【间充质干细胞专栏】

间充质干细胞成骨和成脂分化调控机制研究

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摘 要：间充质干细胞（MSCs）是人体内参与免疫平衡、维持组织器官的稳态和功能以及组织损伤修复的一类重要成体干细胞。MSCs具有自我更新能力和多向分化潜能，国际干细胞协会将MSCs向脂肪、成骨等细胞分化的能力作为其重要的检测标准。作为骨细胞和脂肪细胞的共同来源，MSCs在成骨和成脂分化之间相互协调和相互竞争，并在多种调控因素作用下保持着微妙的平衡。对MSCs成骨、成脂分化的信号通路、调控因素进行分析，并对其分化诱导方法以及鉴定方法进行总结，以期为MSCs基础研究及临床应用提供参考依据。

关键词：间充质干细胞；成骨分化；成脂分化；调控机制；信号通路
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Regulation mechanism of osteogenic differentiation and adipogenic differentiation in mesenchymal stem cells

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Abstract: Mesenchymal stem cells (MSCs) are an important adult stem cells, which participate in immune balance, maintain the homeostasis and function of tissues and organs, and repair damaged tissues. Mesenchymal stem cells have the self-renewal and multi-differentiation potential, able to differentiating into multiple cell types, such as bone, fat and other cells. International Stem Cell Association regards the ability as an important standard of MSCs. As common progenitor cells of osteoblasts and adipocytes, MSCs delicately balanced their differentiation commitment. This review analyzes the signaling pathways and regulatory factors of MSCs osteogenic and adipogenic differentiation. The Induced differentiation methods and identification methods are also summarized, so as to provide reference for basic research and clinical application of MSCs.

Key words: mesenchymal stem cells; osteogenic differentiation; adipogenic differentiation; regulation mechanism; signaling pathway

1970年Friedenstei首次利用骨髓培养出能够诱导分化为成骨细胞的成纤维样多能细胞[1]，随后Martin于1981年在鼠幼胚中分离出类似多能细胞系[2]。自此陆续有学者从人骨髓、脂肪、脐带血、羊水、胰腺、牙髓等组织分离出干细胞[3-6]。随着人多能间充质干细胞（MSCs）在许多生物医学领域发挥越来越重要的作用，国际细胞治疗学会对MSCs提出了通用标准[7-8]：首先，MSCs必须具有黏塑性，即MSCs能够在标准培养条件下，黏附生长在组织培养瓶上；其次，MSCs必须能够表达其特异性的表面标志物，包括阳性标志物CD105、CD73和CD90以及阴性标志物CD45、CD34、CD14、CD11b、CD79a和HLA-DR；再次，MSCs必须具有多能分化潜能，即MSC在体外具有向成骨、成脂以及成软骨分化的能力[8]。这一标准为MSCs的临床前以及临床研究提供鉴定依据。诱导成骨的因素常抑制成脂分化，

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而诱导成脂的因素常抑制成骨分化，因成骨分化和成脂分化相关因子之间的相互作用在MSCs细胞分化命运决定中发挥着重要作用，本研究对MSCs成骨、成脂分化的信号通路、调控因素进行分析，并对其分化诱导方法以及鉴定方法等进行总结，以期为MSCs基础研究及临床应用提供参考依据。

1 MSCs成骨、成脂分化信号通路

MSCs作为成骨细胞和脂肪细胞共同的祖细胞，在多种信号调控下，其向成骨、成脂细胞的分化处于一种平衡状态。这些调控都是通过不同的信号通路来激活转录因子进而发挥作用（图1），其中BMP、Wnt以及Hippo等信号通路在MSCs分化调控中发挥主要作用。

1.1 BMP信号通路

骨形态发生蛋白（bone morphogenetic protein，BMP）是转化生长因子-β（TGF-β）超家族中的多功能生长因子[9]。BMP信号通路主要是通过Smad和MAPK途径触发细胞反应[10]。BMP信号通路通过与BMP-I和BMP-II受体结合进而激活BMP信号，磷酸化的Smad 1/5/8与Smad 4形成复合物后进入细胞核内并与转录因子结合，以细胞类型特异的方式调节特异性基因的转录[11-12]。在BMP信号激活下，由Smad和MAPK途径调控Runx2/Cbfa1和PPARγ基因的表达，而特异性转录因子表达水平的改变则直接影响MSCs的成骨、成脂分化能力[13]。因此BMP信号通路在MSCs成骨、成脂分化过程中起到双重的调控作用。

1.2 Wnt信号通路

Wnt是分泌性糖蛋白家族。Wnt信号通路主要是通过典型的Wnt/β-catenin和非典型的Wnt－cGMP/Ca^{2+}信号通路来调节MSCs向成骨、成脂细胞的分化[14]。Wnt与跨膜受体FZD和核心受体IRP5/6结合通过抑制Axin/GSK3/APC复合物使得β-catenin在细胞核内稳定积累[15]。β-catenin与淋巴增强因子结合因子/T细胞因子结合能够抑制PPARγ基因的表达来抗MSCs向成脂细胞分化；通过上调Runx2/Cbfa1基因的表达来促进MSCs向成骨细胞的分化[16]。因此Wnt信号通路在MSCs分化过程中起到了促成骨细胞和抗脂肪细胞生成的重要作用。

1.3 Hippo信号通路

Hippo信号由衔接蛋白和抑制激酶组成，并且是果蝇和哺乳动物之间高度保守的信号通路[17-19]。经典的Hippo信号通路是由MST1/2和LATS1/2激酶与SAV1和MOB1磷酸化并抑制YAP和TAZ发挥功能的[20]。当LATS1/2的PY基序与YAP和TAZ的WW结构域相互作用时，使得YAP/TAZ磷酸化并定位于细胞质内，同时β-TRCP依赖性蛋白酶降解；YAP/TAZ去磷酸化后转移至细胞核内，作为其他转录因子的转录辅激活剂调控细胞的成骨、成脂分化[20-22]。Hippo信号通路通过TAZ的WW结构域与RUNX2的PY基序结合上调RUNX2、ALP以及Osterix的表达，从而诱导MSCs向成骨细胞分化[23]。相较于TAZ而言，YAP的作用更为复杂，不但可以

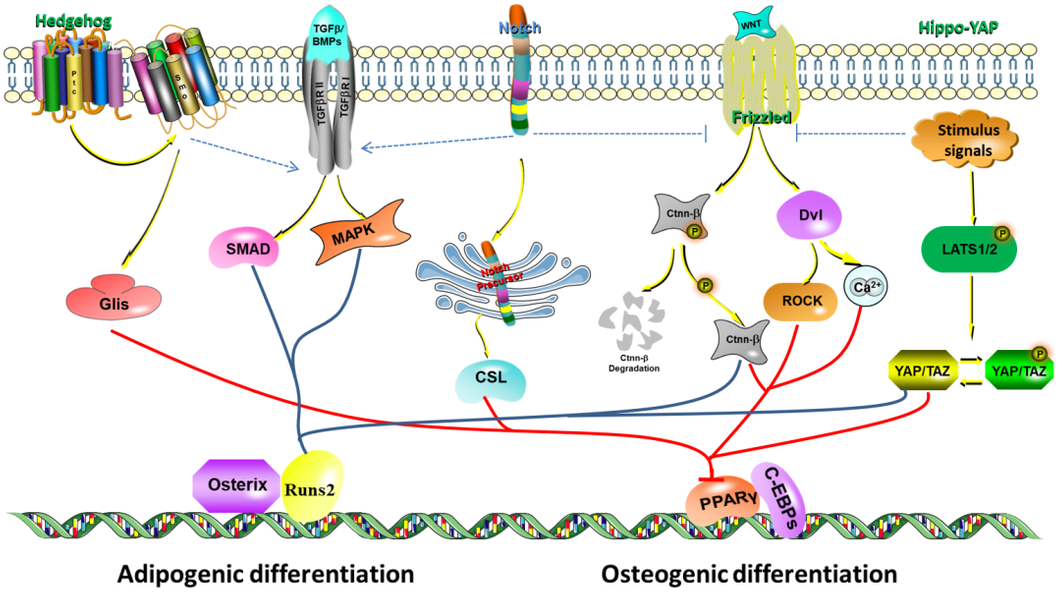

图1 间充质干细胞成骨、成脂分化机制示意图

Fig. 1 Schematic diagram of osteogenic and adipogenic differentiation mechanism of mesenchymal stem cells

作为RUNX2的阻遏物，还能够在YAP过表达时促进MSCs的成骨分化[24-25]。在成脂分化过程中，Hippo信号通路在TAZ的WW结构域与PPARγ的PY基序结合后，通过抑制PPARγ的转录活性从而抑制成脂分化[26]。而YAP则通过诱导Wnt拮抗剂来减少成骨信号，进而促进MSCs的成脂分化[27]。

1.4 其他相关信号通路

除上述BMP、Wnt和Hippo信号通路外，还有一些参与MSCs分化调控的信号通路。如Notch信号通路可通过阻断PPARγ和C/EBPα的表达来抑制MSCs向成脂细胞的分化[28]。除此之外，Notch信号通路还可以通过抑制Wnt/β-catenin信号通路来降低MSCs向成骨细胞的分化[29]。Hedgehogs信号通路通过抑制PPARγ和C/EBPα的表达及脂质积累来阻止MSCs向成脂细胞分化[30]。同时Hedgehogs信号通路通过与BMP信号通路相互作用来调控Smad进而促进MSCs向成骨细胞分化[31]。其他的信号分子如FGF、IGF等也参与MSCs的分化调控[32]。值得注意的是这些信号通路在MSCs分化过程中并不是单独地发挥作用，而是在特定条件下相互作用共同调控MSCs的成骨、成脂分化过程。

2 MSCs成骨、成脂分化调控

转录因子是各种信号通路的直接或间接靶点，而RUNX2、PPARγ、YAP/TAZ等多个转录因子在MSCs向成骨细胞和脂肪细胞的分化过程中起着重要的作用。此外，包括microRNA、物理刺激等因素也对MSCs的成骨及成脂分化具有重要调控作用。

2.1 RUNX2

Runx2作为最早的成骨标志物之一，在新骨形成过程中发挥着控制细胞增殖和分化的重要作用[33]。在成骨细胞分化过程中，目前研究的大多数信号通路都是以Runx2为靶点，通过上调Runx2促进MSCs向未成熟成骨细胞分化，同时抑制其向脂肪细胞的分化[34]。Runx2由PI和PII启动子启动表达[35]，并且弱表达于未分化的间充质细胞中，表达上调于前成骨细胞中，在未成熟成骨细胞中达到最高表达水平，而在成熟成骨细胞中表达下调[36]。Runx2与CBFB形成的异源二聚体能够增强结合DNA的能力和蛋白质的稳定性[37]。最近有研究表明，蛋白质翻译后修饰能够调节Runx2进而调控MSCs向成骨、成脂细胞的分化[38]。

2.2 PPARγ

PPARγ是促进MSCs成脂分化的关键转录因子，其不仅能够调控脂肪生成，同时具有抗骨母细胞生成的作用[39]。通常所有的前脂肪信号通路都与PPARγ相关，并且过表达PPARγ能够使成纤维母细胞有效分化为成熟脂肪细胞，而敲除PPARγ的研究表明PPARγ是体内外脂肪生成所必需的[40]。此外，C/EBP能够促进前脂肪细胞的成脂分化[41]。磷酸化的C/EBP诱导C/EBPβ激活PPARγ和C/EBPα转录表达[41]。而PPARγ和C/EBPα在整个成脂分化过程中都保持较高水平的表达，并在脂肪细胞的整个生命过程中持续表达[9]。

2.3 YAP/TAZ

14-3-3结合蛋白TAZ是YAP的一个副同源物，是具有PDZ结合基序的转录辅激活因子，能够抑制PPARγ依赖性基因转录，并且辅激活Runx2依赖性基因转录[42-45]。小鼠的TAZ SiRNA转染C2C12细胞的研究表明TAZ调控Runx2刺激的骨钙素基因的表达是成骨细胞分化的重要内源性调节因子；同时也证明了TAZ是PPARγ诱导基因表达的转录阻遏物，并且是MSCs成脂分化程序的内源性抑制剂[26]。然而YAP/TAZ并不是Hippo信号通路调节的唯一决定因素，在坚硬的基质上，当YAP/TAZ消耗殆尽时MSCs成骨分化受到抑制，而在相同基质上敲除YAP/TAZ时则促进MSCs的成脂分化[25]。除此之外，YAP/TAZ还能够与其他的信号通路如Wnt、TGF、TNF-α、Eph-Ephrin等相互作用来共同调控MSCs的成骨、成脂分化过程[46-50]。

2.4 其他因素

除上述转录因子之外，成纤维细胞生长因子（FGFs）、miRNAs和物理因素也能够影响MSCs的成骨、成脂分化。FGFs是细胞增殖、分化的有效调节因子，而不同FGF成员对MSCs分化具有不同的调控作用。FGF7可通过激活ERK-Runx2信号通路刺激干细胞向成骨细胞分化[51]，而bFGF则可通过上调PPARγ的水平从而促进成脂分化[52]。

miRNA在MSCs分化方向中也发挥重要调控作用，不同的miRNAs参与MSCs分化调节的作用不同，例如miR204、miR211、miR637过表达能够在促进成脂分化的同时抑制成骨细胞的分化[53-54]。miR21不仅能够促进人脐血干细胞的成骨分化，而且可以促进人脂肪来源的干细胞的成脂分化[55]。miR138、miR335抑制MSCs的成骨、成脂分化[56-57]。此外，机械信号在MSCs的谱系分化中也发挥重要的作用。例如振荡流体流动（OFF）能够通过上调Runx2、PPARγ等的表达来调控MSCs的成骨、成脂

分化[58]；动态压迫（DC）能够增加骨髓间充质干细胞（BMSCs）中成骨基因的表达进而促进成骨分化[59-60]；而拉伸应变则能够促进BMP2、成骨基因的表达以及钙沉积从而促使MSCs向成骨细胞分化[61-63]。因此，机械信号作为MSCs分化的关键调节因素，能够很好地阐明各种物理因素对MSCs成骨、成脂分化的调控，进而可以更好地服务于再生医学相关的研究领域。

3 MSCs成骨、成脂的分化诱导及验证

3.1 MSCs成骨分化诱导方法

MSCs向成骨细胞分化过程中转录因子Runx2的表达进一步促进ALP、Osterix、Col1a1、OPN、BSP、OCN的表达。这些表达的连续上调将促进成骨细胞成熟和矿化细胞外基质的沉积[64-66]。在体外培养体系中常用的MSCs成骨分化诱导补充剂为100 nmol/L地塞米松（DEX）、50 mmol/L抗坏血酸-2-磷酸（As-2-P）和10 mmol/L β-甘油磷酸（β-GP）[67]。As-2-P不仅可以促进胶原细胞外基质的形成和成骨细胞相关蛋白的合成，而且能够上调碱性磷酸酶和骨钙素mRNA的表达从而促进成骨分化[68]。β-GP是蛋白磷酸酶抑制剂，在基质矿化研究中充当磷酸基团供体，有助于成骨细胞Ca^{2+}的沉积，促进矿化结节的形成[69-70]。DEX是广泛应用的糖皮质激素，在体外诱导成骨分化可提高碱性磷酸酶（ALP）活性、骨钙素（OC）和骨唾液蛋白（BSP）的表达水平[71]。研究表明，1 μmol/L的DEX不仅抑制MSCs的成骨分化，促进成脂分化。而且能够降低细胞活性，提高活性氧水平，促进细胞凋亡[72]。

近年来的研究表明除化学药物外，细胞因子、中药及提取物和物理因素等均影响成骨细胞的分化。例如2 μmol/L锶能够促进成骨细胞分化和矿化形成，并且抑制脂肪细胞的过度生成[73]。H_2S通过Wnt信号通路来保护成骨细胞免受H_2O_2或DEX诱导的细胞损伤[74]。生长分化因子5（GDF-5）是骨组织工程的一个显著因素，而DEX/GDF-5则能增强ALP的活性和钙沉积[75]。类胰岛素一号生长因子（IGF-1）不仅能够促进骨形态发生蛋白9（BMP9）诱导的成骨分化，同时通过PI3K/AKT途径降低高浓度DEX对BMP9诱导的成骨分化的抑制作用[76]。紫草在体外能够诱导BMSCs分化为成骨细胞并具有促进其成骨的作用[77]。低浓度黄芩苷通过调节OPG和RANKL蛋白的表达来参与骨重塑过程[78]。LDI-glycerol-AA-GP-DEX支架、HBMSCs laden-LPN-GelMA支架等均能够支持成骨分化，促进矿化结节的形成[79-80]。对MSCs成骨分化影响因素的深入研究将会为防治糖皮质激素引起的骨质疏松症提供新的方向。

3.2 MSCs成骨分化验证方法

MSCs成骨分化的鉴定方法主要包括染色法、成骨特异性转录因子表达以及成骨蛋白质表达的检测等。茜素红、Von Kossa是常见的成骨染色方法[81]。茜素红染色法主要是基于茜素磺酸钠能与Ca^{2+}发生显色反应，产生深红色的带色化合物[82]。而Von Kossa染色法主要是利用硝酸银与钙盐发生反应在强光或紫外下生成金属银，使得钙化组织呈黑色或褐色颗粒[83]。也可以应用实时荧光定量PCR（qRT-PCR）技术检测MSCs成骨分化特异性转录因子Runx2、ALP、OPN、OC的表达[84-85]。亦或是通过ELISA检测成骨诱导培养基上清中OC的表达量或Western blotting检测ALP、骨钙蛋白（OCN）等相关成骨分化标志物来鉴定MSCs向成骨细胞的分化程度[86-88]。

3.3 MSCs成脂分化诱导方法

脂肪细胞的分化是一个复杂过程，通过前脂肪细胞过度到充脂细胞再到胰岛素响应脂肪细胞[89]。脂肪细胞分化受PPARγ、C/EBP的调控[90]。在体外培养体系中常用的MSCs成脂分化诱导补充剂为1 μmol/L DEX、10 μg/mL胰岛素、0.5 mmol/L 3-异丁基-1-甲基黄嘌呤（IBMX）[91]。高浓度DEX能够促进脂肪生成，同时抑制成骨分化[92-93]。IBMX调节C/EBPβ，单独或与DEX联合调节PPARγ活性[94-95]。胰岛素广泛用于诱导前脂肪细胞的增殖和分化[96-97]。

此外，研究表明罗格列酮能够促进前脂肪细胞向成熟脂肪细胞的分化，促进脂肪细胞的增殖[98-99]。含硼化合物NaB可抑制人BMSCs成脂分化和脂肪沉积[100]。TGF-β能够抑制前脂肪细胞系中的脂肪生成，当TGF-β过表达时降低了体内脂肪细胞的分化[101]。趋化因子CXCL3通过诱导C/EBPb和C/EBPd以自分泌或旁分泌的方式促进脂肪生成[102]。由骨细胞产生的硬结蛋白可促进3T3-L1细胞的脂肪分化[103]。核蛋白JMJD 6在脂肪分化过程中具有转录后调控C/EBPβ和C/EBPδ来促进脂肪基因的表达，并且具有直接转录激活PPARγ和CEBPα的双重作用[104]。高浓度葡萄糖可抑制β-catenin/TCF-4途径，促进MSCs脂肪生成[105-106]。另外低频磁场导致ADSCs细胞质中脂肪酸含量降低，参与诱导调节脂肪分化程序的信号通路MAPK-ERK1/2的激活与

特异性基因PPARγ和Sox9表达的改变[107]。

3.4 MSCs成脂分化验证方法

MSCs成脂分化的鉴定方法主要包括油红O染色法、成脂转录因子的表达以及相关成脂蛋白表达的检测等[108-110]。油红O染色法是常见的成脂鉴定方法[111-112]，主要是利用油溶性染料油红O对细胞内的脂肪滴进行着色以鉴定脂肪细胞的分化[113]。应用qRT-PCR技术检测成脂转录因子PPARγ和C/EBPβ的表达也可以鉴定MSCs的成脂分化情况[114-115]，相应的检测成脂标志蛋白的表达也可以鉴定成脂细胞的分化程度[116-117]。如应用Western blotting技术检测脂肪分化标志物PPARγ和C/EBPβ蛋白的表达[118-119]；或应用ELISA检测试剂盒检测PPARγ、C/EBPβ和脂联素相关脂肪分化标志蛋白的表达[120-121]；亦或是通过免疫组化方法鉴定PPARγ和C/EBPβ蛋白的表达等[122-123]。

4 展望

MSCs存在于骨髓、脂肪组织、肌肉和肝脏等多种器官的基质中，具有多向分化潜能，能够在特定条件下分化为脂肪细胞、骨细胞和软骨细胞等，是一种多能干细胞。MSCs在损伤后的组织稳态和再生中都发挥着重要的作用，为临床治疗方面提供了更多的选择与方向。近年来，越来越多的研究发现，MSCs具备强大的免疫调节功能和多向分化潜能，因其自我更新和低免疫原性等优点，MSCs被认为是最具临床前景的干细胞。

大量的研究表明，在人类众多疾病的发生和发展中，都伴随着脂肪-成骨平衡的失调，如肥胖、骨硬化和骨质疏松症等疾病。而MSCs能够在多因素的相互作用下向不同细胞谱系分化，从而使机体内的脂肪-成骨分化处于一个动态平衡的稳态。MSCs的分化机制除了与细胞因子、miRNA和物理因素等相关外，所涉及的信号通路和关键转录因子也十分复杂。因此，深入探究MSCs成骨成脂的分化机制，不仅能够对脂肪-成骨平衡起到一定的调节作用，而且能够为干细胞临床应用提供重要的指导意义和新的治疗思路。

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