

微小 RNA、长链非编码 RNA 及二者相互作用调控成骨细胞功能的研究进展

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摘要: 成骨细胞是一类特殊的具有成骨潜能的细胞, 在骨重建及骨稳态维持中发挥重要的作用, 其分化过程受到众多因素的调控。非编码 RNA(non-coding RNAs, ncRNAs), 尤其是微小 RNA(microRNAs) 和长链非编码 RNA(long non-coding RNAs, lncRNAs) 在成骨细胞增殖、分化、矿化和凋亡等多种生理过程中均发挥着重要的作用。近年来研究发现 microRNAs 和 lncRNAs 可以相互调控, 构成复杂的生物学调控网络, 但在成骨细胞中的具体作用机制尚未完全明确。本文概述了 microRNAs、lncRNAs 及两者相互作用调控成骨细胞功能的研究进展。

关键词: 微小 RNA; 长链非编码 RNA; 成骨细胞; 凋亡; 基因

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Research advances in microRNAs and long non-coding RNAs and their interaction to regulate the function of osteoblasts

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Abstract: Osteoblasts are a kind of special cells with osteogenic potential, which play an important role in bone reconstruction and homeostasis. Osteoblast differentiation is regulated by multiple factors. Non-coding RNAs (ncRNAs), especially microRNAs and long non-coding RNAs (lncRNAs), are of great importance in the proliferation, differentiation, mineralization and apoptosis of osteoblasts. It has been proved that microRNAs and lncRNAs can regulate each other and form a complex biological regulatory network. However, the microRNAs-lncRNAs interaction in osteoblasts has not been fully clarified. Recent advances in the microRNAs, lncRNAs and their interactions in osteoblasts are reviewed in this paper.

Keywords: microRNA; long non-coding RNA; osteoblast; apoptosis; gene

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成骨细胞起源于间充质干细胞 (mesenchymal stem cells, MSCs), 是新骨形成的关键细胞, 对骨骼的生长和维持至关重要。成骨细胞在许多骨疾病, 特别是在骨质疏松症、骨发育不良和原发性骨肿瘤的发病机制中起着至关重要的作用^[1]。在骨组织中, 骨形成取决于成熟成骨细胞的数量和功能, 与成骨细胞的形成、寿命和活性密切相关。

而成骨细胞的数量和活性均由细胞转录和表观遗传机制控制, 并受到激素、机械应力和细胞间相互作用等方式调节^[2-4]。

人类基因组中只有 1%~2% 的基因可编码蛋白质, 其余 98% 曾被认为是“垃圾”DNA, 然而越来越多的特异性非编码 RNA(non-coding RNAs, ncRNAs) 被认为是某些生物学过程的关键性调控因子, 包括调控基因表达、细胞周期、染色质重塑和表观遗传修饰等^[5]。ncRNAs 中有一部分是管家 ncRNA(house-keeping non-coding RNA), 包括核糖体 RNA、转运 RNA、胞质小 RNA 和核内小 RNA 等, 这些 RNA 分子直接或间接参与蛋白质的表达。大部分 ncRNA 则是在一定条件下诱导表达, 可以调节蛋白质编码基因的表达, 因而被称

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为调节性 ncRNA(regulatory non-coding RNA), 依据分子大小可分为短链非编码 RNA 和长链非编码 RNA^[5]。短链非编码 RNA 分子小于 50 nt, 主要分为微小 RNA(microRNAs, miRNAs)、内源性小干扰 RNAs(endo-siRNAs) 和 PIWI 相互作用 RNAs(piRNAs)。长链非编码 RNA(long non-coding RNAs, lncRNAs) 则主要指分子大于 200 nt 的调节性非编码 RNA。microRNAs 和 lncRNAs 在成骨细胞蛋白质基因表达中发挥着重要的调节作用, 其相关研究较多也较为深入, 本文就近年来 microRNAs、lncRNAs 及其相互作用调控成骨细胞功能的研究进行综述。

1 microRNAs 调控成骨细胞功能

microRNAs 是长度为 22 nt 左右 (18 ~ 25 nt) 的内源性非编码 RNA, 可通过与特定信使 RNA 3' UTR 区结合形成 miRNA 诱导的沉默复合物, 负向调控基因表达, 从而诱导其降解或抑制其翻译^[6]。哺乳动物 miRNAs 的合成受到精细的调控, 一般来说是在 RNA 聚合酶 II 催化作用下转录产生 miRNA 的初级前体 (pri-miRNAs), 经 RNase III 酶 Droscha 和 pri-miRNA 结合蛋白 DGCR8 组成的微处理器加工为具有发卡结构的前体 miRNAs(pre-miRNAs), 然后通过输出蛋白 (exportin 5) 转运到细胞质中。在细胞质中, pre-miRNAs 被 Dicer 切割为成熟的 RNA 双链, 与 AGO 蛋白结合, 将特定的 miRNA 链整合到 RNA 诱导沉默复合物中, 进而靶向调节靶基因 mRNA 表达^[7]。

1.1 microRNAs 在成骨分化过程中的改变 成骨细胞的分化过程以成骨细胞特异基因的顺序激活为标志, 经过细胞增殖、细胞外基质沉积、成熟和矿化后, 一部分走向凋亡, 另一部分则被周围矿化基质包埋形成骨细胞。在成骨细胞分化过程中, 众多 miRNAs 的表达会发生改变。Oskowitz 等^[8] 研究发现如果抑制人骨髓间充质干细胞 (human bone marrow mesenchymal stem cells, hMSCs) 中 miRNAs 的合成, 其成骨分化能力减弱, 并发现 hMSCs 向成骨细胞分化过程中有 19 个 miRNAs 表达上调。Gong 等^[9] 通过 Satb2 诱导骨髓基质干细胞向成骨细胞分化, 发现了 miR-27a 等 10 个下调的 miRNAs 及 miR-17 等 18 个上调的 miRNAs, 生物信息学分析显示这些 miRNAs 的靶基因参与了多条成骨分化通路, 与骨形成及骨骼发育密切相关。而通过 BMP2 诱导骨髓基质干细胞向成骨细胞分化的过程中有 22 个 miRNAs 表达发生了显著变化, 且其中具有代表性的 miR-133 和 miR-

135 均可促进骨形成^[10]。

1.2 microRNAs 调控成骨细胞分化 成骨细胞分化过程中, miRNAs 表达发生改变, 且在成骨细胞分化基因转录后调控中发挥着关键作用。Runx2 是成骨细胞分化的主开关, 可以调节成骨细胞中 Col I、ALP 和骨桥素等多个分化相关基因的表达, miR-433、miR-103a、miR-628-3p、miR-155、miR-204、miR-205-5p、miR-505 和 miR-30 家族等可以降低间充质干细胞 (mesenchymal stem cells, MSC) 及成骨细胞中 Runx2 的表达并抑制成骨分化^[11-18]。成骨细胞分化过程还受到骨形态发生蛋白 (bone morphogenetic protein, BMP) 信号的调节, miR-542-3p、miR-98 可分别靶向抑制 BMP-7 及 BMP-2^[19-20], miR-222-3p、miR-155、miR-106b-5p 和 miR-17-5p 则可通过特异性阻碍 Smad5 的翻译中断 BMP 信号通路, 从而导致成骨抑制^[21-23]。同时部分 miRNAs 可通过靶向成骨分化过程中的一些关键基因抑制成骨细胞分化, 如 miR-637 靶向 Osterix^[24]、miR-186 靶向 SIRT6^[25]、miR-143 靶向 k-RAS^[26]、miR-3077-5p 和 miR-705 靶向 HOXA10^[27], miR-214 抑制 ATF4 和成纤维细胞生长因子受体 1 (fibroblast growth factor receptor 1, FGFR1)^[28-29]。另有研究表明, miRNAs 不仅可以通过靶向成骨因子抑制成骨细胞分化, 还可通过靶向成骨抑制因子促进成骨细胞分化。组蛋白脱乙酰酶 (histone deacetylase, HDAC) 是一种特殊的蛋白质, 能够负向调控 Runx2 等基因的表达, 并可受到 miRNAs 调控^[30]。miR-449a 可抑制成骨细胞 HDAC1 的表达, 维持组蛋白乙酰化状态, 刺激 Runx2 基因表达^[31]。miR-233 可抑制小鼠 MC3T3-E1 前成骨细胞中的 HDAC-2 表达, miR-873-3p 可抑制大鼠成骨细胞 UMR106-01 中 HDAC4 的表达, 进而促进成骨细胞分化^[32-33]。miR-143 则可通过抑制 HDAC7 促进成骨细胞分化, 且可以促进内皮细胞血管生成, 小鼠体内过表达 miR-143 可有效改善骨质丢失及老年性骨质疏松^[34]。BMP 途径的下游调节因子 Smad 1、Smad 5、Smad 6 和 Smad 7 可结合 Smad 泛素调节因子 1 (Smad ubiquitination regulatory factor-1, Smurf-1) 诱导 E3 泛素连接酶依赖性蛋白降解^[35]。miR-590-5p 通过靶向 Smad7, miR-503、miR-15b 和 miR-17 则通过靶向 Smurf-1, 间接保护 Runx2 降解减少而促进成骨细胞分化^[36-39]。Hsc70 相互作用蛋白/STIP1 同源性的 C 末端和含有蛋白 1 的 U-Box(CHIP/STUB1) 可通过促进 Runx2 蛋白降解而负调控成骨细胞分化,

miR-764-5p 则可抑制 CHIP 蛋白翻译而促进成骨分化^[40]。He 等^[41]研究发现, miR-20b 可通过抑制 PPAR γ 、Bambi 和 Crim1 在多个阶段激活 BMPs/Runx2 信号通路而促进成骨。miR-335-5p 则可通过下调 Dickkopf 相关蛋白 1 上调 β -catenin 表达, 激活 Wnt 信号通路, 促进成骨分化^[42]。另有一项研究表明, miR-29b 对成骨分化具有正性调控作用, 可直接下调已知的成骨细胞分化抑制因子 HDAC4、TGF- β 3 和 ACVR2A 等, 并可抑制胶原合成, 促进骨质矿化^[43]。

1.3 microRNAs 调控成骨细胞增殖和凋亡
miRNAs 同时也能调控成骨细胞的增殖和凋亡^[44]。研究发现, miR-17-92 基因簇可促进成骨细胞增殖, 抑制其凋亡^[45-46]。microRNA-23a 则可通过调节 Fas 的表达, 抑制小鼠成骨细胞凋亡^[47]。相反, miR-182 可通过抑制 FoxO1 减少成骨细胞增殖, 促进其凋亡^[48]。Wei 等^[49]研究发现, miR-34b/c 基因敲除小鼠模型中成骨细胞数量增加, 细胞实验进一步证实 miR-34b/c 通过抑制细胞周期蛋白 D1 积累来抑制成骨细胞的增殖。miR-542-3p 的过表达则可通过抑制 BMP-7 及其下游 PI3K/survivin 通路促进成骨细胞凋亡^[19]。

2 lncRNAs 调控成骨细胞功能

lncRNAs 是长度超过 200 nt 的非编码 RNA, 大多数由 RNA 聚合酶 II 转录产生, 具有 mRNA 的结构特征 (5' 帽式结构和 3' polyA 尾), 但没有长阅读框架。lncRNAs 可以定位于胞核和胞质中, 通过多种机制调节基因表达。定位于细胞核的 lncRNAs, 主要参与蛋白质编码基因表达的转录调控和表观遗传调控等; 而定位于细胞质的 lncRNAs, 则主要参与转录后基因调控过程, 尤其是与 microRNAs 相互调控^[5]。

2.1 lncRNAs 在成骨分化过程中的改变 通过高通量技术检测发现, lncRNAs 在成骨分化过程中表达发生改变。Zuo 等^[50]研究发现, 骨髓间充质干细胞 C3H10T1/2 诱导分化过程中有 116 个差异表达的 lncRNAs, 并发现了 24 对 lncRNAs 和附近的 mRNAs 协同差异表达。Qiu 等^[51]在 hBMSCs 的成骨分化中发现 433 个持续上调和 232 个持续下调的 lncRNAs。Song 等^[52]在 MSC 成骨分化过程中发现 574 个 lncRNAs 的表达发生显著改变, 其中 TCONS_00046478、TCONS_00027225 和 TCONS_00007697 可能作为 miR-689、miR-544 和 miR-640 的前体调控共表达基因 (Col4A4、Col21A1 和 WNT2) 在成骨分化中发挥作用。Wang 等^[53]在

hBMSCs 成骨分化过程中的 lncRNAs 微阵列分析鉴定出 1206 个差异表达的 lncRNAs, 其中 H19 和 uc022axw.1 可能在成骨过程中起重要作用。Zhang 等^[54]认为 MSC 成骨分化过程中 1408 个差异表达明显的 lncRNAs 中有 6 个核心调控因子 (NR_024031、XR_111050、FR148647、FR406817、FR401275 和 FR374455), 且 XR_111050 具有促进成骨细胞分化的潜能。Xie 等^[55]研究发现强直性脊柱炎患者骨髓 MSC 较正常人 MSC 具有更强的成骨分化能力, 微阵列分析结果显示有 520 个差异表达明显的 lncRNAs, 其中 lnc-ZNF354A-1、lnc-LIN54-1、lnc-FRG2C-3 和 lnc-USP50-2 可能参与了强直性脊柱炎患者骨髓 MSC 的成骨分化异常。在其他类型的细胞中, 如人牙周膜干细胞 (human periodontal ligament stem cells, hPDLSCs)、人脂肪干细胞 (human adipose-derived stem cells, hASCs) 和小鼠成骨细胞系 MC3T3-E1 中, 同样发现成骨分化过程中 lncRNAs 表达的显著差异^[56-59]。

2.2 lncRNAs 调控成骨细胞分化 在成骨细胞中, 目前研究较多的是 lncRNAs 对成骨分化的调控作用^[60-61]。在转录水平, Tang 等^[62]研究发现, BMSCs 中 lncRNA OG 可与异质核糖核蛋白 K 蛋白相互作用激活 BMP 信号通路促进成骨分化。Jin 等^[63]发现在 hASCs 中 lncRNA MIR31HG 可以直接与 I κ B α 相互作用, 参与 NF- κ B 的活化。敲除 MIR31HG 基因不仅能显著促进成骨分化, 还能显著缓解炎症诱导的 hASCs 成骨抑制作用。在老龄化研究中发现 lncRNA Bmncr 可以调节 BMSCs 的命运。Bmncr 作为促进 TAZ 和 ABL 蛋白相互作用的支架, 可以促进 TAZ 和 Runx2/pPARG 转录复合物的组装, 进而促进成骨分化并抑制脂肪生成^[64]。而在成骨相关疾病的研究中同样发现, 多发性骨髓瘤患者的 hBMSCs 成骨分化受到抑制, lncRNA MEG3 表达降低。MEG3 通过直接影响 SOX2 活性而激活 BMP4 的转录活性, 基因敲除 MEG3 可显著降低成骨标志物 Runx2 等的表达^[65]。lncRNAs 也可以在表观遗传水平调控成骨分化, 尤其是通过组蛋白修饰影响基因表达。EZH2 可以通过催化靶基因启动子组蛋白 H3K27 的甲基化抑制靶基因的表达。lncRNA HoxA-AS3 和 lncRNA ANCR 均可与 EZH2 结合引起 H3K27 甲基化, 抑制 MSCs 中 Runx2 的表达, 进而抑制成骨分化^[66-67]。另有研究发现, lncRNAs 可以通过组蛋白乙酰化修饰靶基因, 如 lncRNA AK141205 和 lncRNA-HIF1 α -AS1 可以分别通过促进 CXCL13、

HoxD10 启动子区组蛋白 H4 的乙酰化上调其表达, 进而促进成骨细胞分化^[68-69]。而在转录后水平, 尤其是 lncRNAs 和 microRNAs 相互调控进而影响成骨细胞功能的研究目前较多也较为成熟, 下文将进行重点阐述。

2.3 lncRNAs 调控成骨增殖和凋亡 lncRNAs 同样可以调节成骨细胞的增殖和凋亡。lncRNA DANCR 在 hBMSCs 中的表达减少, 可通过 p38 丝裂原活化蛋白激酶途径抑制成骨细胞增殖^[70]。在 hPDLSCs 中抑制 lncRNA ANCR 表达, 可抑制 GSK3 β 表达, 激活 Wnt 通路促进其增殖^[71]。在 MC3T3-E1 细胞中, lncRNA Crnde 也可以通过 Wnt 通路促进成骨细胞增殖^[72]。而去卵巢骨质疏松大鼠成骨细胞中 lncRNA AK023948 表达明显增多, 通过调节 AKT 磷酸化水平抑制成骨细胞增殖^[73]。此外, 重力敏感的 lncRNA ODSM 在 MC3T3-E1 细胞中不仅能促进成骨分化, 也能抑制其凋亡^[74]。

3 microRNAs 和 lncRNAs 相互作用调控成骨细胞功能

竞争性内源性 RNAs (competitive endogenous RNAs, ceRNAs) 假说是一种重要的功能模式, lncRNAs 可通过与 miRNAs 相互作用作为 ceRNA 调节基因表达, 这类 lncRNAs 亦被称为 miRNA 海绵^[75]。这类 lncRNAs 包含一个或多个 miRNA 的结合位点, 并通过吸附 miRNAs 减少其与靶 mRNA 的结合或加速其降解。反过来, miRNAs 也可以与 lncRNAs 通过 AGO2 途径结合调节 lncRNAs 的表达水平。

目前研究发现 microRNAs 和 lncRNAs 可以相互作用, 协同调节成骨细胞功能。研究发现, lncRNA PGC1 β -OT1 可以通过拮抗 miR-148a-3p 促进赖氨酸特异性脱甲基酶 6b(KDM6B) 的表达, MCF2L-AS1 也可通过结合 miR-33a 促进 Runx2 表达, LOC100506178 和 lncRNA Rhno1 可分别与 miR-214-5p 和 miR-6979-5p 结合促进 BMP2 表达, 进而促进 BMSCs 向成骨细胞分化^[76-79]。Linc-ROR 同样可以通过结合 miR-138 和 miR-145, 促进 ZEB2 的表达, 进而激活 Wnt/ β -catenin 通路, 促进成骨细胞分化^[80]。牙周炎患者 hPDLSCs 中 lncRNA-POIR 表达下降, 与 miR-182 相互抑制, 形成一个网络来调节 FoxO1 表达, 促进 hPDLSCs 向成骨分化^[81]。在 hASCs 的研究中发现 lncRNA-HIF1A-AS2、lncRNA-PCAT1 可分别吸附 miR-665 和 miR-45-5p, 促进 ASC 成骨分化^[82-83]。Linc02349 作为 miR-25-3p 和 miR-33b-5p 的分子海绵可分别调控 SMAD5

和 Wnt10b 的表达, 激活 Dlx5/OSX 途径, 从而促进人脐带源性干细胞向成骨分化^[84]。在 MC3T3-E1 细胞中发现 lncRNA OGRU 可通过竞争性结合 miR-320 促进 Hoxa10 蛋白表达, 进而促进成骨细胞分化^[85]。相反的, Yang 等^[86] 在骨质疏松患者的骨组织中及去卵巢骨质疏松小鼠的 BMSC 和骨组织中观察到 lncRNA-ORLNC1 表达升高。进一步研究发现 lncRNA-ORLNC1 可以作为 ceRNA 结合 miR-296, 影响靶基因 Pten 的表达, 抑制成骨分化。骨质疏松患者血清中 lncRNA H19 表达显著减少, 体外研究发现 lncRNA H19 可以通过下调 miR-19b-3p 表达而显著抑制 BMSCs 细胞增殖和成骨分化^[87]。在小鼠 MC3T3-E1 前成骨细胞中, miR-139-3p 可以抑制成骨细胞分化、促进成骨细胞凋亡, 并且可以与 lncRNA ODSM 相互调控^[88]。而 lncRNA KCNQ1OT1 可以与 miR-701-3p 相互作用, 通过调控 FGFR3 表达促进 MC3T3-E1 细胞增殖、迁移, 减少凋亡^[89]。Bu 等^[90] 发现 lncRNA TSIX 可以负性调节 miR-30a-5p 表达, 促进成骨细胞凋亡。此外, He 等^[91] 研究发现 miR-141 可以通过下调 lncRNA H19 和 miR-675 的表达, 抑制成骨细胞增殖, 促进成骨细胞凋亡。

lncRNAs 还可作为具有 miRNAs 的前体分子发挥调控功能。Huang 等^[92] 研究发现, lncRNA H19 外显子可以编码 miR-675, 抑制 TGF- β 1 的 mRNA 和蛋白表达, 减少 Smad3 的磷酸化水平, 进而通过下调组蛋白去乙酰化酶 4/5 的表达, 增加成骨标志基因 Runx2 等的表达, 促进成骨分化。

4 结语

综上所述, microRNAs 和 lncRNAs 在成骨细胞中构成了复杂的相互作用网络, 可以调控成骨细胞功能, 影响骨形成, 对骨稳态的维持至关重要。然而目前仍有一些问题需要解决, 如成骨细胞中不同 microRNAs 和 lncRNAs 相互作用的研究较少, 成骨细胞众多发挥作用的 ncRNA 中最为关键的分子尚不十分明确。进一步研究应阐明成骨细胞中 microRNAs 和 lncRNAs 的作用机制, 探寻其关键通路和靶点, 可以更好地帮助研究骨质疏松、骨发育不良等疾病的发病机制, 并可能为这些疾病提供新的治疗药物。

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