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·综述·

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环状 RNA 及其作为疾病标志物的潜能

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摘要 环状 RNA(circular RNA, circRNA)是一种共价闭合环状结构的内源性非编码 RNA 分子,不具有 5′端帽子和 3′端 poly(A)尾巴结构,具有广泛性、保守性、组织特异性以及稳定性等特性;根据序列来源的不同可分为 3 种类型:外显子 circRNA、内含子 circRNA、外显子-内含子 circRNA;随着生物信息学的快速发展和高通量测序技术的不断革新,目前已经在真核细胞中发现大量内源性 circRNA,主要分布于细胞质中,其独特的序列结构,使它具有 microRNA 海绵、调节选择性剪接或转录、与 RNA 结合蛋白结合、滚动翻译和衍生假基因等功能;它参与癌症、糖尿病、神经系统疾病和动脉粥样硬化等疾病发生、发展过程。众多研究显示 circRNA 会成为新型的疾病临床诊断标志物或人类疾病治疗的潜在靶点,该评述较为详细地从 circRNA 的形成、特性、生物学功能、研究方法、研究数据库以及和疾病的关系等方面来阐述 circRNA 的最新研究进展,方便研究人员进行 circRNA 研究。

关键词 环状 RNA;疾病;数据库
中图分类号 Q2

Circular RNA and Its Potential as a Disease Marker

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Abstract Circular RNA (circRNA) is an endogenous noncoding RNA molecule with covalently closed cyclic structure which does not have 5′ end cap and 3′ poly (A) tail. It is found to be evolutionary conservative and stable with tissue specificity. According to the different sources of the sequences, circRNA can be divided into three types: exon circRNA, intron circRNA, and exon-intron circRNA. With the rapid development of bioinformatics and continuous innovation of high-throughput sequencing technology, a large number of endogenous circRNA, which was mainly distributed in the cytoplasm, has been found in eukaryotic cells. CircRNA, which has unique sequence structure, acts as microRNA sponge, regulates selective splicing or transcription, binds to RNA-binding proteins and produces pseudogenes by rolling translation. It is involved in cancer, diabetes, nervous system diseases, atherosclerosis and other diseases. Numerous studies have shown that circRNAs may become potential targets and clinical diagnostic markers for human disease. This review is a detailed study of the latest progress of circRNA research, including its formation, characterization, biological function, research methods, research database and its relationship to the disease.

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Key words circular RNA; disease; database

环状 RNA(circular RNA, circRNA)是一种调节基因表达的内源性非编码 RNA(ncRNA)[1],在上世纪 70 年代至 80 年代,circRNA 首次作为某些 RNA 病毒的基因组被发现,ncRNA 包括长链非编码 RNA(lncRNA)、微小 RNA(microRNA)和环状 RNA(circRNA)[2]。与线性 RNA 形成方式不同,如 mRNA(Fig. 1 a),circRNA 根据序列的形成和组合方式的不同可分为 3 种类型(Table 1):外显子 circRNA(exonic circRNA, ecRNA)(Fig. 1 b, c)、内含子 circRNA(circular intronic RNA, ciRNA)(Fig. 1 d)、外显子-内含子 circRNA(exon-introncircRNA, EIciRNA)(Fig. 1c)。与 ncRNA 一样,circRNA 序列和结构也决定了它的生物学功能,某些 circRNA 可以作为 microRNA 海绵来调节其他相关 RNA 的表达水平[3,4]。其利用 microRNA 的应答元件来结合 microRNA,并阻止 microRNA 对其目标基因表达的抑制作用[5]。它主要在细胞质中存在,并且在人体中具有高度稳定性,在不同生物中也被鉴定出来[6],真核生物中被广泛地表达和进化[7,8],特别是人类和小鼠。在许多疾病中,如神经系统疾病、动脉粥样硬化、糖尿病和癌症,circRNA 也扮演着非常重要的角色[9,10]。circRNA 在生物体内有特殊的特性。目前,circRNA 通过许多其他分子来调节宿主基因线性 mRNA 的产生,如拼接因子、RNA 聚合酶 II[11]、核小核糖核酸蛋白颗粒(snRNP)[12,13]和 microRNA[14],这些相互作用促进或抑制相应的线性信使 RNA 的转录。

Table 1 Characteristics of different types of circRNAs

Name	Type	Location	Joint site	Sequence feature	Function
ecRNA	Exon	Cytoplasms	3′-5′ phosphodiester bond	Participate in cyclization exons containing the reverse complementary sequence of introns and selective cyclization	Function as miRNA sponges; interact with rbp; participate in translation
ciRNA	Intron	Nucleus	2′-5′ phosphodiester bond	Enrichment of 5′ splice site contains 7 GU motif, enrichment of 3′ branch site contains 11 C motif	Regulation of gene transcription
EIciRNA	Exon-intron	Nucleus	3′-5′ phosphodiester bond	Participate in cyclization exons containing the reverse complementary sequence of introns and selective cyclization	Regulation of gene transcription

ecRNA: Exon circRNA; ciRNA: Intron circRNA; EIciRNA: Exon-intron circRNA

1 环状 RNA 的生成机制

CircRNA 是一种不具有 5′端帽子和 3′端 poly(A)尾巴结构的共价闭合环状 RNA 分子,Jeck 等提出 2 种不同的外显子环化的模型:内含子配对环化(intron-pairing driven circularization)(Fig. 2a)和套索驱动环化(Lariat-driven circularization)[11](Fig. 2b)。前者认为侧翼内含子通过反向互补序列配对形成环状结构,然后剪切去除内含子并连接外显子,最终形成 ecRNA,如 circEts-1 和 circSry[15,16]。通常,由内含子形成的许多套索结构很快就会被分支酶降解[17];后者则认为 hnRNA 在转录时 RNA 部分折叠与非相邻的外显子靠近,导致外显子跳跃(exon skipping),形成的套索中间体进一步剪接产生外显子 circRNA(ecRNA)。

并非所有的外显子都能形成 circRNA,在生成的过程中,内部互补序列的两边都可以促进外显子的环化,比如 Alu 元件。然而,Alu 元件可被其他互补重复序列代替[3],表明其生成过程与互补的重复序列的数量有关。另外,侧面内含子和外显子越多越容易发生环化[18]。

某些蛋白质分子参与 RNA 环化过程。例如甘露糖结合凝集素(mannose-binding lectin, MBL)作为 RNA 结合蛋白(RNA binding proteins, RBPs)的侧面连接,以维持环化结构,从而促进第 1 个双子环的形成[19]。起始同源物 KH 结构域 RNA 结合(quaking homolog, KH domain RNA binding, QKI)是通过 2 个单体结合在一起参与外显子环化,然后通过 QKI 蛋白自身进入到 2 个循环剪切位点附近的同源体[20]。但 RNA 编辑酶 ADARs(腺苷酶作用

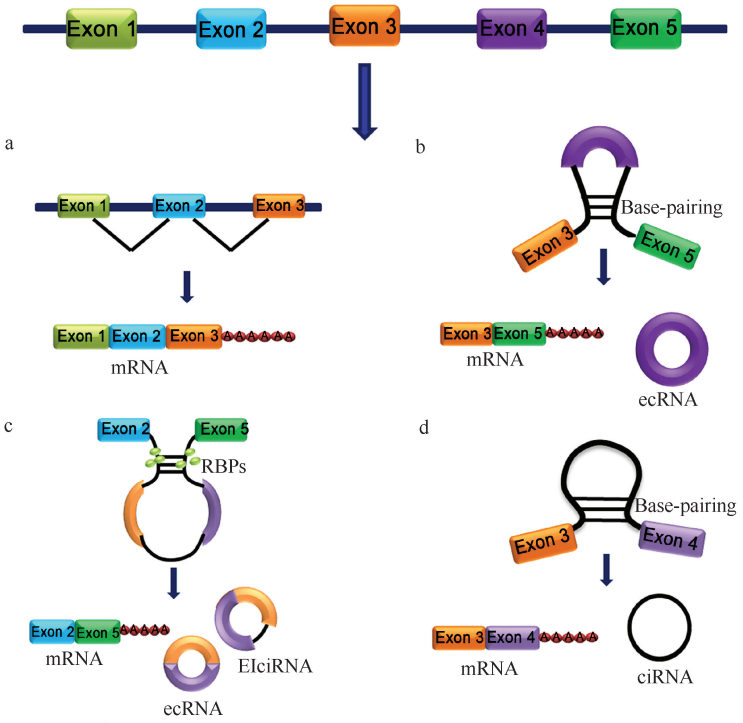

Fig. 1　Classification of circRNAs　ecRNA: Exon circRNA; ciRNA: Intron circRNA; EIciRNA: Exon-intron circRNA

于 RNA),能结合两侧互补双链区域,去除双链的相互作用,从而抑制 circRNA 形成[21]。

2　环状 RNA 的特性

与线性 RNA 相比,circRNA 有几个重要的特性。(1)绝大多数位于细胞质中,只有小部分在细胞核中[22];(2)大部分来自外显子,小部分来自内含子或内含子片段;(3)一些 circRNA 通过微反应元素与 microRNA 相互作用,从而调节目标基因表达[23];(4)大多数 circRNA 内源非编码 RNA(ncRNA)分子调节基因的表达;(5)除了少数 circRNA 在转录中扮演角色之外,大多数在转录前和转录后发挥调控作用[24];(6)circRNA 和线性 mRNA 一样具,有相同的组织特异性和/或发育阶段特异性表达[25];(7)circRNA 在多种类型的细胞外体液中存在,如唾液、血液和尿液,其表达水平比相应线性 mRNA 的表达水平高 10 倍以上[26];(8)circRNA 在不同的物种中显示出古老的进化保守特

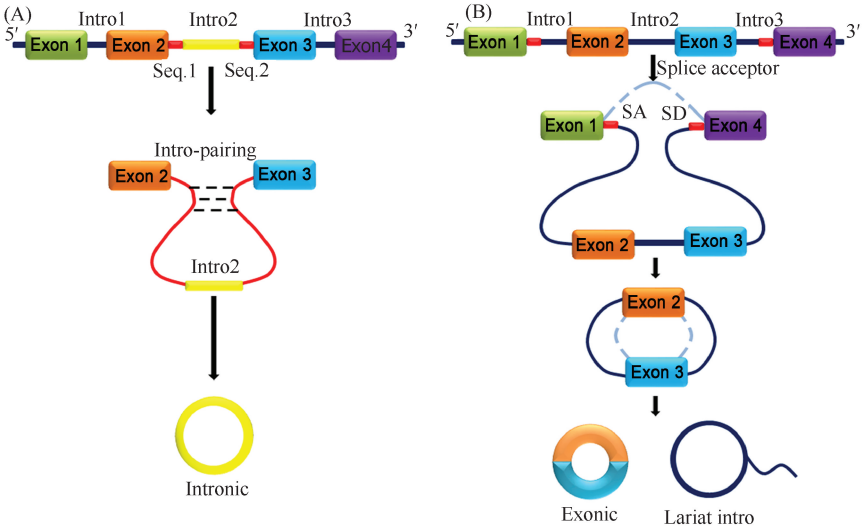

Fig. 2　CircRNA biogenesis　(A) Intron-pairing-driven circularization. (B) Lariat-driven circularization

征[27];(9)不具有 5′-3′极性和多聚腺苷酸尾的共价闭环结构,对 RNA 外切核酸酶或 RNase R 具有高抗性[28],因此 circRNA 的生物学作用更持久。大多数物种的 circRNA 的平均半衰期远远长于半衰期仅为 10 h 的 mRNA,而且自健康人类无细胞唾液(CFS)中已发现超过 400 种 circRNA[29]。

3 环状 RNAs 的功能

CircRNA 可以作为 microRNA(miRNA)海绵、调节选择性剪接或转录、使用滚动翻译、与 RBPs 结合以及 circRNA 可以衍生出假基因。

3.1 CircRNA 作为 miRNA 海绵起作用[25]

CircRNA 包含共有的 miRNA 响应元件(miRNA response element, MRE),结合 miRNA 并阻止它们与靶 mRNA 相互作用(Fig. 3a)。

CiRS-7 (CDR1as)[25, 30] 和 Sry circRNA (circSry)[31] 分别由小脑变性相关蛋白 1(cerebellar degeneration associated protein 1, CDR1)和失调的大鼠睾丸 SRY 产生。在斑马鱼胚胎发育过程中,CDRlas 表达可以减少脑体积并阻碍其发育,注射 miR-7 可恢复正常,说明 CDRlas 可能与 miR-7 结合[32]。来自 *HIPK*3 基因外显子 2 的 circHIPK3 沉默 *HIPK*3 mRNA,显著抑制人类细胞的生长。通过荧光素酶筛选测定出 circHIPK3 通过 18 个潜在结合位点沉默 9 个 miRNA,并直接特异性结合 miR-124 抑制其活性[33](Table 2)。但是生物信息学分析表明,miRNA 结合位点数量多的 circRNAs 不一定有很强的海绵作用,其他 circRNA 则证实了这种情况[34, 35]。

Table 2 circRNAs act as miRNA sponge

Name	Source	Sequence feature	Function
CDR1as[3, 25, 30]	Produced by the cerebellar degeneration associated protein 1 (CDR1)	More than 70 miR-7 binding sites and can form RISC with miR-7 and Ago2 proteins	Promote miR-7 degradation of the target gene
CircSry[3, 31]	Produced by dysregulated rat testis SRY	More than 10 miR-138 binding sites	As a miR-138 sponge
CircHIPK3[33]	Derived from Exon2 of the HIPK3 gene	18 potential binding sites	Affecting nine types of miRNAs that inhibit cell proliferation

3.2 CircRNA 调节选择性剪接或转录

研究表明,circRNA 参与可变剪接和转录的调控(Fig. 3b),可变剪接是 pre-mRNA 通过不同的剪接方式(选择不同的剪接位点)产生不同的 mRNA 剪接异构体的过程,circRNA 对可变剪切能够产生明显的调控作用。例如由剪接因子 MBL (muscleblind)的第 2 外显子产生的 circMbl,其与标准的 pre-RNA 融合竞争。circMbl 及其侧翼内含子具有保守的 MBL 结合位点,被 MBL 强效且特异性抑制。MBL 水平的调节显著影响 circMbl 的形成,这种作用取决于侧翼内含子序列中的 MBL 结合位点[19]。

3.3 CircRNA 与 RNA 结合蛋白相互作用

CircRNA 可能和一些线性非编码 RNA 一样与 RBPs 结合来发挥生物学功能[36]。当它与 RBPs 和核糖核蛋白复合物结合时,可以起到 RNA 结合蛋白海绵作用以及“储存”功能[37],同时形成复合体(Fig. 3c)。EcRNA 可以稳定地与细胞中的某些蛋白质分子特异性结合,作为 RNA 或 DNA 与互补序列结合的支架,为 RNA 结合蛋白、RNA 和 DNA 提供相互作用平台。如 CDRlas 可以与 miRNA 作用因子 Ago2 蛋白结合共同发挥蛋白水解功能[30]。

3.4 CircRNA 使用滚动翻译

近几年研究发现,circRNA 也具有翻译蛋白质的功能:(1)当环状 mRNA 包含内部核糖体进入位点(internal ribosome entry site, IRES)序列且直接与核糖体结合时,可以在真核细胞中被翻译[38, 39];(2)当真核的核糖体 40S 亚基在入口处与 circRNA 结合,可以在体外和体内启动翻译[40, 41];(3)在具有 ORF(开放阅读框)的大肠杆菌无细胞翻译系统中,circRNA 也能有效地翻译产生蛋白质[39],在大肠杆菌中,插入 GFP(绿色荧光蛋白)开放阅读框的 circRNA 可成功转录 GFP[41];(4)某些 circRNA 在人类细胞中翻译时不需要内部核糖体进入位点、poly-A 尾或帽结构的参与,而后者对于常规翻译的内部起始是必不可少的;(5)真核转译系统中的 circRNA 可通过类似于聚合酶反应的滚环扩增(RCA)方式合成蛋白质(Fig. 3d),这表示不需要与 RNA 模板多次结合,circRNA 不仅可以产生长且重复的肽序列,而且还能在给定的时间段内提高其线性对应物的生产力[39]。已经有直接证据证明,天然真核内源性 circRNA 通过腺苷 N6(m6A)的甲基化

来驱动蛋白质翻译[42]。

3.5　CircRNA 衍生出假基因

有研究表明,稳定的 circRNA 分子能被逆转录并整合到基因组中,形成 circRNA 衍生的假基因[43](Fig. 3e)。从小鼠基因组中 circRFWD2 相应的环化位点(外显子 6-外显子 2)分析中发现,33 个"高度可信的 circRFWD2 衍生假基因",9 个"低度可信的 circRFWD2 衍生假基因",以及位于 circRFWD2 外部的 6 个含有假基因的外显子序列。LINE-1 介导的 RNA 逆转录通常需要 Poly(A)的存在,42 个 circRFWD2 相关假基因中的 39 个不含 Poly(A)序列,说明一些 circRFWD2 能基于一种未知的方式被逆转录成 cDNA。因此,在以后研究 circRNA 时要考虑到假基因对实验的干扰。

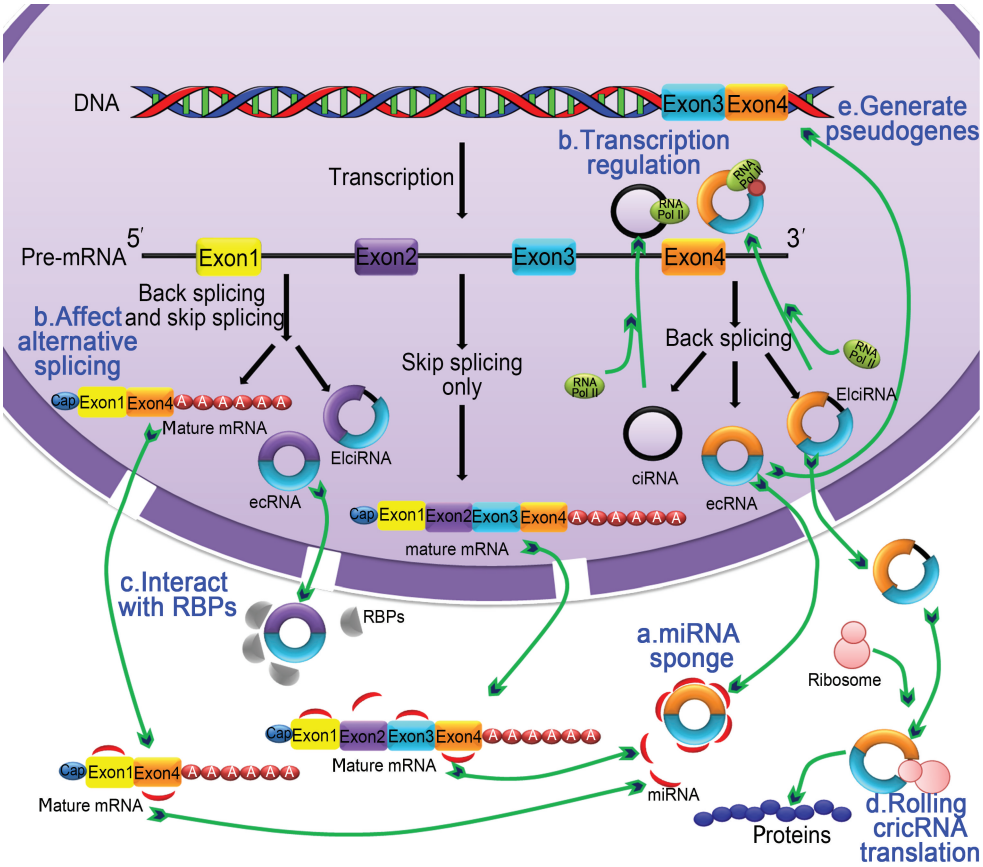

Fig. 3　The main functions of the circRNA　(a) miRNA sponge: circRNA binds miRNA to affect its biological function and regulate miRNA target gene activity; (b) Regulating selective splicing or transcription: stable ciRNAs and EIciRNAs are located in the nucleus, bind to RNAs and promote transcription; circRNAs compete with pre-mRNA splicing to reduce linear mRNA and exclude specificity from pre-mRNA to change the composition of processed mRNA; (c) Interaction with RBPs: circRNA binds RBPs and ribonucleoprotein complexes to prevent these factors from functioning and play a "storage" function; (d) Rolling circle translation: some *in vitro* circRNAs can be translated into proteins by means of a roll loop amplification mechanism; (e) Generate pseudogenes: some circRNAs may be reverse transcribed into cDNA and integrated into the genome, and integration is not yet clear

4　环状 RNA 的研究方法

4.1　分子生物学方法

CircRNA 的环状结构具有高稳定性,可抵抗酶消化,因此可用分子生物学方法进行初步纯化鉴定[44]:(1)用核酸外切酶 R、烟酸磷酸酶、5′末端核酸外切酶等降解大多数线性 RNA 而保留 circRNA,再用 circRNA 特异性引物对酶样品进行定量分析,处理前后可以鉴定或定量 circRNA[44, 45];(2)circRNA 末端没有极性结构,在交联凝胶中迁移速度比长链线性 RNA 慢,与同源基因转录相比,核酸的 circRNA 序列更少,在弱交联凝胶中的迁移速度更慢,因此可以通过 Northern 印迹鉴定 circRNA[46];(3)通过荧光原位杂交技术在亚细胞水平上定位 circRNA,使用 siRNA 或反义寡核苷酸干扰 circRNA 表达来验证 circRNA 功能[12, 24]。

4.2 高通量测序

与传统的分子生物学方法相比,高通量测序和生物信息学的结合为发现新的低丰度的 circRNA 提供了捷径。CircRNA 通过反向剪接产生,而早期 RNA-seq 技术算法,对于区分反向剪接位点和相应环状结构的效率极低。研究人员对测序分析的策略和算法进行了有效的改进:(1)假设基因外显子重排有不同的形式,构建 circRNA 候选序列边界组合,然后与序列数据进行比较[47];(2)序列数据通过不同的序列比对算法,直接与基因组序列匹配;(3)通过设计多个序列剪接,直接从 cDNA 序列中检测 circRNA 分子[48]。目前应用于 circRNA 分子研究的算法有 MapSplice[49]、CircSeq[11]、CIRI[50] 和 CIRCexplorer[22] 等。CIRI 与注释相关算法不仅能检测从内含子或基因间基因组区转录的 circRNA,而且适用于未注释或无注释的真核生物的测序数据。由于 circRNA 不具有 poly(A)结构,传统的寡聚 dT 富集方法无效,使用 ribo-zero kit 去除 rRNA,再用 RNase R 去除线性 RNA,库构建方法能有效富集 circRNA[45, 51]。

5 环状 RNA 研究数据库

随着 circRNA 的研究越来越广泛,circRNA 研究工具的数量和质量不断完善,为了更方便的进行 circRNA 研究本文的收集,可使用一些免费的在线数据库,其中包含从 GenBank 注释或发表文章收集的 circRNA(Table 3)。

Table 3 Free online database

Name	New version	URL	Introduction
starBase v2.0[52]	July 2013	http://starbase.sysu.edu.cn/	Allows users to search for functional classes or procedures
circBase[53]*	December 2015	http://www.circbase.org/	
circ2Traits[54]	December 2013	http://gyanxet-beta.com/circdb	Provides a wealth of information about the genome position of cirRNA-related diseases and several circRNAs
nc2Cancer[55]		http://www.Bioinfo.Tsinghua.edu.cn/nc2Cance	
CircNet[56]*	December 2015	http://circnet.mbc.nctu.edu.tw/	Contains the most circulatory annotations, especially the human circRNA
deepBase v2.0[57]	November 2015	http://biocenter.sysu.edu.cn/deepBase/	
CircInteractome[58]	December 2015	http://circinteractome.nia.nih.gov	With information on the matching of the circRNA with the associated RNA binding protein

* With BLAST Web interface, comparable to the sequence of the circRNA; CircNet is the only database provided in the sample of the circRNA expression

没有哪一种数据库是完美而全面的,每个数据库都有自己的特征,并且更新时间也不同,只有各种数据库的灵活运用才能获取准确而全面的信息。

6 环状 RNA 与疾病

CDR1as 是已知的调节各种疾病如癌症、肿瘤、神经系统疾病、糖尿病和心血管疾病的 circRNA 之一。同样,circMBL 通过与其合成的线性肌肉屏障基因竞争,来调节线性 mRNA 的比例。由于这些分子与关键 miRNA 和基因家族的复杂关系,circRNA 可能在人类疾病的起因和进展中发挥重要作用。

6.1 circRNA 在心血管疾病的作用

6.1.1 *病理性心肌肥大和心力衰竭* MiR-223 是一种内源性调节因子,可以诱导心肌肥大,心力衰竭和心肌细胞肥大[59]。在心脏肥大中,ARC(具有 CARD 结构域的凋亡抑制因子)作为 miR-223 下游靶标介导 miR-223 的功能[60]并作为 miRNA 海绵影响 miRNA 在心肌细胞肥大和凋亡中的表达[3, 25]。心血管关联的 circRNA(HRCR)可以直接结合 miR-223,并作为内源性 miR-223 海绵抑制 miR-223 活性,进而降低 ARC 的表达[60]。因此,通过 HRCR 调节心肌细胞肥大、心脏肥大和心力衰竭。此外,它是抑制相关心血管疾病药物开发的治疗靶标。

6.1.2 *动脉粥样硬化* Burd 等[9]发现,cANRIL(circular antisense non-coding RNA in the INK4 locus)表达受人 INK4a/ARF(inhibitor of CDK4/alternative reading frame)转录本调控,且与动脉粥样硬化风险相关。这种影响与剪切位点的单核苷酸多态性相关,人类全基因组关联分析显示,靠近基因 *INK4/ARF*(CDKN2a/b)的染色质 9p21.3,与动脉粥样硬化的易感性具有单核苷酸多态性(single nucleotide polymorphisms,SNPs)。进一步研究显示,

cANRIL 是基因 *INK4/ARF* 的反义转录物[47],可通过特异的多梳家族复合物来抑制 INK4/ARF 表达[61],从而影响动脉粥样硬化的患病风险,表明 cANRIL 或许与预防和治疗动脉粥样硬化有一定的关联性。

6.1.3 心血管衰老　由转录因子 foxo3 的成员组成的 circ-Foxo3,在老年患者和大鼠的心肌样本中高度表达,这与细胞衰老的标志物相关[62]。已经证明,circFoxo3 的高表达能阻止更多的细胞自 G_1 期转运至 S 期,因此 circ-Foxo3 的表达能抑制细胞增殖和细胞周期进展[63]。Circ-Foxo3 主要分布在细胞质中,与抗衰老蛋白 ID-1 和转录因子 E2F1,以及抗应激蛋白 FAK 和 HIF1α 相互作用,转录因子 ID-1(inhibitor of DNA binding-1),E2F1(E2F transcription factor 1),FAK(focal adhension kinase)和 HIF1a(hypoxia-inducible factor 1 a)仅在进入细胞核时才发挥抗衰老作用,所以 circFoxo3 能阻止这些因子进入细胞核,从而抑制这种抗衰老效应。研究已证明了 circ-Foxo3 和衰老之间的正相关性[62]。通过在细胞质中阻止和重新定位 ID-1、E2F1、FAK 和 HIF1a 并阻止其抗衰老功能,或许利用此发现能更好地进行心脏衰老和心肌保护的相关研究。

6.1.4 心肌梗死　心肌梗死(myocardial infarction,MI)一直是全球死亡和残疾的主要原因之一[64]。在心肌梗死发展过程中,延长心肌缺血有助于心肌细胞死亡过程[65]。Cdr1as 具有作为 miR-7 海绵的功能,可以抑制 miR-7 的活性[59,82,83]。miR-7 a / b 通过负调节 PARP 和减少心肌细胞凋亡来发挥保护作用[66,67],SP1(specificity protein 1)和 PARP(poly ADP-ribose polymerase)是 miR-7 靶基因,在缺氧治疗下它们促进 miR-7 a 诱导的细胞凋亡[65],在心肌梗死发展过程中也发挥促凋亡作用[68,69]。因此,Cdr1as 通过降低 miR-7 a 的活性和促进 miR-7 a 靶基因(如 PARP 和 SP1)在 MI 损伤中的表达,研究表明,Cdr1as / miR-7 a 在心肌梗死诱导的心肌细胞凋亡中具有重要作用。Cdr1as 在心肌细胞中作为 miR-7 a 海绵的功能不仅说明了 circRNA 与心肌梗死有关,而且提供了改善心肌梗死相关损伤的潜在治疗价值。

6.2 circRNA 与神经系统疾病

CircRNA 最早在脑组织中被发现表达,在哺乳动物神经元中的含量很高,在果蝇体内,具有神经调节功能的基因通常含有较长的内含子区,circRNA 表达水平也相对较高,人类大脑中 circRNA 表达水平也高于相应的线性 RNA[21,70],Rybak-wolf 等[27]和 You 等[35]研究了上千种大脑 circRNA 的图谱,显示在神经形成时,多数 circRNA 与相应的线性 RNA 亚型相比表达上调,通过系统鉴定和分析神经组织 circRNA,发现大脑中数千种 circRNA 的序列和表达模式在不同物种之间具有高度保守性。

Lin 等[71]通过建立葡萄糖剥夺/再复氧(oxygen-glucose deprivation/reoxygenation, OGD/R)的 HT22 细胞模型,发现 mmu-circRNA-015947 的表达高于正常细胞,表明 circRNA 表达变化与 OGD/R 诱发神经损伤有关。大量研究显示,miR-7 是能直接调节 a-突触核蛋白和泛素蛋白连接酶 A 的表达,这与阿尔茨海默疾病[72]和帕金森[54]的发生相关。Lukiw[72]发现,在散发型阿尔茨海默病(Alzheimer's disease,AD)的海马 CAl 区域,存在 miRNA-circRNA 系统的失调,当 CDRlas(CiRS-7)表达降低或吸附 miR-7 的能力减弱时,miR-7 的表达会增加并直接导致人类中枢神经系统中的泛素蛋白连接酶 A 表达下调,从而影响中枢神经系统的正常功能,对脑组织造成严重损伤。在肌萎缩性脊髓侧索硬化模型中,去除核脱分支酶后,某些内含子来源的套索状结构聚集在细胞浆中,通过降解 TDP43(43 kD transactivation response DNA-binding protein)而抑制其毒性[73]。因此,某些 ciRNA 参与了肌萎缩性脊髓侧索硬化的发病过程。CircRNA 在神经系统中的机制尚不清楚,但 circRNA 在神经系统疾病中具有重要的研究价值。

6.3 circRNA 在糖尿病中的作用

CDRlas(ciRS-7)在糖尿病的诊疗过程中具有重要作用。胰岛 β 细胞功能受损、胰岛素分泌绝对或相对不足(胰岛素抵抗),会使血糖升高,从而引发糖尿病[74]。研究发现,miR-7 能够负调节胰岛 β 细胞增殖,其在胰岛 β 细胞中的过表达会损伤 β 细胞去分化功能,并导致胰岛素分泌的下调,从而引起糖尿病。另外,miR-7 通过雷帕霉素靶蛋白信号通路抑制胰岛 β 细胞扩增,而 CDRlas(CiRS-7)能够抑制 miR-7 功能,从而刺激胰岛 β 细胞扩增。表明 miR-7 可能影响胰腺 β 细胞的更新,同时也是糖尿病的潜在治疗靶点[75]。

6.4 circRNA 在癌症中的作用

6.4.1 胃癌　Li 等[76]通过 101 例胃癌患者的胃癌组织与癌旁组织的 circRNA,以及 36 例血浆标本

的 circRNA 分析测定,发现 Hsa-circ-002059 在胃癌患者血浆中的水平于术后和术前相比有显著性差异,同时胃癌组织的 Has-circ-002059 水平显著低于邻近的非肿瘤组织,进一步发现它的水平与胃癌的肿瘤阶段显著相关,说明 Hsa-circ-002059 可作为胃癌诊断的生物标志物。

6.4.2 食道癌 Li 等[14]通过 684 例食管鳞状细胞癌患者癌组织及癌旁组织 cir-ITCH 差异表达分析发现,cir-ITCH 在食管鳞状细胞癌患者癌组织的表达水平显著低于癌旁组织。进一步功能分析证实,cir-ITCH 通过发挥 miR-7、miR-17 和 miR-214 分子海绵的作用增强 ITCH 的表达,从而在 Wnt 通路中发挥抑制作用,说明 cir-ITCH 在食管鳞状细胞癌发病过程中发挥重要作用。

6.4.3 肝癌 Xu 等[77]发现,CDRlas 在肝癌组织中的含量与肝的微血管侵犯(microvascular invasion, MVI)发生密切相关。miR-7 在肝癌细胞中,增强其靶基因 *PIK3CD* 和 *p70S6K* 表达能力,通过某些机制来增加微血管侵犯(MVI)的概率,导致癌细胞复发率增加,影响疾病的预后。而当肝癌细胞中的 CDRlas 过表达时,通过吸附 miR-7 来抑制其活性,表明 CDRlas 可作为肝的微血管侵犯生物标志物,并可作为一种新的抑制肝微血管侵犯的治疗靶点。

6.4.4 宫颈癌 在宫颈癌组织中 CDRlas 的表达高于癌旁组织,而 miR-7 在人宫颈癌组织中的表达低于癌旁组织。在宫颈癌细胞 HeLa 和 C33 A 细胞中过表达 CDRlas 后,通过抑制 miR-7 的活性来提高细胞中 miR-7 的靶基因 *FAK* 的表达,黏着斑激酶(focal adhesion kinase,FAK)能够促进宫颈癌细胞的增殖、侵袭及迁移,加剧疾病的恶化。这表明 CDRlas 和 miR-7 以及黏着斑激酶(focal adhesion kinase,FAK)之间的相互作用与宫颈癌的关系密切[78]。针对 CiRS-7 调节网络的分子靶向治疗,有望为宫颈癌的诊断和治疗提供新的途径。深入研究 circRNA 能更好地了解其病理机制,改善相关疾病的预防和诊断。下表是对部分疾病相关的 circRNA 简要总结,供研究人员参考使用(Table 4)。

Table 4 Some known diseases-related CircRNAs

Diseases	CircRNA	Signal path	Features
Heart failure/ Pathological hypertrophy	HRCR	miR-223, ARC	Combined with miR-223 reduced ARC expression
Atherosclerosis	cANRIL	INK4/ARF	Expression of INK4 / ARF was inhibited by specific multi comb comb family complexes
Heart aging	circ-Foxo3	ID1, E2F1, FAK, HIF1a	Prevent transcription factors ID1, E2F1, FAK and HIF1a play a role
Myocardial infarction	CDRlas	miR-7, SP1, PARP	Reduce miR-7 a activity and promote SP1, PARP expression
Alzheimer's disease	CDRlas	miR-7, a-synuclein	The expression of a-synuclein and ubiquitin protein ligase A was regulated by miR-7
Diabetes	CDRlas	miR-7, Islet beta cells	Inhibit miR-7 function, stimulate pancreatic beta cell proliferation
Liver cancer	CDRlas	miR-7, PIK3CD, p70S6K	Inhibition of miR-7 activity, decreased PIK3CD and p70S6K expression
Cervical cancer	CDRlas	miR-7, FAK	Inhibition of miR-7 activity enhances FAK expression
Stomach cancer	Hsa-circ-002059		Regulatory effect
Esophageal cancer	cir-ITCH	miR-7, miR-17, miR-214	As miR-7, miR-17, miR-214 molecular sponge enhances ITCH expression

7 目前研究较为清晰的环状 RNA

随着 circRNA 功能研究的逐步开展,越来越多的研究人员对 circRNA 充满兴趣,这里列举部分研究较为清晰的 circRNA 信息(Table 5),包括名称、功能、信号通路和文献中的研究样本等,供研究人员参考使用。

很多 circRNA 潜在功能正在被研究报道,只有了解 circRNA 的相关功能,才能进一步的挖掘出其他重要功能。

Table 5　Some diseases-related circRNAs

Serial number	Name	Features	Signal path	Sample
1	Heart-related circRNA (HRCR)[60]	As an endogenous miR-223 sponge to inhibit cardiac hypertrophy and heart failure	MiR-223	Mouse cardiomyocytes
2	CircRNA_100290[79]	As a competitive endogenous RNA that regulates CDK6 expression through a member of the spongiform miR-29b family	MiR-29	Human oral squamous cell carcinoma (OSCC)。
3	CircZKSCAN1[80]	And ZKSCAN1mRNA in close cooperation, inhibition of HCC growth, migration and invasion	ZKSCAN1mRNA	Human hepatocellular carcinoma (HCC)
4	Hsa_circ_0005105[81]	The degradation of extracellular matrix (ECM) was promoted by modulating the expression of miR-26 a target NAMPT	MiR-26 a	Osteoarthritis (OA)
5	CircCCDC66[82]	By modulating the oncogene subtype, knockout circCCDC66 inhibited tumor growth and cancer invasion in xenotransplantation and in situ mouse models, respectively	Cell proliferation; immigration; invasion; do not depend on the growth of anchorage	Colorectal cancer (CRC)
6	Hsa_circ_0000190[83]	A new noninvasive biomarker for the diagnosis of gastric cancer		Gastric cancer tissue
7	CircHIAT1[84]	As a metastatic inhibitor of inhibition of AR-enhanced ccRCC cell migration and invasion	MiR-195-5p/29 a-3p/29c-3	AR Enhanced ccRCC cells
8	Circ- Foxo3[62]	Promote heart senescence by regulating a variety of factors associated with stress and aging reactions	ID-1, E2F1, FAK, and HIF1α	Elderly patients and heart samples of mice
9	CircRNA_000203[85]	Enhance the expression of fibrosis related genes	MiR-26b-5p, Col1a2 and CTGF	Mouse cardiac fibroblasts
10	Circ100284[86]	Accelerate cell cycle and lead to malignant transformation	MiR-217; EZH2; cyclin D1and CDK4	HaCaT cells
11	Hsa_circ_0000069[87]	Upregulate and promote cell proliferation, migration and invasion		Colorectal cancer (CRC)
12	Hsa_circ_0054633[88]	For the early stage of diabetes and T2DM to provide a certain diagnostic ability		Type 2 diabetes mellitus (T2DM) patients
13	Has_circ_0067934[89]	Uptake esophageal squamous cell carcinoma, promote proliferation		Esophageal squamous cell carcinoma (ESCC)
14	CDR1as[76, 90]	Can regulate the activity of miRNAs, mRNA and RBP, play a specific biological role	miR-7	HCC and matched non-tumor tissue
		As a risk factor for hepatic microvascular infiltration in hepatocellular carcinoma		Hepatocellular carcinoma
15	cZNF292[91]	Inhibit angiogenesis by inhibiting glioma cell proliferation and cell cycle progression	Wnt/β-catenin	Human glial tumor tube
16	CircRar1[92]	The expression of caspase8 and p38 was up-regulated by mRNA and protein levels by regulating microRNA miR-671 by promoting LncRpa neuronal apoptosis	MiR-671	Lead-induced neurotoxicity in mouse models
17	CircTCF25[93]	Downregulate miR-103 a-3p and miR-107, increase CDK6 expression, promote in vitro and in vitro proliferation and migration	MiR-103 a-3p, MiR-107	Bladder Cancer

Continued Table 5

Serial number	Name	Features	Signal path	Sample
18	Hsa_circRNA_103636[94]	Is reproducible and readily detectable in clinical blood samples and may have the potential as a new class of MDD biomarkers		Severe depression (MDD)
19	Hsa_circ_001569[95]	As a positive regulator of cell proliferation and invasion of colorectal cancer (CRC)	MiR-145	Colorectal cancer (CRC)
20	CircHIPK3[33]	Binds to miR-124 and inhibits miR-124 activity	MiR-124	Normal and cancerous tissue
21	Hsa_circ_0001649[96]	As a new potential biomarker of HCC, may play a role in the carcinogenesis and metastasis of HCC		Human hepatocellular carcinoma (HCC)

8 问题与展望

CircRNA 具有广泛性、保守性、组织特异性以及稳定性等特点,使其在作为疾病筛查和治疗的标志物上具有巨大潜力,并且随着高通量测序和分子生物信息学技术的快速发展,预示着它在未来会成为新型的生物标志物,以及随着 circRNA 在临床疾病如糖尿病、癌症、心血管疾病和神经系统疾病上的应用,为疾病治疗提供重要的研究新思路和治疗靶点。circRNA 的功能研究越深入,相关 circRNA 信息的数据库在数量和质量上也会逐步发展,这为研究 circRNA 提供了前所未有的便捷,但只有多种数据库的结合运用,才能获取最新最全面的 circRNA 信息,从而正确全面的进行 circRNA 的研究。目前 circRNA 的命名尚未统一,更多的生物学功能有待进一步研究,参与疾病相关的详细机制也并不清晰,这说明 circRNA 的研究还有相当长的路要走,但随着研究人员的不懈努力,相信不久的未来,circRNA 的庐山真面目会慢慢呈现在世人面前,或许会有更新更有效的研究方法出现,让 circRNA 的研究变得更加事半功倍。

参考文献(References)

[1] Wilusz JE, Sharp PA.. Molecular biology. a circuitous route to noncoding RNA[J]. Science, 2013, **340**(6131): 440-441

[2] Beermann J, Piccoli MT, Viereck J, *et al*. Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches[J]. Physiol Rev, 2016, **96**(4): 1297-1325

[3] Hansen, TB, Jensen TI, Clausen BH, *et al*. Natural RNA circles function as efficient microRNA sponges[J]. Nature, 2013, **495**(7441): 384-388

[4] Maass PG, Luft FC, Bähring S. Long non-coding RNA in health and diseases[J]. J Mol Med (Berl), 2014, **92**(4): 337-346

[5] Valdmanis PN, Kay MA. The expanding repertoire of circular RNAs[J]. Mol Ther, 2013, **21**(6): 1112-1114

[6] Danan M, Schwartz S, Edelheit S, *et al*. Transcriptome-wide discovery of circular RNAs in Archaea [J]. Nucleic Acids Res, 2012, **40**(7): 3131-3142

[7] Wang PL, Bao Y, Yee MC2, *et al*. Circular RNA is expressed across the eukaryotic tree of lifes[J]. PLoS One, 2014, **9**(6): e90859

[8] Morris KV, Mattick JS. The rise of regulatory RNA[J]. Nat Rev Genet, 2014, **15**(6): 423-437

[9] Burd CE, Jeck WR, Liu Y, *et al*. Expression of linear and novel circular forms of an INK4/ARF-associated non-coding RNA correlates with atherosclerosis risk [J]. PLoS Genet, 2010, **6**(12): e1001233

[10] Wang Y, Mo Y, Gong Z, *et al*. Circular RNAs in human cancer [J]. Mol Cancer, 2017, **16**(1): 25

[11] Jeck WR, Sorrentino JA, Wang K, *et al*. Circular RNAs are abundant, conserved, and associated with ALU repeats [J]. RNA, 2013, **19**(2): 141-157

[12] Li Z, Huang C, Bao C, *et al*. Corrigendum: exon-intron circular RNAs regulate transcription in the nucleus [J]. Nat Struct Mol Biol, 2017, **24**(2): 194

[13] Huang C, Shan G. What happens at or after transcription: Insights into circRNA biogenesis and function [J]. Transcription, 2015, **6**(4): 61-64

[14] Li F, Zhang L, Li W, *et al*. Circular RNA ITCH has inhibitory effect on ESCC by suppressing the Wnt/beta-catenin pathway [J]. Oncotarget, 2015, **6**(8): 6001-6013

[15] Cocquerelle C, Daubersies P, Majérus MA, *et al*. Splicing with inverted order of exons occurs proximal to large introns [J]. EMBO J, 1992, **11**(3): 1095-1098

[16] Dubin RA, Kazmi MA, Ostrer H. Inverted repeats are necessary for circularization of the mouse testis Sry transcript[J]. Gene, 1995, **167**(1-2): 245-248

[17] Rodríguez-Trelles F, Tarrío R, Ayala FJ. Origins and evolution of spliceosomal introns[J]. Annu Rev Genet, 2006, **40**: 47-76

[18] Shen T, Han M, Wei G, *et al*. An intriguing RNA species--perspectives of circularized RNA[J]. Protein Cell, 2015, **6**(12): 871-880

[19] Ashwal-Fluss R, Meyer M, Pamudurti NR, *et al*. circRNA biogenesis competes with pre-mRNA splicing[J]. Mol Cell, 2014, **56**(1): 55-66

[20] Conn SJ, Pillman KA, Toubia J, *et al*. The RNA binding protein quaking regulates formation of circRNAs[J]. Cell, 2015, **160**(6): 1125-1134

[21] Ivanov A, Memczak S, Wyler E, *et al*. Analysis of intron sequences reveals hallmarks of circular RNA biogenesis in animals[J]. Cell Rep, 2015, **10**(2): 170-177

[22] Zhang XO, Wang HB, Zhang Y, *et al*. Complementary sequence-mediated exon circularization [J]. Cell, 2014, **159**(1): 134-147

[23] Yang C, Wu D, Gao L, *et a*l. Competing endogenous RNA networks in human cancer: hypothesis, validation, and perspectives[J]. Oncotarget, 2016, **7**(12): 13479-13490

[24] Zhang Y, Zhang XO, Chen T, *et al*. Circular intronic long noncoding RNAs[J]. Mol Cell, 2013, **51**(6): 792-806

[25] Memczak S, Jens M, Elefsinioti A, *et al*. Elefsinioti, *et al*. Circular RNAs are a large class of animal RNAs with regulatory potency[J]. Nature, 2013, **495**(7441): 333-338

[26] Qu S, Yang X, Li X, *et al*. Circular RNA: A new star of noncoding RNAs[J]. Cancer Lett, 2015, **365**(2): 141-148

[27] Rybak-Wolf A, Stottmeister C, Glažar P, *et al*. Circular RNAs in the mammalian brain are highly abundant, conserved, and dynamically expressed[J]. Mol Cell, 2015, **58**(5): 870-885

[28] Suzuki H, Tsukahara T. A view of pre-mRNA splicing from RNase R resistant RNAs[J]. Int J Mol Sci, 2014, **15**(6): 9331-9342

[29] Bahn JH, Zhang Q, Li F, *et al*. The landscape of microRNA, Piwi-interacting RNA, and circular RNA in human saliva[J]. Clin Chem, 2015, **61**(1): 221-230

[30] Hansen TB, Wiklund ED, Bramsen JB, *et al*. miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA[J]. EMBO J, 2011, **30**(21): 4414-4422

[31] Zhao ZJ, Shen J. Circular RNA participates in the carcinogenesis and the malignant behavior of cancer[J]. RNA Biol, 2017, **14**(5): 514-521

[32] Hansen TB, Kjems J, Damgaard CK. Circular RNA and miR-7 in cancer[J]. Cancer Res, 2013, **73**(18): 5609-5612

[33] Zheng Q, Bao C, Guo W, *et al*. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs[J]. Nat Commun, 2016, **7**: 11215

[34] Guo JU, Agarwal V, Guo H, *et al*. Expanded identification and characterization of mammalian circular RNAs[J]. Genome Biol, 2014, **15**(7): 409

[35] You X, Vlatkovic I, Babic A, *et al*. Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity[J]. Nat Neurosci, 2015, **18**(4): 603-610

[36] Yin QF, Yang L, Zhang Y, *et al*. Long noncoding RNAs with snoRNA ends[J]. Mol Cell, 2012, **48**(2): 219-230

[37] Ashwal-Fluss R, Meyer M, Pamudurti NR, *et al*. circRNA biogenesis competes with pre-mRNA splicing[J]. Mol Cell, 2014, **56**(1): 55-66

[38] Abe N, Matsumoto K, Nishihara M, *et al*. Rolling circle translation of circular RNA in living human cells[J]. Sci Rep, 2015, **5**: 16435

[39] Thompson SR. So you want to know if your message has an IRES?[J]. Wiley Interdiscip Rev RNA, 2012, **3**(5): 697-705

[40] Thomas LF, Sætrom P. Circular RNAs are depleted of polymorphisms at microRNA binding sites[J]. Bioinformatics, 2014, **30**(16): 2243-2246

[41] Wang Y, Wang Z. Efficient backsplicing produces translatable circular mRNAs[J]. RNA, 2015, **21**(2): 172-179

[42] Yang Y, Fan X, Mao M, *et al*. Extensive translation of circular RNAs driven by N6-methyladenosine[J]. Cell Res, 2017. **27**(5): 626-641

[43] Liu J, Liu T, Wang X, *et al*. Circles reshaping the RNA world: from waste to treasure[J]. Mol Cancer, 2017, **16**(1): 58

[44] Jeck WR, Sharpless NE. Detecting and characterizing circular RNAs[J]. Nat Biotechnol, 2014, **32**(5): 453-461

[45] Suzuki H, Zuo Y, Wang J, *et al*. Characterization of RNase R-digested cellular RNA source that consists of lariat and circular RNAs from pre-mRNA splicing[J]. Nucleic Acids Res, 2006, **34**(8): e63

[46] Tabak HF, Van der Horst G, Smit J, *et al*. Discrimination between RNA circles, interlocked RNA circles and lariats using two-dimensional polyacrylamide gel electrophoresis[J]. Nucleic Acids Res, 1988, **16**(14a): 6597-6605

[47] Salzman J, Chen RE, Olsen MN, *et al*. Cell-type specific features of circular RNA expression[J]. PLoS Genet, 2013, **9**(9): e1003777

[48] Hoffmann S, Otto C, Doose G, *et al*. A multi-split mapping algorithm for circular RNA, splicing, trans-splicing and fusion detection[J]. Genome Biol, 2014, **15**(2): R34

[49] Wang K, Singh D, Zeng Z, *et al*. MapSplice: accurate mapping of RNA-seq reads for splice junction discovery[J]. Nucleic Acids Res, 2010, **38**(18): e178

[50] Gao Y, Wang J, Zhao F. CIRI: an efficient and unbiased algorithm for de novo circular RNA identification[J]. Genome Biol, 2015, **16**: 4

[51] Ebbesen KK, Kjems J, Hansen TB. Circular RNAs: identification, biogenesis and function[J]. Biochim Biophys Acta, 2016, **1859**(1): 163-168

[52] Li JH, Liu S, Zhou H, *et al*. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data[J]. Nucleic Acids Res, 2014, **42**(Database issue): D92-97

[53] Glažar P, Papavasileiou P, Rajewsky N. circBase: a database for circular RNAs[J]. RNA, 2014, **20**(11): 1666-1670

[54] Hancock, JM. Circles within circles: commentary on Ghosal et al. (2013) "Circ2Traits: a comprehensive database for circular RNA potentially associated with disease and traits"[J]. Front Genet, 2015, **5**: 459

[55] Cheng Z, Liu K, Yan Z, *et al*. nc2Cancer: a database for cancer-associated human ncRNAs[J]. Chin J Bioinformatics, 2015, **13**(2): 77-84

[56] Liu YC, Li JR, Sun CH, *et al*. CircNet: a database of circular RNAs derived from transcriptome sequencing data[J]. Nucleic Acids Res, 2016, **44**(D1): D209-215

[57] Zheng LL, Li JH, Wu J, *et al*. deepBase v2.0: identification, expression, evolution and function of small RNAs, LncRNAs and circular RNAs from deep-sequencing data[J]. Nucleic Acids Res. 2016, **44**(D1): D196-202

[58] Dudekula DB, Panda AC, Grammatikakis I, *et al*. CircInteractome: A web tool for exploring circular RNAs and their interacting proteins and microRNAs[J]. RNA Biol, 2016, **13**(1): 34-42

[59] Wang YS, Zhou J, Hong K, *et al*. MicroRNA-223 displays a protective role against cardiomyocyte hypertrophy by targeting cardiac troponin I-interacting kinase[J]. Cell Physiol Biochem, 2015, **35**(4): 1546-1556

[60] Wang K, Long B, Liu F, *et al*. A circular RNA protects the heart from pathological hypertrophy and heart failure by targeting miR-223[J]. Eur Heart J, 2016, **37**(33): 2602-2611

[61] Jacobs JJ, Kieboom K, Marino S, *et al*. The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus[J]. Nature, 1999, **397**(6715): 164-168

[62] Du WW, Yang W, Chen Y, *et al*. Foxo3 circular RNA promotes cardiac senescence by modulating multiple factors associated with stress and senescence responses[J]. Eur Heart J, 2017, **38**(18): 1402-1412

[63] Du WW, Yang W, Liu E, *et al*. Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2[J]. Nucleic Acids Res, 2016, **44**(6): 2846-2858

[64] Benjamin EJ, Blaha MJ, Chiuve SE, *et al*. Heart disease and stroke statistics-2017 update: a report from the American heart association[J]. Circulation, 2017, **135**(10): e146-603

[65] Geng HH, Li R, Su YM, *et al*. The circular RNA Cdr1as promotes myocardial infarction by mediating the regulation of miR-7a on its target genes expression[J]. PLoS One, 2016, **11**(3): e0151753

[66] Zhao YJ, Wang JH, Fu B, *et al*. Effects of 3-aminobenzamide on expressions of poly (ADP ribose) polymerase and apoptosis inducing factor in cardiomyocytes of rats with acute myocardial infarction[J]. Chin Med J (Engl), 2009, **122**(11): 1322-1327

[67] Fan X, Weng X, Zhao Y, *et al*. Circular RNAs in cardiovascular disease: an overview[J]. Biomed Res Int, 2017, **2017**: 5135781

[68] Li B, Li R, Zhang C, *et al*. MicroRNA-7a/b protects against cardiac myocyte injury in ischemia/reperfusion by targeting poly

(ADP-ribose) polymerase[J]. PLoS One, 2014, **9**(3): e90096
[69] Read DE, Gupta A, Ladilov Y, *et al.* miRNA signature of unfolded protein response in H9c2 rat cardiomyoblasts[J]. Cell Biosci, 2014, **4**(1): 56
[70] Westholm JO, Miura P, Olson S, *et al.* Genome-wide analysis of drosophila circular RNAs reveals their structural and sequence properties and age-dependent neural accumulation[J]. Cell Rep, 2014, **9**(5): 1966-1980
[71] Lin SP, Ye S, Long Y, *et al.* Circular RNA expression alterations are involved in OGD/R-induced neuron injury[J]. Biochem Biophys Res Commun, 2016, **471**(1): 52-56
[72] Lukiw WJ. Circular RNA (circRNA) in Alzheimer's disease (AD)[J]. Front Genet, 2013, **4**: 307
[73] Armakola M, Higgins MJ, Figley MD, *et al.* Inhibition of RNA lariat debranching enzyme suppresses TDP-43 toxicity in ALS disease models[J]. Nat Genet, 2012, **44**(12): 1302-1309
[74] Kim MK, Shin HM, Jung H, *et al.* Comparison of pancreatic beta cells and alpha cells under hyperglycemia: Inverse coupling in pAkt-FoxO1[J]. Diabetes Res Clin Pract, 2017, **131**: 1-11
[75] Wang Y, Liu J, Liu C, *et al.* MicroRNA-7 regulates the mTOR pathway and proliferation in adult pancreatic beta-cells[J]. Diabetes, 2013, **62**(3): 887-895
[76] Li P, Chen S, Chen H, *et al.* Using circular RNA as a novel type of biomarker in the screening of gastric cancer[J]. Clin Chim Acta, 2015, **444**: 132-136
[77] Xu L, Zhang M, Zheng X, *et al.* The circular RNA ciRS-7 (Cdr1as) acts as a risk factor of hepatic microvascular invasion in hepatocellular carcinoma[J]. J Cancer Res Clin Oncol, 2017, **143**(1): 17-27
[78] Lee BY, Timpson P, Horvath LG, *et al.* FAK signaling in human cancer as a target for therapeutics[J]. Pharmacol Ther, 2015, **146**:132-149
[79] Chen L, Zhang S, Wu J, *et al.* circRNA_100290 plays a role in oral cancer by functioning as a sponge of the miR-29 family[J]. Oncogene, 2017, **36**(32): 4551-4561
[80] Yao Z, Luo J, Hu K, *et al.* ZKSCAN1 gene and its related circular RNA (circZKSCAN1) both inhibit hepatocellular carcinoma cell growth, migration, and invasion but through different signaling pathways[J]. Mol Oncol, 2017, **11**(4): 422-437
[81] Wu Y, Zhang Y, Zhang Y, *et al.* CircRNA hsa_circ_0005105 up regulates NAMPT expression and promotes chondrocyte extracellular matrix degradation by sponging miR-26a[J]. Cell Biol Int, 2017, **41**(12): 1283-1289
[82] Hsiao KY, Lin YC, Gupta SK, *et al.* Non-coding effects of circular RNA CCDC66 promote colon cancer growth and metastasis[J]. Cancer Res, 2017, **77**(9): 2339-2350
[83] Chen S, Li T, Zhao Q, *et al.* Using circular RNA hsa_circ_0000190 as a new biomarker in the diagnosis of gastric cancer[J]. Clin Chim Acta, 2017, **466**: 167-171
[84] Wang K, Sun Y, Tao W, *et al.* Androgen receptor (AR) promotes clear cell renal cell carcinoma (ccRCC) migration and invasion via altering the circHIAT1/miR-195-5p/29a-3p/29c-3p/CDC42 signals[J]. Cancer Lett, 2017, **394**: 1-12
[85] Tang CM, Zhang M, Huang L, *et al.* CircRNA_000203 enhances the expression of fibrosis-associated genes by derepressing targets of miR-26b-5p, Col1a2 and CTGF, in cardiac fibroblasts[J]. Sci Rep, 2017, **7**: 40342
[86] Xue J, Liu Y, Luo F, *et al.* Circ100284, via miR-217 regulation of EZH2, is involved in the arsenite-accelerated cell cycle of human keratinocytes in carcinogenesis[J]. Biochim Biophys Acta, 2017, **1863**(3): 753-763
[87] Guo JN, Li J, Zhu CL, *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[J]. Onco Targets Ther, 2016, **9**: 7451-7458
[88] Zhao Z, Li X, Jian D, *et al.* Hsa_circ_0054633 in peripheral blood can be used as a diagnostic biomarker of pre-diabetes and type 2 diabetes mellitus[J]. Acta Diabetol, 2017, **54**(3): 237-245
[89] Xia W, Qiu M, Chen R, *et al.* Circular RNA has_circ_0067934 is upregulated in esophageal squamous cell carcinoma and promoted proliferation[J]. Sci Rep, 2016. **6**: 35576
[90] Peng L, Yuan XQ, Li GC. The emerging landscape of circular RNA ciRS-7 in cancer (Review)[J]. Oncol Rep, 2015, **33**(6): 2669-2674
[91] Yang P, Qiu Z, Jiang Y, *et al.* Silencing of cZNF292 circular RNA suppresses human glioma tube formation via the Wnt/beta-catenin signaling pathway[J]. Oncotarget, 2016, **7**(39): 63449-63455
[92] Nan A, Chen L, Zhang N, *et al.* A novel regulatory network among LncRpa, CircRar1, MiR-671 and apoptotic genes promotes lead-induced neuronal cell apoptosis[J]. Arch Toxicol, 2017, **91**(4): 1671-1684
[93] Zhong Z, Lv M, Chen J. Screening differential circular RNA expression profiles reveals the regulatory role of circTCF25-miR-103a-3p/miR-107-CDK6 pathway in bladder carcinoma[J]. Sci Rep, 2016, **6**: 30919
[94] Cui X, Niu W, Kong L, *et al.* hsa_circRNA_103636: potential novel diagnostic and therapeutic biomarker in Major depressive disorder[J]. Biomark Med, 2016, **10**(9): 943-952
[95] Xie H, Ren X, Xin S, *et al.* Emerging roles of circRNA_001569 targeting miR-145 in the proliferation and invasion of colorectal cancer[J]. Oncotarget, 2016, **7**(18): 26680-26691
[96] Qin M, Liu G, Huo X, *et al.* Hsa_circ_0001649: A circular RNA and potential novel biomarker for hepatocellular carcinoma[J]. Cancer Biomark, 2016, **16**(1): 161-169