,88] Besides, hsa_circ_001988, hsa_circ_002059, hsa_circ_0000096 and has_circ_0067934 were found and may become the diagnostic biomarker of cancers.[74,89—92] In summary, these findings indicate that circRNAs are potentially involved in cancer initiation and progression. However, most research cited above lack the clear demonstration of the molecular mechanism, and further insights into their association with cancer would be warranted. Thus, clinical implications of the circRNAs as new clinical diagnostic and prognostic markers need further studies.

Conclusion and perspectives

Circular RNAs are re-emerging as important regulatory factors of biological and pathological processes. Although more and more functional circRNAs are being gradually revealed, there are still thousands of circRNAs for which the functions remain unknown. Specially, our understanding of circRNAs on metabolic processes including glucose and lipid metabolism is still at its infancy and many questions remain unanswered.

Firstly, according to the above review, many circRNAs expression exhibit tissue- and development-specific manner, more tran-factors or splicing factors with tissue- or development-specificity need to be explored not just MBL and QKI. In addition, profiling of circRNA in different tissues or different development stages should be construct and function of key circRNAs are necessary to elucidate. Secondly, adipose tissue, muscle and liver are major metabolic organ to maintain whole body energy homeostasis. The functional circRNAs in these metabolic tissues are the key point for finding treatmental target of metabolic diseases such as type 2 diabetes, insulin resistance and non-alcoholic fatty liver. It is emergent project to identify the key circRNAs and further elucidate their functions in metabolic tissue. Third, exploring the detail mechanism is always difficult study, the same with circRNAs. So it worth us more effort to gain deeper insight into the molecular mechanisms. Four, due to the properties of circRNAs, it is not difficult to predict that circRNAs can act as ideal, novel and promising diagnostic markers for many diseases. With deeper study of circRNAs, more features will be discovered, the relationship between circRNA and corresponding diseases will be more and more clear, the pos-

sibility of circRNAs as diagnostic biomarker will be true soon in the future.

Conflicts of interest

There is no conflict of interest.

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