长链非编码RNA对干细胞成骨分化的影响

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摘要 随着骨疾病的研究逐渐深入到分子机制水平, 近年来, 对干细胞分化和自我更新能力的研究为多种骨疾病治疗提供了新的视角。长链非编码RNAs(long non-coding RNAs, lncRNAs)是一类转录长度超过200 nt的RNA分子, 它们不直接参与蛋白质的编码, 而是通过参与染色质重构、DNA甲基化、组蛋白修饰并作为miRNA的前体, 来调节细胞的增殖和分化过程。最新研究表明, lncRNAs在维持骨代谢的动态平衡中发挥关键性的调控作用, 并通过多种途径参与干细胞向成骨分化的过程。因此, 该文通过综述国内外lncRNAs调节多种干细胞向成骨分化的相关研究, 阐述lncRNAs诱导不同干细胞成骨分化的研究进展, 为进一步探索lncRNAs在调节干细胞的功能和机制及干细胞疗法对骨代谢相关疾病治疗和预防中提供更加可靠的理论依据。

关键词 LncRNAs; 干细胞; 成骨分化

The Effect of lncRNAs on Osteogenesis Differentiation of Stem Cells

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Abstract With the development of science and technology, the studies on bone diseases research have gradually penetrated into molecular mechanism. In recent years, stem cells differentiation and self-renewal ability provides a new perspective for treatment of a variety of bone diseases. Long non-coding RNAs (lncRNAs) are a class of RNAs molecules with a length of more than 200 nt. They are not directly involved in the protein coding. They are involved in chromatin remodeling, DNA methylation and histone modification, and serve as precursors of miRNA to regulate cell proliferation and differentiation. Latest investigation shows that lncRNAs play a key role in maintaining the dynamic balance of bone metabolism, and is involved in the process of stem cell differentiation to osteogenesis through various ways. Therefore, this article reviews the studies on the regulation of multiple stem cells to osteogenic differentiation by lncRNAs. The progress of lncRNAs on differentiation of bone induced by different stem cells, to further explore the function and mechanism of lncRNAs in regulating stem cells, and stem cell therapy provides a more reliable theoretical basis for the treatment and prevention of bone metabolic diseases.

Keywords lncRNAs; stem cells; osteogenesis differentiation

随着世界人口老龄化进程的加快, 骨性疾病的发病率逐年上升[1]。骨性疾病的发生是由于骨骼内动态平衡失衡导致的。其中, 骨骼动态平衡是由成骨细胞主导的骨形成和破骨细胞主导的骨吸收失

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衡导致, 当骨吸收大于骨形成则会导致骨退行性疾病, 因此, 有关干细胞向成骨分化成为研究重点。随着研究水平的深入, 研究发现成骨分化受多种非编码RNA的调节, 通过Wnt/β-catenin、骨形态发生蛋白(bone morphogenetic protein, BMP)等骨代谢信号通路来调节*RUNX2*(Runt-related transcription factor 2)、*OCN*(osteocalcin)、*ALP*(alkaline phosphatase)等成骨分化相关基因的转录, 进而调节成骨分化的进行[2]。人类基因组中编码基因约占2%, 而91%的基因序列为非编码RNAs(non-coding RNAs, ncRNAs)[3]。其中, 一种长度超过200 nt的ncRNAs, 占ncRNAs总量的80%以上, 它们不直接参与蛋白质的编码, 称为长链非编码RNAs(long non-coding RNAs, lncRNAs)[4]。近年来, 关于lncRNAs结构、功能、作用机制的研究取得了实质性的突破。有研究表明, lncRNAs参与染色质重构、DNA甲基化、组蛋白修饰并作为miRNA的前体, 进而调节细胞的增殖和分化过程[5]。

干细胞(stem cells, SCs)是一种具有特定分化潜能及自我更新能力的细胞, 可分化为成骨细胞、脂肪细胞、软骨细胞、肌细胞、脂肪细胞和纤维细胞等, 在生物学和再生医学领域受到广泛关注[6]。干细胞疗法已被用于癌症的治疗、受损组织的修复和各种退行性疾病的治疗[7]。体内成骨细胞主要来自骨髓间充质干细胞(bone marrow derived stroma cells, BMSCs)[8], BMSCs向成骨分化可作为成骨细胞的分化的金标准[9]。体内具有良好的组织再生和修复潜能的脂肪间充质干细胞、牙髓干细胞、牙周干细胞等, 通过体外诱导也可分化为成骨细胞[10-14], 因此, 干细胞疗法也被应用于各种骨性疾病中[15]。

目前已有研究发现, lncRNAs可以调控干细胞向成骨细胞的分化和增殖[16], 对骨形成或骨吸收过程产生关键性的影响。细胞外的机械和分子信号精确地调节干细胞向成骨分化的转录及转录后各个水平, 调节成骨分化进程[17]。因此, 本文通过综述lncRNAs对干细胞成骨分化影响的最新研究(表1), 为进一步探究长链非编码RNA对骨代谢的作用和机制的研究奠定理论基础, 以此来更好地预防并治疗各类骨性疾病。

1 LncRNAs调节BMSCs成骨分化

BMSCs具有强大的增殖和多向分化能力, 其较强的成骨分化潜能可保持骨坏死组织的修复能力, 在适宜的体外或体内环境通过多种途径可诱导BMSCs分化为成骨细胞、软骨细胞、造血细胞等多种细胞, 因此成为目前骨组织工程研究领域的理想细胞[18]。

1.1 LncRNAs调控BMSCs成骨分化的差异性表达

LncRNAs在调节BMSCs成骨分化前后表达具

表1 LncRNAs调控干细胞成骨分化作用及途径

Table 1 LncRNAs regulates the differentiation and pathway of stem cells

干细胞类型 Stem cell types	信号通路 Pathways	功能 Functions	参考文献 References
BMSCs	lncRNA ak028326/CXCL13	Promote	[22]
	lnc RNA XR-11050/RUNX2	Promote	[24]
	lncRNA HoxA-AS3/EZH2/H3K27me3	Inhibition	[30]
	lncRNA MEG3/miR-133a-3p	Inhibition	[26]
	lncRNA H19/miR-675/TGF-β1 lncRNA H19/miR-675/Smad3/HDAC	Promote/inhibition	[27]
	lncRNA H19/miR-141, miR-22/β-catenin lncRNA H19/miR-675- 5p/β-catenin	Promote Inhibition	[28]
DTSCs	lncRNAs FR249114, FR299091 and ENST00000450004/NF-κB, TFIIB and NR3C1	–	[37]
	lncRNA-POIR/miR-182/TCF-4/Wnt lncRNA-POIR/miR-182/FoxO1	Inhibition/promote	[38]
	lncRNA ANCR/β-catenin	Promote	[41]
	lncRNA MODR/miR-454/RUNX2	Promote	[42]
ASCs	lncRNA MIAT/TNF	Inhibition	[45]
	lncRNA MIR31HG/p65/IκBα lncRNA MIR31HG/NF-κB	Promote/inhibition	[46]

有差异性。Wang等[19]诱导人BMSCs成骨分化, 通过lncRNAs芯片和相关生物信息分析, 得出在成骨分化中有1 206条lncRNAs表达有差异性: 687条上调、519条下调, 其中lncRNAs(H19和uc022axw.1)表达上调, 在成骨分化进程中发挥重要作用。Xie等[20]诱导BMSCs成骨分化10天, lncRNA芯片及相关分析得出520条lncRNAs和665条mRNA差异性表达, 包括TGF-β(transforming growth factor-β)的64个信号通路有明显差异, 4个具有明显差异表达的lncRNA(lnc-ZNF354A-1、lnc-LIN54-1、lnc-FRG2C-3和lnc-USP50-2)使BMSCs成骨分化异常, 加速脊髓灰质炎的病变进程。Cui等[21]在炎性环境中诱导BMSCs成骨分化, 通过lncRNAs芯片分析发现, 在2 033条lncRNAs中, 641条下调和1 392条上调, 并筛选出NONHSAT009968。通过抑制NONHSAT009968表达发现, 成骨分化标志物RUNX2、OCN(osteocalcin)、OPN(osteopontin)和COL1A1(type 1 collagen α1)表达量增加, 表明抑制lncRNAs NONHSAT009968表达可促进BMSCs成骨分化, 并改善炎性环境对成骨分化的抑制作用。Zuo等[22]使用BMP2(bone morphogenetic protein 2)诱导小鼠间充质干细胞系C3H10T1/2向成骨分化, 发现lncRNAs ANR_027652和DLK1(delta-like 1 homologue)表达均上调, lncRNAs 0231和EGFR(epidermal growth factor receptor)表达均下调。因此, lncRNAs H19 uc022axw.1、lncRNA(ZNF354A-1、LIN54-1、FRG2C-3和USP50-2)、lncRNAs NONHSAT009968、lncRNAs ANR_027652等差异性表达对BMSCs向成骨分化过程中起着重要的调节作用。

1.2　LncRNAs直接调控BMSCs成骨分化

LncRNAs在调控BMSCs成骨分化过程中, 可直接作用于骨形成标记蛋白, 进而来调节成骨分化过程。Cao等[23]在高糖环境下诱导小鼠BMSCs成骨分化, 通过ak028326或CXCL13过表达降低了高糖环境对BMSCs成骨分化的抑制影响, 进一步分析得出, CXCL13可正调控ak028326基因表达。RUNX2蛋白在成骨细胞分化的多种信号途径中起中心作用, 是非常重要的标记性蛋白, 作为成骨分化的显著标记物[24]。Zhang等[25]通过诱导人BMSCs向成骨分化, 并进行lncRNAs芯片检测发现, lncRNA XR-11050在成骨分化的过程中表达上调, lncRNA XR-11050过表达促进了RUNX2的表达, 进而影响下游骨形成相关蛋白基因的表达, 促进BMSCs向成骨分化。因此, 以上结果证明, lncRNA ak028326和lncRNA XR-11050分别通过调控CXCL13和RUNX2表达, 进而促进BMSCs的成骨分化。

1.3　LncRNAs通过下游miRNA作用于BMSCs成骨分化

目前, 关于增强子和miRNA非编码RNA参与调控干细胞向成骨分化的报道很多, 随着研究的逐渐深入, lncRNA与miRNA协同调控BMSCs成骨分化报道也明显增多。

最新的研究证明, lncRNAs与microRNAs(miRNAs)作为相互竞争的内源性RNAs(ceRNAs)参与调节MSC的分化[26]。LcnRNAs通过调控竞争性结合的miRNAs, 进而调节转录后水平, 影响BMSCs成骨分化。Song等[27]诱导BMSCs成骨分化28天, 使用高通量RNA测序技术分析发现32种lncRNAs是miRNA(miR-689、miR-640、miR-601和miR-544)前体, 217条lncRNAs与miRNA之间存在同性共表达的关系, 并通过调节肿瘤、ECM受体和黏着斑来影响骨髓间充质干细胞的成骨分化。Wang等[28]分别诱导去卵巢小鼠和绝经后骨质疏松病人的BMSCs向成骨分化, 发现lncRNA MEG3和miR-133a-3p表达均增加, 且两者呈正相关。BMSCs向成骨细胞分化中MEG3过表达时, miR-133a-3p介导的成骨分化表达下调, 同时伴随SLC39A1(solute carrier family39)显著下降[28]。因此, 上述结果证明, lncRNA MEG3促进了miR-133a-3p的表达, 抑制SLC39A1及成骨分化基因表达, 抑制BMSCs成骨分化, 加速骨质疏松症发生。Huang等[29]发现, BMSCs成骨分化lncRNA H19表达上调, H19过表达促进体外人BMSCs成骨分化和体内异位骨形成, 抑制H19表达抑制成骨分化和体内异位骨形成。进一步研究发现, H19联合miR-675可促进人BMSCs成骨分化, 进而调节下游转化TGF-β1 (transforming growth factor-β1)表达[29]。同时, H19可抑制Smad3(drosophila mothers against decapentaplegic)磷酸化进程, 下调HDAC(histone deacetylase)基因和蛋白表达水平, 进而增加了成骨分化标记基因表达。因此, 他们证明了H19可通过促进H19/miR-675/TGF-β1通路和抑制H19/Smad3/HDAC通路来调控hMSCs成骨分化。Liang等[30]研究发现, 在hMSCs的成骨分化过程中lncRNA H19表达上调。进一步研究发现, lncRNA H19作为miR-141

和miR-22的海绵, 抑制其表达, 进而促进骨生成和Wnt/β-catenin通路的表达。此外, 还发现了lncRNA H19与miR-675-5p之间的一种新的负反馈调节, lncRNA H19是miR-675-5p直接靶向基因, 并对抗成骨细胞分化, 抑制MSCs成骨分化。LcnRNAs通过调控增强子的表达影响BMSCs向成骨分化。Zhao等[31]研究发现, 当抑制h19的表达后, miR-675表达降低, NOMO1蛋白表达受到抑制, 进而抑制了BMSCs的成骨分化进程。Zhu等[32]在诱导人BMSCs成骨分化过程中发现lncRNA HoxA-AS3的抑制作用。通过抑制lncRNA HoxA-AS3表达, 发现成骨转录基因*RUNX2*、*COL1A1*等表达增加, 进一步研究发现, HoxA-AS3、EZH2及H3K27me3三者联合调控RUNX2的表达, 抑制BMSCs的成骨分化[32]。Shang等[33]上调BMSC向成骨和成脂分化中lncRNA TCONS_00041960的表达, 发现TCONS_00041960通过与miR-204-5p和miR-125a-3p内源性竞争进而促进RUNX2及GILZ(anti-adipogenic gene glucocorticoid-induced leucine zipper)的表达。因此, 他们证明, 新的lncRNA TCONS_00041960通过TCONS_00041960-miR-204-5p/miR-125a-3p-RUNX2/GILZ调节BMSCs向成骨分化的重要作用。LcnRNAs可以直接参与BMSCs成骨分化, 也可以通过调节增强子和miRNA的表达来调节BMSCs向成骨分化的进程, 进而来调节骨形成和骨吸收, 维持骨量的恒定或加速骨性疾病的发生。

2 LncRNAs调节其他干细胞成骨分化

多能干细胞在不同的解剖位置有不同的生物活性, 维持多能分化状态依赖数千个基因的表达和调控。体外诱导多能干细胞技术为直接从人体组织细胞再生无限多能干细胞提供有效的手段[34]。许多研究表明, lncRNAs在多种生物过程中发挥重要的作用, 包括细胞分化、转录、印迹、染色体修饰等方面的作用, 与调节细胞分化也有密切关系[35]。

2.1 LncRNAs调节牙周组织干细胞成骨分化

牙周组织干细胞(dental tissue-derived stem cells, DTSCs)是来自干细胞具有自我增殖和多向分化潜能的牙齿组织, 其中包含牙髓干细胞(dental pulp stemcells, DPSCs)、牙周韧带干细胞(periodontal ligament stem cells, PDLSCs)、上颌窦膜干细胞(maxillary sinus membrane stem cells, MSMSCs)及根尖牙乳头干细胞(stem cells from the apical papilla, SCAP)。DTSCs是牙组织工程中重要的多能干细胞, 离体可以通过不同的方法诱导DTSCs向成骨细胞、脂肪细胞和软骨细胞分化[36-37], 在体诱导可重新生成牙髓和牙本质组织。Dong等[38]通过研究BMSCs和PDLSCs lncRNA和miRNA表达谱及lncRNA生物信息学分析发现, lncRNAs 457条表达上调, 513条表达下调, 证明了DTSCs增殖和分化过程中lncRNAs作为重要的调节因子。

Jia等[39]体外诱导DTSCs多向分化, 发现下调lncRNAs ANCR表达时, DTSCs向成骨细胞、脂肪细胞和神经细胞的分化得到促进, 但是DPSCs和SCAP增殖影响较小, 证明lncRNA ANCR可促进DTSCs成骨分化。Yi等[40]诱导老年和青年DPSCs成骨分化, 发现青年组较老年组DPSCs中lncRNAs有389条上调, 172条下调; mRNA有247条上调, 57个基因下调。进一步的生物信息分析发现, FR249114、FR299091和ENST00000450004作为核心lncRNAs, 调节NF-κB、TFIIB(transcription initiation factor IIB)和NR3C1(nuclear receptor subfamily 3 group C member 1)表达, 调控细胞周期和RNA运输, 进而调控DPSCs成骨分化。Wang等[41]诱导牙周炎病人PDLSCs成骨分化, 通过lncRNAs芯片分析发现, 牙周炎患者成骨分化增强时lncRNA-POIR表达明显降低。离体和在体实验发现, lncRNA-POIR和miR-182为内源性竞争关系, 导致靶基因FoxO1(forkhead box O1)表达受阻。其中, FoxO1和TCF-4(T cell factor-4)通过Wnt/β-catenin[42]和NF-κB[43]信号通路调节PDLSCs成骨分化。因此, 以上结果证明了lncRNA-POIR抑制miR-182表达, 并通过TCF-4抑制Wnt/β-catenin信号通路促进FoxO1的表达, 促进PDLSCs成骨分化。Jia等[44]诱导PDLSCs成骨分化中lncRNA ANCR表达下调, 通过相关实验分析得出抑制lncRNA ANCR表达后DKK1(dickkopf-related protein 1)、GSK-3β(glycogen synthase kinase-3β)和β-catenin表达增加, Wnt信号通路表达增加, 因此, 通过抑制lncRNA ANCR表达促进Wnt信号通路表达, 进而促进PDLSCs成骨分化过程。Weng等[45]体外诱导MSMSCs成骨分化, 过表达lncRNA MODR后MSMSCs成骨分化增加, 抑制lncRNA MODR表达, 使成骨分化标记物RUNX2表达降低。进一步分析得出, lncRNA MODR和miR-454为内源性竞争关系, 通过抑制miR-454表达, 使miR-454/RUNX2途径中RUNX2表达受到抑制, 因

此, lncRNA MODR通过抑制miR-454表达, 促进MSMSCs成骨分化。

DTSCs向成骨分化主要是通过Wnt/β-catenin和NF-κB信号通路来调节, lncRNA作为重要的调节因子, 通过不同的途径产生调节作用: 其中lncRNA ANCR通过直接作用于Wnt/β-catenin信号通路相关细胞因子, 调节RUNX2表达, 抑制DTSCs成骨分化; 此外, lncRNA POIR和MODR抑制miR-182和miR-454的表达调节Wnt/β-catenin和NF-κB信号通路, 进而促进DTSCs成骨分化。

2.2 LncRNAs调节脂肪间充质干细胞成骨分化

脂肪间充质干细胞(adipose-derived stromal cells, ASCs)与BMSCs相比是一种更加丰富和容易获得的细胞群, 且具有多能分化的潜能, 因此将其纳入软组织重建疗法中。但由于ASCs在体外易向脂肪分化, 因此体外诱导成骨分化相对较难, 因此相关研究较少[46]。Wang等[47]诱导正常ASCs成骨分化, 发现2 775条lncRNAs和2 439条mRNA表达存在协同性, 通路富集分析表明, 32个通路上调而7个通路表达下调。Jin等[48]诱导ASCs成骨分化, 发现lncRNA MIAT表达下调。通过离体和在体抑制lncRNA MIAT实验, 发现ASCs成骨分化增加, 而且MIAT可增加TNF介导的炎症反应。因此, 通过抑制lncRNA MIAT表达可促进ASCs成骨分化并抑制成骨分化中炎症反应得到证明。Jin等[49]为证明, lncRNA MIR31HG在人ASCs成骨分化中的作用, 进行沉默MIR31HG表达实验, 发现ASCs成骨分化得到促进, 同时成骨分化炎症反应受到抑制。通过进一步实验发现, 启动子p65与MIR31HG结合并促进MIR31HG的表达, 调节抑制蛋白IκBα(I-kappa-B-alpha)并参与NF-κB活化, 进而建立NF-κB调节通路, 破骨细胞增多使炎症反应增加。因此, 他们证明了抑制lncRNA MIR31HG表达可促进ASCs成骨分化, 同时lncRNA MIR31HG和启动子p65结合, 抑制IκBα表达及增加NF-κB的活化, 进而增加炎症反应。由于目前针对lncRNAs调节ASCs成骨分化的研究较少, 所以有关lncRNAs的发现也比较少, lncRNA MIAT和MIR31HG调控的成骨分化过程及介导的炎症反应具体的信号通路仍需要进一步研究。

3 小结

长链非编码RNA是非编码RNA的一种重要类型。lncRNAs对于多种疾病的调控至关重要, 包括神经、自身免疫和心血管疾病以及癌症等。目前, 国内外lncRNAs的研究多为基础阶段, 通过lncRNAs芯片和测序技术比较干细胞成骨分化中lncRNAs、mRNA表达的差异性, 但也有研究报道, 一些lncRNAs, 如MEG3、H19、MIAT、MODR、POIR、HoxA-AS3及lncRNA-ANCR等, 在正常和异常的条件下调控骨髓间充质干细胞或多能干细胞的成骨分化[50]。LncRNAs可以通过直接作用于干细胞促进成骨分化, 也可以通过调控下游miRNA的表达来调控干细胞成骨分化, 还可以通过与内源性miRNA相互抑制表达, 共同调控干细胞内的成骨分化。通过生物信息分析并得到验证的lncRNAs调控干细胞成骨分化的信号通路主要Wnt/β-catenin、NF-κB、TGF-β1/Smad及TNF信号通路, 进而来调节骨形成和骨吸收组成的骨代谢稳态。根据上述文献报道, lncRNAs可能通过lncRNA-miRNA-mRNA和内源性竞争假说(图1)这两种不同的方式调节成骨分化进程。但有关lncRNAs调节干细胞成骨分化的功能和机制的研究还是非常少, 因此, lncRNAs调节干细胞的成骨分化机制和功能仍然存在很多的未知, 其机制有待更加深入的研究。有关lncRNAs调节骨代谢平衡及其相关机制更是今后的研究热点。

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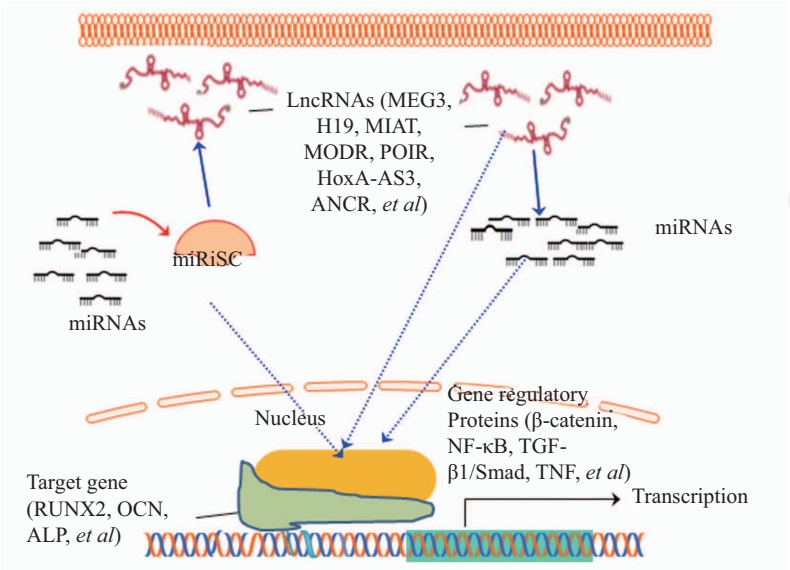

图1 LncRNAs调节干细胞成骨分化途径

Fig.1 LncRNAs regulates the osteogenesis differentiation of stem cells

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