circRNAs在成骨细胞分化中的调控作用

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摘要 环状RNAs(circular RNAs, circRNAs)是一类新型内源性非编码RNAs, 在调节生长发育、疾病发展等方面具有重要的生物学功能。新近研究证实, circRNAs参与调控牙周膜干细胞和骨髓干细胞等的成骨细胞分化。该文就当前circRNAs在成骨细胞分化中的最新研究进展作一综述, 以帮助开发骨科疾病新疗法。

关键词 环状RNA; 牙周膜干细胞; 骨髓干细胞; 成骨细胞分化

Regulation of circRNAs in Osteogenic Differentiation

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Abstract circRNAs (circular RNAs), a novel class of endogenous noncoding RNAs, play a key role in regulating growth and development of diseases. Recent studies confirmed that circRNAs were involved in regulating osteogenic differentiation of PDLSCs (periodontal ligament stem cells) and BMSCs (bone marrow stem cells). This review focuses on the latest research progress of circRNAs in osteogenic differentiation, to help develop new therapies for bone diseases.

Keywords circRNAs; PDLSCs; BMSCs; osteogenic differentiation

环状RNAs(circular RNAs, circRNAs)是一类新型长链非编码RNAs, 较经典含5′端和3′端poly(A)结构的线性RNAs, circRNAs是共价闭合的单链环状结构[1-2](图1)。1976年, 研究者通过电子显微镜在类病毒中首次观察到circRNAs的存在[3], 此后相继在真菌、人、小鼠、大鼠及其他有机体中发现circRNAs的踪迹[4-7]。然而, 由于缺乏可靠的检测方法和已知功能的缺失, 长久以来circRNAs被认为是RNA剪接错误的副产品, 无生物学功能且未引起研究者的重视。由于21世纪转录组测序(RNA-seq)技术的更新和生物信息学的发展, circRNAs的神秘面纱被揭开, 2012年及2013年, 两项影响世界的研究相继报道, circRNAs在哺乳动物体内保守存在且发挥重要功能[1,8], 由此拉开研究circRNAs的热潮。本文就当前circRNAs在成骨细胞分化中的最新研究作一综述。

1 circRNAs的生物学特性及功能

研究发现, 较经典mRNAs分子剪接合成, circRNAs主要由前体mRNA(pre-mRNA)反向剪接生成的一类共价闭合环状特殊RNA结构[1-2,9], 被视为共转录产物, 且在真核细胞中保守存在, 具有组织、疾病和细胞发育阶段的表达特异性和强抗核酸外切酶活性[10-11]。根据circRNAs形成的结构[12](图1), circRNAs可分为4种类型: 外显子环状RNAs(circular exon RNAs, ecircRNAs)、内含子环状RNAs(circular intronic RNAs, ciRNAs)、外显子−内

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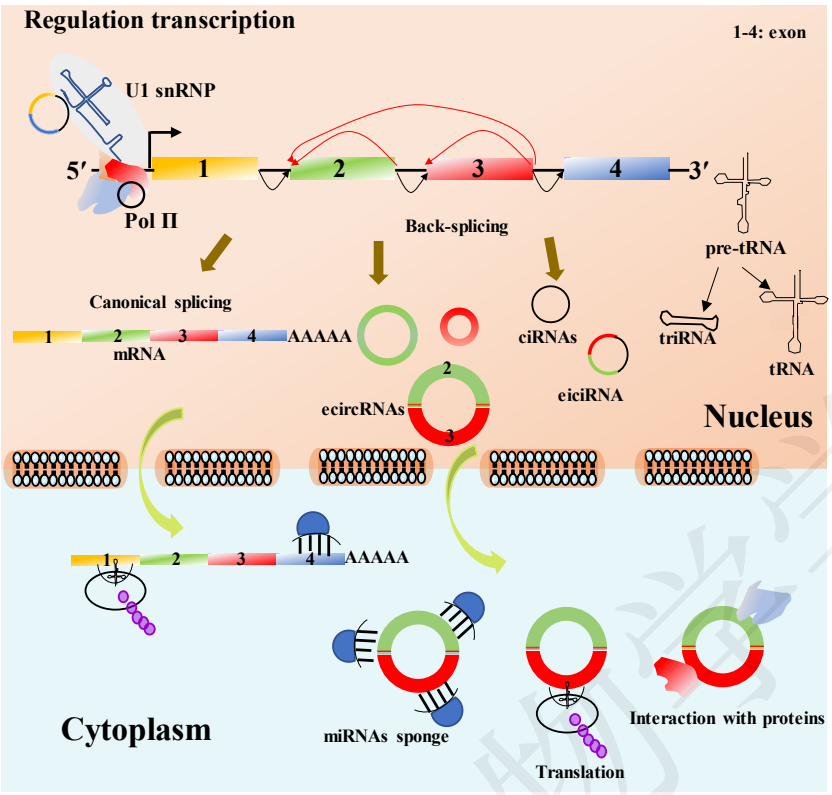

图1 circRNAs的生成、分类及主要功能(根据参考文献[12]修改)

Fig.1 Synthesis, classification and function of circRNAs (modified from reference [12])

含子环状RNAs(exonic-intronic circular RNAs, eiciRNAs)和基因间环状RNAs(intergenic regions circular RNAs)[13]。随着研究的推进, circRNAs的功能不断被揭示。(1) circRNAs吸附miRNAs调控miRNAs靶基因mRNAs的翻译[1], 如circKDM4C在乳腺癌中通过吸附miR-548p, 调节其靶基因*PBLD*(phenazine biosynthesis like protein domain), 抑制肿瘤的进展并减弱阿霉素的耐药性[14]; circHECTD1通过吸附miR-1256激活β-catenin/c-Myc信号通路促进谷氨酰胺分解进而促进胃癌的进展[15]。(2) circRNAs与RNA结合蛋白(RNA binding protein, RBP)结合调节RNA剪接和转录[16], 如盲肌蛋白(muscle blind protein, MBL)能够直接结合circ-MBL, 通过促进circ-MBL的产生来减少自身mRNA的产生。当在果蝇S2细胞系及人HEK293细胞系中过表达*MBL*后会产生更多的circ-MBL, 敲除*MBL*后, circ-MBL则显著减少[17]。(3) circRNAs与mRNAs结合形成双链RNA结构调节mRNAs的稳定性[18], 如研究发现, 小鼠*Fmn*基因对肢体发育起关键作用, 在转录过程中经反式剪接可产生ecircRNAs。当敲除*Fmn*基因编码序列上游的剪接受体后, ecircRNAs则不产生。虽然后代小鼠驱体和四肢发育正常, 但其表现出不同于*Fmn*等位基因突变的渗透性肾发育不全特征, 无繁殖能力[19]。(4) 少数circRNAs能够翻译多肽[20]抑制肿瘤的侵袭和迁移, 如circFBXW7能编码一种新的21 kDa的多肽(FBXW7-185aa), 癌细胞中高表达的FBXW7-185aa可延缓细胞周期循环抑制脑胶质瘤细胞的增殖。

2 circRNAs在PDLSCs成骨分化中的调控作用

牙周膜干细胞(periodontal ligament stem cells, PDLSCs)具有自我更新和多向分化的能力, 参与牙骨质、齿槽骨、周围神经和血管的再生[21], 被认为是牙周骨再生的良好种子细胞[22]。近年来, lncRNAs和miRNAs被证实在调控PDLSCs向成骨分化中发挥重要作用, 如miR-146a、miR-17和miR-22被证实在转录后水平控制其靶基因的表达调节PDLSCs的成骨分化[23-25]; lncRNA-ANCR被证实通过Wnt信号通路调控PDLSCs的成骨分化[26]。相较于lncRNAs和miRNAs, circRNAs是一类新型非编码RNA, 有较强的抗核酸外切酶活性, 在哺乳动物细胞中具有更高的稳定性和序列保守性[27]。

最近, ZHENG等[28]通过RNA-seq分析PDLSCs成骨分化中circRNAs的表达变化发现, circRNAs在PDLSCs成骨分化和生物矿化过程中具有阶段表达特异性, 提示circRNAs可能在成骨分化和成骨过程中发挥重要作用。进一步生物信息学分析表明, 差异表达的circRNAs的基因在胞质或膜结合囊泡和细胞外基质中显著富集, 提示它们在调节细胞外囊泡的生物发生中功能重大; 此外, circRNAs下游的靶标mRNAs同样表现出在包括细胞外基质组织、细胞分化和骨形态发生蛋白等一系列骨形成相关过程中显著差异。GU等[29]发现, PDLSCs成骨分化中circRNAs的表达显著变化, 其中circRNAs-BANP和circRNAs-ITCH可能吸附miR-34a和miR-146a通过MAPK途径(图2)调控PDLSCs的成骨分化, miRNAs 的靶标mRNAs(PDGFRA和TGFBR2; DUSP1和FAS)在成骨分化、MAPK通路、Wnt通路和干细胞多能性等信号通路中具有重要的功能。此外, LI等[30]揭示, PDLSCs成骨分化中circRNA CDR1as显著高表达。体外细胞功能研究发现, 敲除CDR1as的表达抑制了碱性磷酸酶活性、茜素红S染色和成骨基因的表达, 碱性磷酸酶和茜素红S染色是被用来检测成骨细胞活性和矿物沉积。体内研究通过显微CT和组织学分析进一步发现, CDR1as的敲除导致骨形成显著减少。机制研究发现, CDR1as通过抑制miR-7活性, 导致生长分化因子5(growth differentiation factor 5, GDF5)高表达以及Smad1/5/8和p38-MAPK的磷酸化(图2), 揭示了成骨分化的新机制, 为牙周组织和骨再生的治疗提供新的思路和策略。

3 circRNAs在MC3T3-E1成骨分化中的调控作用

成骨细胞分化受到一系列激素、细胞因子和转录因子的严格调控。其中一种重要的细胞因子——BMP2(bone morphogenetic protein 2)被证实在胚胎发生、细胞生长和分化、骨发育和骨折修复等多种生命过程中发挥重要作用[31-33]。如BMP2通过活化小鼠胚胎成骨细胞前体细胞(mouse embryo osteoblast precursor cells, MC3T3-E1)中p38/Smad5信号通路和调节成骨基因的转录来调节成骨活性[21]。BMP2通过β-catenin积累和GSK-3β表达抑制刺激Wnt信号通路, 增强2型糖尿病大鼠骨髓间充质干细胞的骨再生[34]。最近, QIAN等[35]通过RNA-seq对BMP2诱导的MC3T3-E1成骨分化阶段circRNAs的表达发现, 相较对照组, BMP2组中158个circRNAs差异表达, 74个上调表达, 84个下调表达。BMP2组circRNAs-5846、circRNAs-19142和circRNAs-10042显著高表达, 其中

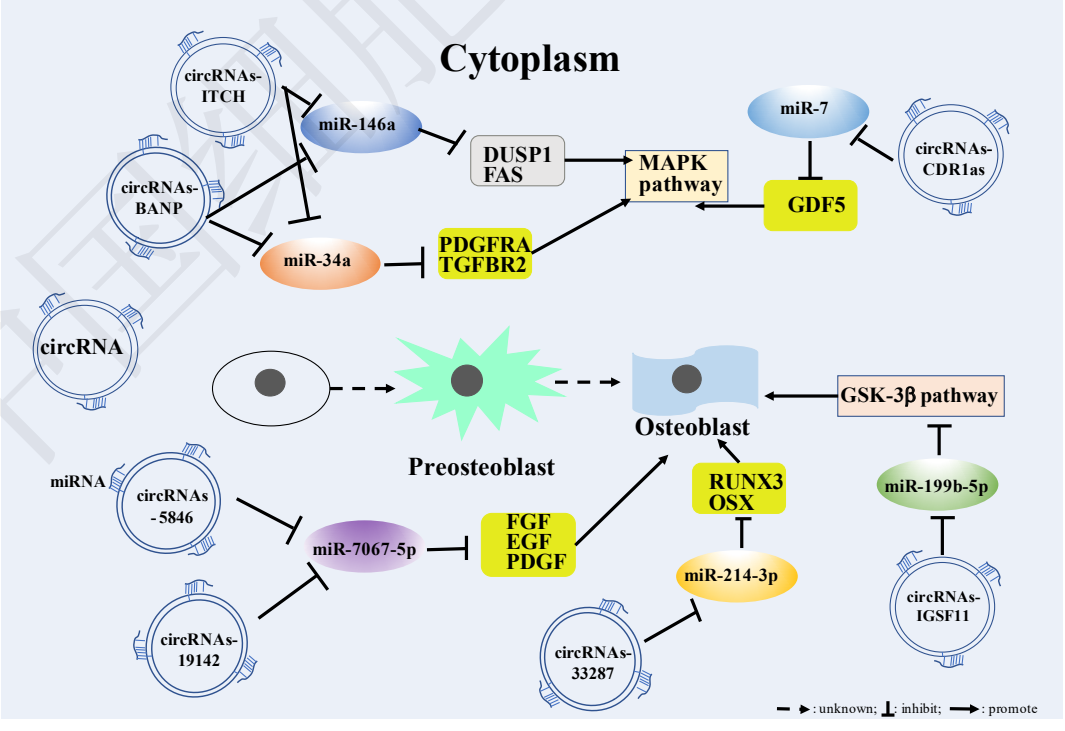

图2 circRNAs在成骨细胞分化中的调控机制

Fig.2 Regulation mechanism of circRNAs in osteogenic differentiation

circRNAs-5846和circRNAs-19142分别可吸附51和21个miRNAs, 共同靶向miR-7067-5p, 而Go和Pathway通路分析显示, miR-7067-5p的靶标mRNAs基因显著参与FGF(fibroblast growth factor)、EGF(epidermal growth factor)、PDGF(platelet-derived growth factor)和Wnt信号通路以及细胞生长和分化(图2)。这表明, circRNAs-19142和circRNAs-5846不仅与发育过程正调控的生物学过程密切相关, 而且与参与细胞生长和分化的FGF、EGF、PDGF和Wnt信号通路也密切相关, 提示BMP2可能通过circRNAs-19142或circRNAs-5846靶向的miR-mRNA诱导成骨分化。

PENG等[36]通过RNA-seq发现, 在BMP2处理组上颌窦膜干细胞(maxillary sinus membrane stem cells, MSMSCs)中50个circRNAs异常表达, 32个上调, 18个下调。其中circRNAs-33287显著表达上调, 随后荧光素酶实验证实, circRNAs-33287能直接吸附结合miR-214-3p。进一步实验证实, 在MSMSCs成骨分化中, circRNAs-33287的缺失显著降低了成骨标志物RUNX3(runt-related transcription factor 3)、OSX(osterix)和碱性磷酸酶的表达, 相反circRNAs-33287的高表达促进了MSMSCs的成骨分化。此外, 研究证实, miR-214-3p可以靶向RUNX3, 抑制MSMSCs的成骨分化, RUNX3的过表达则可部分逆转这种抑制。当MSMSCs被移植到生物松质骨小颗粒支持物Bio-OSS时, circRNAs-33287的高表达显著增强了新骨的形成。这证实, circRNAs-33287能抑制miR-214-3p分子的活性, 并通过激活靶基因*RUNX3*促进的MSMSCs的成骨分化和骨形成, 提示BMP2可能通过刺激circRNAs-33287的产生, 抑制miR-214-3p的活性并促进*RUNX3*的表达来诱导成骨细胞分化(图2), 提示circRNAs可能是一种新的生物标记物或治疗靶点, 用于调节成骨细胞分化和骨形成。

4 circRNAs在BMSCs成骨分化中的调控作用

骨髓干细胞(bone marrow stem cells, BMSCs)是一种被广泛应用于组织工程的多能干细胞, 是成骨细胞的主要来源, 被证实能增强骨修复。此前报道非编码RNA在转录水平和转录后水平严格调控BMSCs的成骨分化[37-39]。最近, ZHANG等[40]通过RNA-seq筛选与BMSCs成骨分化早期相关的circRNAs, 结果发现, 第0天与第7天相比circRNAs差异显著。进一步分析表明, 显著差异的circRNAs亲本基因与细胞发育和细胞黏附密切相关。此外通过生信分析发现circRNAs可调节miRNAs, 并进一步验证了下调的circRNAs-IGSF11与miR-199b-5p之间的负相关作用, 证实circIGSF11在BMSCs成骨过程中下调, 并通过调节GSK-3β信号通路进一步促进成骨细胞分化(图2)。同样地, miR-199b-5p的增加能显著促进钙化结节的形成, 与此同时circRNAs-IGSF11的敲除增加了miR-199b-5p的表达, 促进BMSCs的成骨分化, 表明circRNAs-IGSF11能释放miR-199b-5p的活性介导BMSCs的成骨作用。

5 展望

circRNAs是近年来被揭示的具有重要生物学功能的一类新型RNA分子, 虽然之前被视为转录噪音或转录错误产物而被忽略。然而, 随着RNA-seq和生物信息学的快速发展, circRNAs引起了研究者的广泛关注, 已然成为当前国际研究焦点和热点。

circRNAs自然的闭环结构、较高的表达水平、稳定较强的抗逆性、进化保守性及表达特异性, 使其成为多种疾病的理想诊断生物标志物和治疗靶标。当前研究发现, circRNAs吸附miRNA, 调节其靶基因的表达, 在包括增殖、侵袭、迁移、转移和耐药等多种恶性行为中发挥作用。值得注意的是circRNAs在细胞质中的拷贝数与其功能作用密切相关, 如circSCAF11在胶质瘤组织和细胞系中显著高表达, 与患者预后密切负相关, 敲低circSCAF11后, 可显著抑制胶质瘤细胞的增殖、侵袭和迁移[41]; circSLC8A1在膀胱癌组织和细胞系中显著低表达, 与膀胱癌的病理分期和组织学分级相关, 体内外过表达该分子后均抑制膀胱癌细胞的迁移、侵袭和增殖[42]。此外, 值得庆幸的是逐渐有研究证实, circRNAs通过靶向参与成骨细胞分化的关键转录因子或信号通路, 对成骨细胞分化有重要影响。阐明这些调节性RNA在成骨中的作用对于开发成骨、治疗骨丢失和促进骨折修复的新疗法至关重要。

尽管证明了circRNAs其自身在人类疾病信号通路中的重要作用, 但仍有更多的细节有待阐明。上述大多数研究结果都是通过体外研究获得, 但为了实现将这些研究结果转化为人类临床应用的最终目标, 还需要对活体模型进行更深入的研究, 需要制定有效和安全的治疗方法。

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