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·综 述·

microRNA 调控间充质干细胞成骨分化的研究进展

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【摘要】间充质干细胞是一类体内广泛存在的多能干细胞,具有多向分化潜能,为多种组织器官提供保护及损伤修复。近年来,间充质干细胞向成骨细胞诱导分化用于治疗骨代谢等相关性疾病已成为热点。microRNA(miRNA)作为生物体内重要的小分子非编码 RNA,通过转录后调控影响基因表达,参与各种生命过程。研究显示,多种不同的 miRNA 在间充质干细胞向成骨分化过程中起着核心调节作用。本文结合相关文献,对 miRNA 调控间充质干细胞成骨分化的作用及机制归纳总结,以帮助全面了解靶向 miRNA 治疗骨代谢性相关疾病的潜能。

【关键词】间充质干细胞;成骨分化;microRNA;成骨细胞;骨疾病

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Research progress on microRNA in regulation of osteogenic differentiation of mesenchymal stem cells

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【**Abstract**】Mesenchymal stem cells (MSCs), a kind of pluripotent stem cells, widely exist in organisms and have the potential to differentiate into multiple cell types and protect and repair damaged tissues. Recently, inducing MSCs to differentiate into osteoblasts for treating bone metabolism-related diseases has gained a lot of interest. MicroRNA(miRNA) as a small non-coding RNA has been found to participate in diverse life processes by modulating gene expression at the post-transcriptional level. As demonstrated, multiple miRNA could play core roles in regulating osteogenic differentiation of mesenchymal stem cells. In this review, we summarize functions and mechanisms of miRNA in the process of MSCs differentiation, and discuss its potential in treatment of metabolism-related bone diseases via inducing osteogenic differentiation.

【**Key words**】mesenchymal stem cells; osteogenic differentiation; microRNA; osteoblasts; bone disease

间充质干细胞(mesenchymal stem cell, MSCs)是一类非胚胎来源的成体多能干细胞,存在于骨髓、肌肉、脂肪、脐带、皮肤等多种组织中[1]。除具备高度自我更新能力外,在一定诱导条件下,MSCs 可实现向成骨细胞、脂肪细胞、成软骨细胞及肌肉细胞等多向分化[2-3]。研究表明,MSCs 几乎不表达与人类白细胞抗原识别有关的共刺激分子及主要组织相容性复合物[4],具有较低的免疫原性;并可在组织

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损伤及炎症反应中,分泌大量趋化因子并向损伤部位迁移,进而发挥炎症调控及组织修复功能[5]。基于以上特性,MSCs被视为可用于修复衰老或病变引起的组织器官损伤的"理想种子细胞"[6]。

成骨细胞可分泌有机骨基质,促进骨基质矿化,维持骨稳态[7]。成骨细胞生成障碍或功能失调可引起骨组织微结构破坏、骨形成缺陷,导致骨代谢疾病如骨质疏松和骨关节炎等。而MSCs分化为成骨细胞是成骨细胞形成的主要途径[8],其在骨形成和功能维持中起着关键作用。大量研究表明,miRNA可直接或间接地调控MSCs成骨分化过程[8-9]。本文通过对MSCs成骨分化过程中miRNA的作用和机制进行总结,认识miRNA在这一过程中的调控功能,深入理解与此过程相关的骨代谢疾病发病机制。

1 MSCs成骨分化机制

MSCs依次分化为成骨祖细胞、成骨细胞,随后经过多种细胞外基质的矿化,成骨细胞逐渐成为成熟的骨细胞[10]。过去的几十年里,大量的分泌因子和转录因子已被确定可调节成骨发生。成骨细胞特异的Runt-相关转录因子2(RUNX family transcription factor 2, RUNX2)及SP7转录因子(Sp7 transcription factor, OSTERIX)对于MSCs向成骨细胞分化及功能性成骨细胞的形成都是必需的[11]。另外,成骨细胞的分化成熟也涉及Wnt[12]、BMP信号通路[13]及PI3K/Akt信号通路[14]等。

2 miRNA的形成及其生物学功能

miRNA是一类长度约为19~22个核苷酸(nucleotide, nt)的非编码RNA。在细胞核中,miRNA由原始miRNA(pri-miRNA)经核酸酶Drosha切割成短的约70 nt的前体miRNA(pre-miRNA),其具有茎环结构[15],通常包含-5p、-3p,且3′端有2 nt的突出。随即pre-miRNA由输出蛋白5转运至胞质,再由胞质中的Dicer酶等将其切割为约20 nt的双链RNA。双链RNA与RNA诱导沉默复合物结合,在发挥作用的过程中,一条链被剪切降解,另一条链则被选择为约20 nt的功能链[16]。miRNA与mRNA分子3′非翻译区(untranslated region, UTR)互补的靶位点结合,当两者不完全互补时,主要影响翻译过程,而对mRNA的稳定性没有影响,动物细胞内大多采用此调控方式[17];当两者完全互补时,则miRNA发挥剪切功能,特异性切割mRNA,最终导致翻译抑制或目标mRNA降解[18]。最近的研究表明,miRNA通过靶向各种参与MSCs自我更新和分化的基因直接或间接调节MSCs的成骨分化[19-21]。

3 miRNA调控MSCs成骨分化相关转录因子

MSCs成骨分化的过程受多种转录因子的调控,例如RUNX2、OSTERIX、特异AT序列结合蛋白2(SATB homeobox 2, SATB2)和远端同源异型盒(distal less homeobox, DLX)等,其中RUNX2及其下游OSTERIX转录因子是最重要的成骨细胞特异性转录因子,调控成骨祖细胞向成骨细胞分化及成熟的过程[11]。

3.1 RUNX2

RUNX2是Runxx家族成员之一[22],被认为是成骨分化和骨形成过程最关键的转录因子[23],在骨组织的形成和重建中发挥重要作用。小鼠RUNX2敲除可引起成骨细胞成熟过程停滞,导致骨发生完全缺乏[24]。Zhang等[25]发现11个靶向RUNX2的miRNA(miR-23a、miR-30c、miR-34c、miR-133a、miR-135a、miR-137、miR-204、miR-205、miR-217、miR-218和miR-338),在不同的MSCs相关细胞呈谱系特异性表达模式,且在成骨分化过程中呈现与RUNX2相反的表达模式。其中10种miRNA(除miR-218外)都被证实可显著抑制成骨分化,且此过程可被其对应的anti-miRNA逆转。这些结果表明调控RUNX2的功能性miRNA群体形成一个复杂的系统,影响成骨细胞形成。最近,研究通过对萎缩性非愈合骨折患者与正常愈合骨折患者差异表达的miRNA进行筛选,miR-628-3p和miR-654-5p在非愈合骨折患者中高表达,且在成骨分化过程持续高表达[25-26]。功能实验证实miR-628-3p可抑制MSCs成骨分化,进一步生物信息学分析及双荧光报告素酶(Luciferase)实验发现miR-628-3p与RUNX2的3′UTR有两个靶位点结合,过表达miR-628-3p使RUNX2的表达在mRNA和蛋白水平均降低。表1详细展示了影响RUNX2的表达从而调节MSCs成骨分化的miRNAs。

表 1　影响 Runx2 调节 MSCs 成骨分化的 miRNAs
Tab.1　miRNAs in regulating MSCs osteogenic differentiation through RUNX2

miRNAs	细胞系	靶向基因	正向(+)/负向(-)调控成骨分化	发表时间	参考文献
miR-23b	人骨髓 MSCs	RUNX2	-	2018	[27]
miR-133a-5p	MC3T3-E1	RUNX2	-	2018	[28]
miR-628-3p	MG63	RUNX2	-	2017	[26]
miR-221	C2C12	RUNX2	-	2017	[29]
miR-222-3p	人骨髓 MSCs	SMAD5/RUNX2	-	2016	[30]
miR-205	大鼠骨髓 MSCs	SATB2/RUNX2	-	2015	[31]
miR-194	鼠骨髓 MSCs	STAT1/RUNX2	+	2015	[32]
miR-31	大鼠骨髓 MSCs	SATB2/RUNX2	-	2013	[33]
miR-764-5p	MC3T3-E1	CHIP/RUNX2	+	2012	[34]
miR-23a, miR-30c, miR-34c, miR-133a, miR-135a, miR-137, miR-204, miR-205, miR-217, miR-338	MC3T3-E1	RUNX2	-	2011	[25]
miR-204	ST2	RUNX2	-	2010	[20]
miR-2861	ST2	HDAC5/RUNX2	+	2009	[35]

3.2　OSTERIX

OSTERIX 是一种含锌指的转录因子,在发育的骨骼中特异性表达,它的特异性缺失可导致小鼠骨形成能力丧失[36]。大量研究表明 OSTERIX 在成骨分化和骨形成过程中发挥重要作用[37]。miR-143 是 MC3T3-E1 细胞成骨分化的抑制因子,且在分化过程表达降低。OSTERIX 是 miR-143 的一个直接靶基因,抑制 OSTERIX 的表达,MC3T3-E1 成骨分化性能减弱,过表达 OSTERIX 则可部分恢复 miR-143 的抑制作用。表明 miR-143 作为一个新的 OSTERIX 的调控子在成骨分化过程发挥重要作用[38]。Yang 等[39]发现,miR-93 是成骨细胞矿化过程中表达下调最为明显的 miRNA。在小鼠原代培养的成骨细胞中过表达 miR-93 可减弱成骨细胞的矿化。Luciferase 实验显示 miR-93 直接靶向 OSTERIX 的编码区,电泳迁移率变动分析及染色质免疫沉淀证实 OSTERIX 与 miR-93 的启动子结合。另外,过表 OSTERIX 会减少 miR-93 的转录,反之 miR-93 转录增加。这些结果表明 miR-93 作为成骨细胞的重要调节因子,通过新型的 miR-93/OSTERIX 调节反馈环来发挥其调控作用。

同样,miR-96 也是以 OSTERIX 为靶基因来调节成骨细胞分化。miR-96 在老年骨质疏松症患者的血清及老年人、小鼠来源的骨髓 MSCs 都高表达。过表达 miR-96 可抑制骨髓 MSCs 的成骨分化,反之则促进。通过静脉注射在幼鼠体内过表达 miR-96 会引起成骨形成受损,导致低骨量。在老年小鼠中,对 miR-96 的抑制可减轻年龄相关性骨丢失[40]。骨质疏松和脆性骨折的风险增加是衰老的特征表现之一,这一结果有助于此类骨代谢疾病的研究和治疗。

3.3　其他成骨分化转录因子

许多其他成骨分化转录因子也被证实在 miRNA 影响下调控 MSCs 成骨分化。SATB2 在成骨细胞形成过程中起着至关重要的作用,同时它也可以通过提高 RUNX2 的活性协同调控成骨细胞的增殖和分化[41]。miR-31 被证实可通过靶向 SATB2 抑制人 MSCs 成骨分化[42],同时也可以和 RUNX2 及 SATB2 形成调节环影响大鼠骨髓 MSCs 的成骨分化[33]。DLX 基因可调节多种细胞分化,包括成骨细胞[43]。Qadir 等[44]发现,miR-124 可通过靶向 DLX2、DLX3 和 DLX5 抑制体外间充质干细胞(骨髓 MSCs、MC3T3-E1 和 C2C12)成骨分化及体内骨形成。

4　miRNA 通过靶向成骨信号通路调节 MSCs 成骨分化

在体内,骨的发育和平衡涉及多种信号通路对相关基因的激活或抑制进行紧密调节。研究发现 Wnt、BMP、PI3K/Akt、TGF-β、Notch 等信号通路均在 MSCs 成骨分化过程中发挥重要作用,而这些通路中的关键效应分子可受 miRNA 调控,进而影响骨稳态。

4.1　Wnt 信号通路

Wnt 信号通路包括 β-catenin 依赖的经典 Wnt 信号通路和非 β-catenin 依赖的非经典 Wnt 信号通路(包括 Wnt/Ca+通路等),对包括骨在内的机体组织的发育和稳态维持至关重要[45]。目前,成骨分化

过程的机制研究主要集中于β-catenin依赖的经典Wnt信号通路[46]。miR-26a可通过抑制糖原合酶激酶3β(glycogen synthase kinase 3 beta, GSK3β)的表达激活Wnt信号通路转导,从而促进骨髓来源的MSCs成骨分化[47]。同时,miR-26a也可通过抑制GSK3β表达促进脂肪来源的间充质干细胞(ASCs)成骨分化[48]。表明miR-26a可通过抑制GSK3β表达促进不同组织来源的MSCs成骨分化。miR-218可通过直接靶向Wnt信号通路的阻遏物分泌脆性相关蛋2和Dickkopf相关蛋白2,增强Wnt/β-catenin信号活性从而促进人ASCs向成骨分化。而激活Wnt/β-catenin信号通路可促进miR-218的表达,反之则抑制。这种反馈调节系统揭示了miRNA可作为信号放大器在成骨分化过程中与信号分子相互作用[49],有效发挥其调节功能。

近期关于非经典Wnt信号通路转导对成骨细胞分化影响的研究也越来越多[50]。Li等[51]发现过表达miR-26a-5p抑制成骨分化,反之则促进。Luciferase显示miR-26a-5p和Wnt家族成员5A(Wnt family member 5A, Wnt5A)的3′UTR结合。过表达miR-26a-5p会抑制Wnt5A的表达,导致Wnt/Ca^{2+}信号通路抑制从而引起鼠来源的ASCs成骨分化减弱。随后Duan等[52]发现骨质疏松患者骨组织中miR-16-2*的表达与骨形成相关基因(RUNX2、OSTERIX等)呈负相关。人骨髓来源MSCs成骨分化过程中miR-16-2*的上调使成骨分化减弱,而miR-16-2*的下调则增强了这一过程,Wnt5A是这一过程的直接靶基因。

4.2 BMP信号通路

BMPs是TGF-β超家族的成员,在骨骼发育和骨组织形成中起着重要作用,BMP信号通路的中断会导致骨骼形成异常[53]。BMPs家族成员中的BMP2、BMP6、BMP7和BMP9等都可促进成骨分化和骨形成,其中BMP2和BMP7已被应用于胫骨骨折和脊柱融合的临床治疗[54-55]。

BMP2是最常被研究的BMPs成员之一[56],可调控MSCs成骨分化过程中的成骨细胞成熟阶段。Liu等[57]通过体外实验发现miR-106b可抑制MSCs成骨分化。miR-106b在糖皮质激素诱导的骨质疏松小鼠模型中表达升高,而抑制miR-106b的表达可通过促进骨形成和抑制骨吸收减轻骨质疏松对小鼠的不良影响,BMP2是这一过程的直接靶基因。此外,过表达miR-378可以促进BMP2诱导的C2C12细胞成骨分化[58],而过表达miR-370可减弱BMP2诱导的MC3T3-E1细胞成骨分化[59]。

BMP7即成骨细胞蛋白质-1,在骨组织中表现出较强的合成代谢活性,同时被证实可以促进MSCs的成骨分化[60]。软骨肿瘤特异抑制因子miR-542-3p在美迪紫檀素诱导的成骨分化过程中显著低表达。过表达miR-542-3p可抑制成骨分化,抑制miR-542-3p的表达则能促进成骨细胞特异性基因的表达及碱性磷酸酶活性和基质矿化。生物信息学预测和验证发现miR-542-3p和BMP7的3′UTR结合。另外,动物实验发现沉默miR-542-3p的表达可使卵巢切除小鼠的骨形成、骨密度和骨强度增加[61]。

4.3 PI3K/Akt信号通路

PI3K/Akt是近年来发现的调节成骨细胞分化和骨形成的一个重要信号通路[14],已被广泛证明在MSCs成骨细胞分化过程发挥重要作用[62]。Liu等[63]发现丝氨酸蛋白酶抑制剂(Vaspin)可抑制MC3T3-E1的成骨分化,此过程同时伴随miR-34c的显著性表达升高及PI3K/Akt信号通路激活。降低miR-34c的表达,导致Vaspin对成骨分化的抑制作用减弱。而用PI3K/Akt信号通路的特异性阻断剂处理MC3T3-E1,也可减弱Vaspin的成骨抑制作用同时降低miR-34c的表达。且降低miR-34c的表达,反过来可促进PI3K/Akt的活化。因此,在MC3T3-E1成骨分化过程中PI3K/Akt和miR-34c构成一个回路,控制各自的表达,这也可能是Vaspin抑制MC3T3-E1成骨细胞分化的潜在机制。

少数miRNA被发现对成骨分化有积极的调控作用。其中miR-216a在人ASCs细胞成骨分化过程高表达。功能实验及分子信号通路研究表明,miR-216a通过靶向E3泛素蛋白连接酶CBL影响PI3K/Akt通路,拮抗地塞米松对成骨细胞生成的抑制作用,促进体外成骨细胞分化和体内异位骨形成[64]。

5 展 望

miRNA作为核心元件通过调控转录因子及细胞内信号通路促进或抑制MSCs成骨分化,提示可通过靶向核心miRNA选择性调控MSCs成骨分化,进一步开发miRNA抑制剂或模拟体药物,达到治疗

成骨细胞缺乏或功能障碍相关疾病的目的。同时，大量的研究表明，循环血液中的 miRNA 可作为多种人类疾病诊断及预后判断等的标志物[65-66]，因此 MSCs 成骨分化相关的 miRNA 分泌于体液中也可能作为骨代谢性疾病的标志分子，帮助筛选高危人群及早期诊断，达到预防及早期治疗的目的，降低人群发病率、患者预后。当前仍需更多的研究工作对此进行挖掘和探索。此外，最新的研究已发现长链非编码 RNA（lncRNA）和环状 RNA（circRNA）等也在 MSCs 成骨分化中发挥重要作用[67-68]，而 miRNA 可与 lncRNA 及 circRNA 形成竞争性内源性 RNA 互作网络[69-70]，未来的研究应更多关注这类 miRNA 竞争模式在 MSCs 成骨分化中的功能及应用价值。

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