HIF-1α在骨组织细胞代谢及骨疾病中的调控作用

刘莉菲[1,2] 仝晓阳[1] 郭健民[1] 陈熙[3] 杨杰[4]*

([1]上海体育学院运动科学学院, 上海 200438; [2]中国医科大学人民医院, 辽宁省人民医院康复医学科, 沈阳 110016; [3]温州医科大学体育科学学院, 温州 325035; [4]上海体育学院附属竞技体育学校, 上海 200438)

摘要 低氧诱导因子-1α(HIF-1α)是调节细胞对低氧应答的关键因子, 可在氧含量降低时被激活, 能够调节氧代谢、糖酵解等多种生理活动。骨代谢主要包括骨形成和骨吸收作用, 均受到氧浓度等多种因素的调控。HIF-1α在细胞代谢、骨组织生理及病理过程的调控中起着重要的作用, 能够增加骨组织的低氧耐受能力, 调节骨形成和矿化过程。该文主要综述了HIF-1α对成骨细胞、破骨细胞、骨髓间充质干细胞、软骨细胞等骨组织细胞的调控, 对骨血管形成过程的影响, 以及对肿瘤骨转移、股骨头坏死、异位骨化等病理过程的调节作用, 为探讨HIF-1α对骨代谢的调控和相关疾病的治疗提供参考。

关键词 低氧诱导因子-1α; 骨代谢; 骨形成

Regulatory Effects of HIF-1α in Bone Cell Metabolism and Bone Diseases

LIU Lifei[1,2], TONG Xiaoyang[1], GUO Jianmin[1], CHEN Xi[3], YANG Jie[4]*

([1]*School of Kinesiology, Shanghai University of Sport, Shanghai 200438, China*; [2]*Rehabilitation Department, People's Hospital of China Medical University, People's Hospital of Liaoning Province, Shenyang 110016, China*; [3]*School of Sports Science, Wenzhou Medical University, Wenzhou 325035, China*; [4]*Affiliated School of Elite Sport, Shanghai University of Sport, Shanghai 200438, China*)

Abstract HIF-1α (hypoxia-inducible factor-1α) is a key factor that regulates the response of cells to hypoxia. It can be activated when the oxygen content is reduced, and regulates various physiological activities such as oxygen metabolism and glycolysis. Bone metabolism mainly includes bone formation and bone resorption, both of which are regulated by various factors such as oxygen concentration. HIF-1α plays an important role in the regulation of cell metabolism, as well as bone tissue physiology and pathological processes, which can increase the hypoxia tolerance of bone tissue, and regulate bone formation and mineralization. This article mainly reviews the physiological regulation process of HIF-1α on the metabolism and angiogenesis of osteoblasts, osteoclasts, bone marrow mesenchymal stem cells, chondrocytes and other bone cells. The effects of HIF-1α on the formation of bone vessels, bone metastasis, femoral head necrosis and heterotopic ossification are also reviewed. This review provides a theoretical reference for exploring the regulation of HIF-1α on bone metabolism and the treatment of related diseases.

Keywords hypoxia-inducible factor-1α; bone metabolism; bone formation

收稿日期: 2020-10-25 接受日期: 2020-12-15

国家自然科学基金(批准号: 81702235)、温州市应用基础研究项目(批准号: Y20190019)和上海市人类运动能力开发与保障重点实验室(上海体育学院)项目(批准号: 11DZ2261100)资助的课题

*通讯作者。Tel: 021-65507771, E-mail: yangjiea704@126.com

Received: October 25, 2020 Accepted: December 15, 2020

This work was supported by the National Natural Science Foundation of China (Grant No.81702235), the Wenzhou Applied Basic Research Project (Grant No.Y20190019), and the Project of Key Laboratory for the Development and Protection of Human Athletic Ability in Shanghai (Shanghai University of Sport) (Grant No.11DZ2261100)

*Corresponding author. Tel: +86-21-65507771, E-mail: yangjiea704@126.com

URL: http://www.cjcb.org/arts.asp?id=5474

骨代谢主要由骨形成和骨吸收作用共同调控，受到机械刺激、炎症和氧浓度等多种因素的影响[1-2]。研究表明，适当的低氧刺激能够调节骨形成过程，其不仅可以促进骨折愈合，修复骨坏死区域，还能够调节肿瘤的骨转移[3-5]。在针对低氧的诸多研究中，低氧诱导因子-1α(hypoxia-inducible factor-1α, HIF-1α)受到广泛关注，它是调节细胞对低氧应答的关键因子，不仅可以增加骨组织的低氧耐受能力，还能够促进成骨和矿化过程[6]。另外，HIF-1α还具有调节骨组织细胞代谢、软骨内成骨和血管形成等作用[7-9]。在这篇综述中，我们讨论了HIF-1α对多种骨组织细胞的代谢、血管形成和一些骨疾病病理过程的调节作用，为探讨HIF-1α对骨代谢的调控和相关疾病的治疗提供理论参考。

1 HIF-1α简介

HIF蛋白是一种核转录因子，主要包含HIF-1、HIF-2和HIF-3三个子类别。HIF-1是HIFs家族的主要转录因子，由α和β亚基组成，其中HIF-1α由826个氨基酸构成，是一种分子量为120 kDa的蛋白质[10]，可在低氧条件下调节细胞的内环境，增强细胞低氧适应性，维持细胞稳定[11]。在常氧条件下，HIF-1α在氧依赖性降解域中被羟基化，然后通过泛素–蛋白酶体途径被降解[12]；在低氧状态下，HIF-1α的羟基化受到抑制，非羟基化的HIF-1α在细胞质中积聚，然后转运到细胞核中并与HIF-1β发生二聚反应，从而引起氧适应性调节[11,13]。

与其他组织相比，骨骼处于相对低氧的微环境中[14]。低氧可在骨形成的早期阶段诱导HIF-1α产生[15]，HIF-1α信号传导限制了细胞耗氧量，以避免有害的活性氧自由基(reactive oxygen species, ROS)积累并保持氧化还原平衡，此外还能诱导调节自身耗能方式，减少能量损耗，增加细胞存活率[16]。有研究发现，缺乏*HIF-1α*基因小鼠的骨小梁体积显著减少，骨形成速率降低，皮质骨结构改变[17]。相反，在骨缺损局部使用HIF-1α模拟物，可以促进愈伤组织再生和成骨作用[18-19]，这表明HIF-1α能够调节骨代谢，促进骨形成。

2 HIF-1α对骨组织细胞的调控

骨组织细胞主要包括成骨细胞、破骨细胞、软骨细胞等，成骨细胞和软骨细胞主要由骨髓间充质干细胞(bone marrow mesenchymal stem cell, BMSC)分化而来，破骨细胞主要来源于单核细胞[20]。不同类型的骨组织细胞共同协作，使骨形成和骨吸收作用维持动态平衡，保持骨重塑稳态[21]。多数骨组织细胞为氧敏感细胞，低氧条件时会激活HIF-1α信号通路，调节细胞自身功能以适应低氧，进而调节骨代谢[7,22-23]。不同类型的细胞对于HIF-1α的敏感性不同，如成骨细胞对HIF-1α活化更敏感，而破骨细胞对HIF-1α抑制更为敏感[24]，这也使得骨组织细胞在不同的氧环境下发挥不同功能。

2.1 成骨细胞

HIF-1α对成骨细胞的调控分为常氧和低氧条件两种情况。在常氧条件下，过表达*HIF-1α*可以增强成骨细胞活性，促进增殖和成骨作用[17,25]，而敲低*HIF-1α*表达可以抑制成骨细胞活性和增殖能力，并通过抑制叉形头转录因子1(forkhead boxclass O1, FoxO1)的表达，降低Runt相关转录因子2(Runt-related transcription factor 2, Runx2)及碱性磷酸酶(alanine phosphatase, ALP)等成骨标志物的表达，还可以增加ROS和细胞凋亡水平，导致骨皮质结构紊乱以及骨形成率和骨量的显著降低[26]。

在低氧条件下，HIF-1α表达增加，使得成骨细胞的生长受到抑制，细胞活性降低[27]。将MC3T3-E1成骨细胞系置于低氧环境中，发现其生长速度减慢[28]。进一步实验表明，在低氧条件下敲低*HIF-1α*会使成骨细胞活性降低更加显著，而过表达*HIF-1α*会通过抑制低氧诱导的细胞凋亡，而改善细胞活性[28]。也有研究发现，过表达*HIF-1α*可以通过丙酮酸脱氢酶激酶1(pyruvate dehydrogenase kinase 1, PDK1)/蛋白激酶B(protein kinase B, PKB/AKT)/哺乳动物雷帕霉素靶蛋白(mammalian target of rapamycin, mTOR)通路增加MC3T3-E1细胞的成骨能力[29]。与此结果相反的是，另一些实验表明，在低氧条件下敲低*HIF-1α*表达后，细胞增殖活性增强，且HIF-1α可能通过抑制成骨细胞线粒体活性或Wnt/β-catenin信号通路传导而抑制成骨细胞活性[27,30]。这提示，HIF-1α对成骨细胞的调控可能因氧浓度的改变而发挥不同的作用。

2.2 破骨细胞

研究表明，低氧可以促进破骨细胞生成[31]，主要通过以下几个路径。首先，有研究发现，低氧刺激使得HIF-1α表达增加，加速了前体细胞的融合过程[32]，或通过激活蛋白酪氨酸激酶2(Janus kinase 2, JAK2)/信

号转导和转录激活因子3(signal transduction and transcription activator 3, STAT3)信号通路来增加细胞核因子-κB受体活化因子配体(receptor activator of nuclear factor κB ligand, RANKL)的表达, 从而促进破骨细胞生成[33]。其次, HIF-1α还通过调节糖酵解过程, 增强破骨细胞功能, 具体表现在: 低氧刺激使得破骨细胞消耗ATP, 从而增加细胞摄取葡萄糖的需求, 而抑制糖酵解可减少破骨细胞的形成并降低其活性[34]。HIF-1α通过增加RANKL表达使得糖酵解酶大量增加, 从而满足了破骨细胞对葡萄糖的需求并促进增殖[35]。除此之外, 也有研究指出, HIF-1α还可将破骨细胞的代谢模式调整为无氧呼吸, 从而加快低氧条件下的ATP产生, 满足代谢需求[36]。这种HIF-1α介导的代谢途径是一种适应性机制, 可以在短期内快速吸收骨骼, 促进骨骼重塑。值得注意的是, HIF-1α对破骨细胞的调节作用与成骨细胞还有着密切的关系, 有研究发现, 与缺乏HIF-1α的成骨细胞相比, 共培养的破骨细胞具有更高的骨吸收活性[24]。深入研究发现, 成骨细胞中HIF-1α的活化通过与启动子结合而促进白介素-33(interleukin-33, IL-33)的表达, 进一步通过IL-33-miR-34a-5p-Notch1通路抑制破骨细胞的生成[37]。

2.3 骨髓间充质干细胞

与HIF-1α对成骨细胞的调节作用不同, HIF-1α对BMSC的作用多为正向的调节, 如低氧可以显著诱导BMSC增殖、迁移和成骨分化[38], HIF-1α的上调显著增加了BMSC的存活率、增殖迁移能力和成骨能力[39-40], 并可通过HIF/Ca^{2+}/NO/ROS通路促进成软骨分化, 进而加速骨缺损愈合[41]。在低氧条件下, HIF-1α上调可保护BMSC免受H_2O_2、低氧和血清剥夺的影响, 防止因低氧和氧化应激条件引起的细胞死亡和凋亡[42-43], 并通过整合素α4的上调以及Rho相关螺旋蛋白激酶1(Rho-associated kinase 1, ROCK1)的下调来减少BMSC的迁移[44]。另一项研究表明, HIF-1α还能保护BMSC免受氧–葡萄糖剥夺带来的继发损伤, 并通过激活腺苷酸活化蛋白激酶(AMP-activated protein kinase, AMPK)/mTOR信号通路以及调节自噬而促进BMSC的存活[45]。

2.4 软骨细胞

软骨细胞由BMSC分化而来, 成熟后转变为肥大性软骨细胞, 之后再逐渐矿化形成骨组织, 这种软骨内成骨的过程是骨骼形成的一种主要方式[46]。在这一过程中, 扩张的生长板中心氧浓度逐渐降低, HIF-1α的局部激活对于软骨细胞的正常功能和矿化至关重要[47]。

在低氧条件下, HIF-1α的表达上调, 增加了软骨细胞的低氧耐受性, 抑制了细胞凋亡并促进了细胞外基质合成, 从而提高了软骨细胞的存活率[48-49]。在软骨细胞中敲除脯氨酸羟化酶-2(prolyl hydroxylase domain-containing protein 2, *PHD2*)基因, 制造*HIF-1α*高表达模型小鼠后发现, HIF-1α蛋白水平升高, 从肥大软骨细胞到矿化骨基质的转化率提高, 产生高骨量表型, 小鼠的股骨中骨干的皮质厚度和组织矿物质密度也显著增加[50-51]。在对小鼠骨性关节炎的研究中发现, HIF-1α通过抑制核转录因子κB信号传导降低了分解代谢基因基质金属蛋白酶(matrix metalloproteinase 13, *Mmp13*)的表达, 或通过调节OPG-RANKL-RANK信号通路, 而减少了关节软骨的破坏[52-53]。糖酵解是软骨细胞中最重要的能量产生途径, 葡萄糖氧化对于充分的增殖和蛋白质合成是必要的, 而这些途径均受到HIF-1α的调控, 由此可见, HIF-1α可使软骨细胞骨化期间的氧化葡萄糖代谢保持良好的能量平衡, 维持软骨细胞的正常功能[54]。

3 HIF-1α对骨血管形成的调控

血管生成与骨组织形成在骨骼生长和重塑过程中紧密相连。骨折修复过程在很大程度上取决于骨折部位新血管的形成情况, 并且已经证实血管生成因子对于骨折愈合的开始和维持至关重要[19]。实验发现, 在骨骼再生中的血管生成–骨生成耦合过程中, HIF-1α可通过促进血管生成来提高骨愈合能力[19]。但有研究表示, HIF-1α的这种调节作用仅限于3~4周龄的年轻小鼠[55]。

在对成骨细胞进行*HIF-1α*基因敲除的小鼠的研究中发现, 小鼠小梁骨体积减少, 骨形成速率降低, 皮质骨结构改变, 并且通过血管造影术测得的长骨血管发育减慢, 成骨细胞中血管内皮生长因子(vascular endothelial-derived growth factor, VEGF)表达明显降低[17]。另一项研究表明, 在BMSC中过表达*HIF-1α*可以使得VEGF浓度显著增加, 并且能够有效地诱导内皮细胞迁移, 改善低氧条件下血管生成过程[56]。进一步研究发现, 低氧可以通过HIF-1α诱导的VEGF促进Runx2的表达, 并可能通过调节Wnt/β-catenin通路和自噬过程而在骨形成过程中发挥积极作用[57-58]。另有研究表明, HIF-1α能通过调节基质细胞衍生因

子-1(stromal cell-derived factor-1, SDF-1)/VEGF通路来改善组织缺血引起的氧化应激反应[59], 并调节骨关节炎成骨细胞中的VEGF表达[60]。除了VEGF, 其他血管生成因子, SDF-1、碱性成纤维细胞生长因子(basic fibroblastic growth factor, bFGF)、血管生成素-1(angiopoietin-1, ANGPT-1)和干细胞因子(stem cell factor, SCF)等, 也在*HIF-1α*过表达时显著增加[61]。这说明HIF-1α可能是通过调节VEGF等血管生成因子而对骨血管的生成起到调控作用。

4 HIF-1α对骨性疾病的调控

HIF-1α能够促进正常机体的骨形成和血管形成, 同样也被发现促进了一些肿瘤在骨组织中的转移过程, 并且在股骨头坏死和异位骨化(heterotopic ossification, HO)等疾病中也发挥了调节作用。

由于肿瘤细胞的增殖率高, 肿瘤组织的血管生成异常, 约50%的实体肿瘤会存在低氧情况[62]。在常氧条件下, 肿瘤抑制因子VHL(von Hippel-Lindau)可以结合HIF-1α并导致其失活; 但在低氧条件下, HIF-1α无法与VHL结合, 致使形成HIF-1稳定复合物, 促进了肿瘤组织的血管生成、糖代谢改变、癌症扩散和骨转移[63-64]。一些研究表明, 无论是在原发性骨肿瘤(如骨肉瘤、软骨肉瘤等), 还是在骨转移瘤中, HIF-1α的表达与总生存期和无症状生存期呈负相关, 而与骨肿瘤的严重程度(包括临床分期、转移和微血管密度)呈正相关, 由此推断HIF-1α可能是其预后不良的有效预测因素[9,65-66]。而在对乳腺癌骨转移的治疗研究中发现, HIF-1α抑制剂可以减少破骨细胞的骨吸收作用, 增强成骨细胞的活性, 最终减缓骨转移进程并提高生存率, 为骨转移的治疗提供新的方向[67]。

股骨头坏死是由于血液供应破坏而导致的股骨头低氧性损伤。研究发现, 在糖皮质激素诱发的股骨头坏死模型中, 疾病早期尚未出现骨量损失和微结构破坏时, 就检测到了HIF-1α、VEGF的表达下降和骨血管分布的减少, 表明HIF-1α可能参与了低氧引起的股骨头坏死的发病机制[68]。进一步研究发现, 使用3,4-二羟基苯甲酸乙酯(一种PHD2抑制剂), 能够使HIF-1α和VEGF的表达增多, 骨细胞、软骨细胞和骨髓细胞的凋亡减少, 毛细血管网形成增加, 从而减少骨小梁空洞的产生和骨微结构坏死的发生, 增加股骨头骨小梁厚度和局部骨密度[69]。另一项研究将过表达*HIF-1α*基因的BMSC植入股骨头坏死区, 发现局部的微血管和骨小梁的数量和体积显著增加, 骨再生能力增加, 表明HIF-1α可能会成为治疗股骨头坏死的新方法[4]。

HO是指在骨骼外软组织(如肌肉、肌腱和韧带)中成熟层状骨的异常形成, 患者多存在外伤和遗传等危险因素[70]。近期研究发现, HO的产生除了与组织损伤相关的炎症有关外, 还可能与创伤后的持续低氧状态和高HIF-1α活性相关[71]。高稳定性的HIF-1α通过调节骨形态发生蛋白(bone morphogenetic protein, *BMP*)、*VEGF*等基因的表达来促进软骨和成骨细胞的增殖, 并促进软骨内和骨膜内成骨, 导致HO的形成[72-73]。在小鼠HO模型中, 使用HIF-1α抑制剂PX-478或雷帕霉素可以显著减少HO的形成[74], 因此, HIF-1α也可能成为预防和治疗HO的靶标。

5 小结与展望

HIF-1α通过直接调节多种骨组织细胞、促进骨血管生成、改变糖酵解途径等方式对骨组织的生长和重塑过程进行调控, 从而对骨相关肿瘤、股骨头坏死、HO等病理过程产生重要影响。HIF-1α不仅是判断骨转移预后的预测因子, 也为疾病的精准治疗提供了新的方向。一些与HIF-1α相关的治疗方法也在逐步得到更多关注, 如高压氧疗法可以通过降低HIF-1α表达, 减轻类风湿关节炎的炎性反应和关节肿胀[75-76]; HIF-1α的模拟物氯化钴制备出的骨填充材料, 因其具有较好的促成骨作用[77], 被发现在促进牙槽骨再生方面具有很好的生物活性[78]。随着研究的不断深入和拓展, 人们对HIF-1α的探索也将深入到各个学科领域, 为病因的探索和疾病的治疗提供新的思路。

参考文献 (References)

[1] CUNNINGHAM H C, WEST D W D, BAEHR L M, et al. Age-dependent bone loss and recovery during hindlimb unloading and subsequent reloading in rats [J]. BMC Musculoskelet Disord, 2018, 19(1): 223.

[2] YELLOWLEY C E, GENETOS D C. Hypoxia signaling in the skeleton: implications for bone health [J]. Curr Osteoporos Rep, 2019, 17(1): 26-35.

[3] DURAND M, COLLOMBET J M, FRASCA S, et al. *In vivo* hypobaric hypoxia performed during the remodeling process accelerates bone healing in mice [J]. Stem Cells Transl Med, 2014, 3(8): 958-68.

[4] DING H, GAO Y S, HU C, et al. HIF-1alpha transgenic bone

marrow cells can promote tissue repair in cases of corticosteroid-induced osteonecrosis of the femoral head in rabbits [J]. PLoS One, 2013, 8(5): e63628.

[5] JOHNSON R W, SOWDER M E, GIACCIA A J. Hypoxia and bone metastatic disease [J]. Curr Osteoporos Rep, 2017, 15(4): 231-8.

[6] JIANG X, ZHANG Y, FAN X, et al. The effects of hypoxia-inducible factor (HIF)-1alpha protein on bone regeneration during distraction osteogenesis: an animal study [J]. Int J Oral Maxillofac Surg, 2016, 45(2): 267-72.

[7] HIRAGA T, KIZAKA-KONDOH S, HIROTA K, et al. Hypoxia and hypoxia-inducible factor-1 expression enhance osteolytic bone metastases of breast cancer [J]. Cancer Res, 2007, 67(9): 4157-63.

[8] LI H, LI X, JING X, et al. Hypoxia promotes maintenance of the chondrogenic phenotype in rat growth plate chondrocytes through the HIF-1alpha/YAP signaling pathway [J]. Int J Mol Med, 2018, 42(6): 3181-92.

[9] PEZZUTO A, PERRONE G, ORLANDO N, et al. A close relationship between HIF-1alpha expression and bone metastases in advanced NSCLC, a retrospective analysis [J]. Oncotarget, 2019, 10(66): 7071-9.

[10] WANG G L, SEMENZA G L. Purification and characterization of hypoxia-inducible factor 1 [J]. J Biol Chem, 1995, 270(3): 1230-7.

[11] SEMENZA G L. Hypoxia-inducible factors in physiology and medicine [J]. Cell, 2012, 148(3): 399-408.

[12] SALCEDA S, CARO J. Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes [J]. J Biol Chem, 1997, 272(36): 22642-7.

[13] RATCLIFFE P J. HIF-1 and HIF-2: working alone or together in hypoxia [J]? J Clin Invest, 2007, 117(4): 862-5.

[14] SPENCER J A, FERRARO F, ROUSSAKIS E, et al. Direct measurement of local oxygen concentration in the bone marrow of live animals [J]. Nature, 2014, 508(7495): 269-73.

[15] HUANG J, LIU L, FENG M, et al. Effect of CoCl(2) on fracture repair in a rat model of bone fracture [J]. Mol Med Rep, 2015, 12(4): 5951-6.

[16] STEGEN S, DEPREZ S, EELEN G, et al. Adequate hypoxia inducible factor 1alpha signaling is indispensable for bone regeneration [J]. Bone, 2016, 87: 176-86.

[17] SHOMENTO S H, WAN C, CAO X, et al. Hypoxia-inducible factors 1alpha and 2alpha exert both distinct and overlapping functions in long bone development [J]. J Cell Biochem, 2010, 109(1): 196-204.

[18] OH S M, SHIN J S, KIM I K, et al. Therapeutic effects of HIF-1alpha on bone formation around implants in diabetic mice using cell-penetrating DNA-binding protein [J]. Molecules, 2019, 24(4): 760.

[19] KOMATSU D E, HADJIARGYROU M. Activation of the transcription factor HIF-1 and its target genes, VEGF, HO-1, iNOS, during fracture repair [J]. Bone, 2004, 34(4): 680-8.

[20] HADJIDAKIS D J, ANDROULAKIS I I. Bone remodeling [J]. Ann N Y Acad Sci, 2006, 1092: 385-96.

[21] RAGGATT L J, PARTRIDGE N C. Cellular and molecular mechanisms of bone remodeling [J]. J Biol Chem, 2010, 285(33): 25103-8.

[22] WAN C, SHAO J, GILBERT S R, et al. Role of HIF-1alpha in skeletal development [J]. Ann N Y Acad Sci, 2010, 1192: 322-6.

[23] TAMAMA K, KAWASAKI H, KERPEDJIEVA S S, et al. Differential roles of hypoxia inducible factor subunits in multipotential stromal cells under hypoxic condition [J]. J Cell Biochem, 2011, 112(3): 804-17.

[24] SHAO J, ZHANG Y, YANG T, et al. HIF-1alpha disturbs osteoblasts and osteoclasts coupling in bone remodeling by up-regulating OPG expression [J]. In Vitro Cell Dev Biol Anim, 2015, 51(8): 808-14.

[25] WANG Y, WAN C, DENG L, et al. The hypoxia-inducible factor alpha pathway couples angiogenesis to osteogenesis during skeletal development [J]. J Clin Invest, 2007, 117(6): 1616-26.

[26] XU G. HIF-1-mediated expression of Foxo1 serves an important role in the proliferation and apoptosis of osteoblasts derived from children's iliac cancellous bone [J]. Mol Med Rep, 2018, 17(5): 6621-31.

[27] CHEN D, LI Y, ZHOU Z, et al. Synergistic inhibition of Wnt pathway by HIF-1alpha and osteoblast-specific transcription factor osterix (Osx) in osteoblasts [J]. PLoS One, 2012, 7(12): e52948.

[28] XU G, XUE M, WANG H, et al. Hypoxia-inducible factor-1alpha antagonizes the hypoxia-mediated osteoblast cell viability reduction by inhibiting apoptosis [J]. Exp Ther Med, 2015, 9(5): 1801-6.

[29] XU W N, ZHENG H L, YANG R Z, et al. HIF-1alpha regulates glucocorticoid-induced osteoporosis through PDK1/AKT/mTOR signaling pathway [J]. Front Endocrinol, 2019, 10: 922.

[30] LECHLER P, KLEIN S M, PRANTL L, et al. Hypoxic down-regulation of cellular proliferation and loss of phenotype stability in human osteoblasts is mediated by HIF-1alpha [J]. Clin Hemorheol Microcirc, 2011, 49(1/4): 279-86.

[31] ARNETT T R, GIBBONS D C, UTTING J C, et al. Hypoxia is a major stimulator of osteoclast formation and bone resorption [J]. J Cell Physiol, 2003, 196(1): 2-8.

[32] HULLEY P A, BISHOP T, VERNET A, et al. Hypoxia-inducible factor 1-alpha does not regulate osteoclastogenesis but enhances bone resorption activity via prolyl-4-hydroxylase 2 [J]. J Pathol, 2017, 242(3): 322-33.

[33] ZHU J, TANG Y, WU Q, et al. HIF-1alpha facilitates osteocyte-mediated osteoclastogenesis by activating JAK2/STAT3 pathway *in vitro* [J]. J Cell Physiol, 2019, 234(11): 21182-92.

[34] KNOWLES H J. Distinct roles for the hypoxia-inducible transcription factors HIF-1alpha and HIF-2alpha in human osteoclast formation and function [J]. Sci Rep, 2020, 10(1): 21072.

[35] TANG Y, ZHU J, HUANG D, et al. Mandibular osteotomy-induced hypoxia enhances osteoclast activation and acid secretion by increasing glycolysis [J]. J Pathol, 2019, 234(7): 11165-75.

[36] MORTEN K J, BADDER L, KNOWLES H J. Differential regulation of HIF-mediated pathways increases mitochondrial metabolism and ATP production in hypoxic osteoclasts [J]. J Pathol, 2013, 229(5): 755-64.

[37] KANG H, YANG K, XIAO L, et al. Osteoblast hypoxia-inducible factor-1alpha pathway activation restrains osteoclastogenesis via the interleukin-33-microRNA-34a-Notch1 pathway [J]. Front

Immunol, 2017, 8: 1312.
[38] HUANG J, DENG F, WANG L, et al. Hypoxia induces osteogenesis-related activities and expression of core binding factor alpha1 in mesenchymal stem cells [J]. Tohoku J Exp Med, 2011, 224(1): 7-12.
[39] ZHANG J, FENG Z, WEI J, et al. Repair of critical-sized mandible defects in aged rat using hypoxia preconditioned BMSCs with up-regulation of Hif-1alpha [J]. Int J Biol Sci, 2018, 14(4): 449-60.
[40] CHENG M S, YI X, ZHOU Q. Overexpression of HIF-1alpha in bone marrow mesenchymal stem cells promote the repair of mandibular condylar osteochondral defect in a rabbit model [J]. J Oral Maxillofac Surg, 2020, 16(20): 31244-1.
[41] CHEN C, YAN S, QIU S, et al. HIF/Ca(2+)/NO/ROS is critical in roxadustat treating bone fracture by stimulating the proliferation and migration of BMSCs [J]. Life Sci, 2020, 264: 118684.
[42] KIANI A A, KAZEMI A, HALABIAN R, et al. HIF-1alpha confers resistance to induced stress in bone marrow-derived mesenchymal stem cells [J]. Arch Med Res, 2013, 44(3): 185-93.
[43] CHEN C, LI H, JIANG J, et al. Inhibiting PHD2 in bone marrow mesenchymal stem cells via lentiviral vector-mediated RNA interference facilitates the repair of periodontal tissue defects in SD rats [J]. Oncotarget, 2017, 8(42): 72676-99.
[44] CHOI J H, LEE Y B, JUNG J, et al. Hypoxia inducible factor-1alpha regulates the migration of bone marrow mesenchymal stem cells via Integrin alpha 4 [J]. Stem Cells Int, 2016, 2016: 7932185.
[45] LV B, LI F, HAN J, et al. Hif-1alpha overexpression improves transplanted bone mesenchymal stem cells survival in rat MCAO stroke model [J]. Front Mol Neurosci, 2017, 10: 80.
[46] YANG L, TSANG K Y, TANG H C, et al. Hypertrophic chondrocytes can become osteoblasts and osteocytes in endochondral bone formation [J]. Proc Natl Acad Sci USA, 2014, 111(33): 12097-102.
[47] ARO E, KHATRI R, GERARD-O'RILEY R, et al. Hypoxia-inducible factor-1 (HIF-1) but not HIF-2 is essential for hypoxic induction of collagen prolyl 4-hydroxylases in primary newborn mouse epiphyseal growth plate chondrocytes [J]. J Biol Chem, 2012, 287(44): 37134-44.
[48] HUANG Z, WANG Y, MA K. Expression and significance of hypoxia-inducible factor 1alpha in endplate chondrocytes of rats [J]. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi, 2017, 31(3): 351-6.
[49] FERNANDEZ-TORRES J, ZAMUDIO-CUEVAS Y, MARTINEZ-NAVA G A, et al. Hypoxia-Inducible Factors (HIFs) in the articular cartilage: a systematic review [J]. Eur Rev Med Pharmacol Sci, 2017, 21(12): 2800-10.
[50] CHENG S, XING W, POURTEYMOOR S, et al. Conditional deletion of prolyl hydroxylase domain-containing protein 2 (Phd2) gene reveals its essential role in chondrocyte function and endochondral bone formation [J]. Endocrinology, 2016, 157(1): 127-40.
[51] STREET J, BAO M, DEGUZMAN L, et al. Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover [J]. Proc Natl Acad Sci USA, 2002, 99(15): 9656-61.
[52] OKADA K, MORI D, MAKII Y, et al. Hypoxia-inducible factor-1 alpha maintains mouse articular cartilage through suppression of NF-kappaB signaling [J]. Sci Rep, 2020, 10(1): 5425.
[53] KOVACS B, VAJDA E, NAGY E E. Regulatory effects and interactions of the Wnt and OPG-RANKL-RANK signaling at the bone-cartilage interface in osteoarthritis [J]. Int J Mol Sci, 2019, 20(18): 4653.
[54] STEGEN S, LAPERRE K, EELEN G, et al. HIF-1alpha metabolically controls collagen synthesis and modification in chondrocytes [J]. Nature, 2019, 565(7740): 511-5.
[55] RIDDLE R C, LESLIE J M, GROSS T S, et al. Hypoxia-inducible factor-1alpha protein negatively regulates load-induced bone formation [J]. J Biol Chem, 2011, 286(52): 44449-56.
[56] RAZBAN V, LOTFI A S, SOLEIMANI M, et al. HIF-1alpha overexpression induces angiogenesis in mesenchymal stem cells [J]. Biores Open Access, 2012, 1(4): 174-83.
[57] XU Q, LIU Z, GUO L, et al. Hypoxia mediates Runt-related transcription factor 2 expression via induction of vascular endothelial growth factor in periodontal ligament stem cells [J]. Mol Cells, 2019, 42(11): 763-72.
[58] HE X, HAN Z, JIANG W, et al. Hypoxia improved vasculogenesis in distraction osteogenesis through Mesenchymal-Epithelial transition (MET), Wnt/beta-catenin signaling pathway, and autophagy [J]. Acta Histochem, 2020, 122(6): 151593.
[59] WANG K, DAI X, HE J, et al. Endothelial overexpression of metallothionein prevents diabetes-induced impairment in ischemia angiogenesis through preservation of HIF-1alpha/SDF-1/VEGF signaling in endothelial progenitor cells [J]. Diabetes, 2020, 69(8): 1779-92.
[60] LI L, PANG Y, ZHANG L, et al. Triiodothyronine potentiates angiogenesis-related factor expression through PI3K/AKT signaling pathway in human osteoarthritic osteoblasts [J]. Iran J Basic Med Sci, 2020, 23(6): 819-25.
[61] ZOU D, ZHANG Z, HE J, et al. Blood vessel formation in the tissue-engineered bone with the constitutively active form of HIF-1alpha mediated BMSCs [J]. Biomaterials, 2012, 33(7): 2097-108.
[62] SONI S, PADWAD Y S. HIF-1 in cancer therapy: two decade long story of a transcription factor [J]. Acta Oncol, 2017, 56(4): 503-15.
[63] RANKIN E B, GIACCIA A J. Hypoxic control of metastasis [J]. Science, 2016, 352(6282): 175-80.
[64] BENDINELLI P, MARONI P, MATTEUCCI E, et al. Cell and signal components of the microenvironment of bone metastasis are affected by hypoxia [J]. Int J Mol Sci, 2016, 17(5): 706.
[65] LUO D, REN H, ZHANG W, et al. Clinicopathological and prognostic value of hypoxia-inducible factor-1alpha in patients with bone tumor: a systematic review and meta-analysis [J]. J Orthop Surg Res, 2019, 14(1): 56.
[66] LI Y, ZHANG W, LI S, et al. Prognosis value of hypoxia-inducible factor-1alpha expression in patients with bone and soft tissue sarcoma: a meta-analysis [J]. Springerplus, 2016, 5(1): 1370.
[67] DUNN L K, MOHAMMAD K S, FOURNIER P G, et al. Hypoxia and TGF-beta drive breast cancer bone metastases through parallel signaling pathways in tumor cells and the bone microenvironment [J]. PLoS One, 2009, 4(9): e6896.
[68] WEINSTEIN R S, HOGAN E A, BORRELLI M J, et al. The pathophysiological sequence of glucocorticoid-induced osteone-

crosis of the femoral head in male mice [J]. Endocrinology, 2017, 158(11): 3817-31.

[69] FAN L, LI J, YU Z, et al. Hypoxia-inducible factor prolyl hydroxylase inhibitor prevents steroid-associated osteonecrosis of the femoral head in rabbits by promoting angiogenesis and inhibiting apoptosis [J]. PLoS One, 2014, 9(9): e107774.

[70] LEGOSZ P, DRELA K, PULIK L, et al. Challenges of heterotopic ossification-Molecular background and current treatment strategies [J]. Clin Exp Pharmacol Physiol, 2018, 45(12): 1229-35.

[71] AGARWAL S, LODER S, BROWNLEY C, et al. Inhibition of Hif1alpha prevents both trauma-induced and genetic heterotopic ossification [J]. Proc Natl Acad Sci USA, 2016, 113(3): E338-47.

[72] LIN L, SHEN Q, XUE T, et al. Heterotopic ossification induced by Achilles tenotomy via endochondral bone formation: expression of bone and cartilage related genes [J]. Bone, 2010, 46(2): 425-31.

[73] HUANG Y, WANG X, LIN H. The hypoxic microenvironment: a driving force for heterotopic ossification progression [J]. Cell Commun Signal, 2020, 18(1): 20.

[74] WANG H, LINDBORG C, LOUNEV V, et al. Cellular hypoxia promotes heterotopic ossification by amplifying BMP signaling [J]. J Bone Miner Res, 2016, 31(9): 1652-65.

[75] HARNANIK T, PRIHARTONO S, JULIANDHY T. Hyperbaric oxygen in animal model of rheumatoid arthritis: analysis of HIF-1alpha, ACPA and IL-17a [J]. Infect Dis Rep, 2020, 12(Suppl 1): 8766.

[76] HARNANIK T, SOEROSO J, SURYOKUSUMO M G, et al. Effects of hyperbaric oxygen on T helper 17/regulatory T polarization in antigen and collagen-induced arthritis: hypoxia-inducible factor-1alpha as a target [J]. Oman Med J, 2020, 35(1): e90.

[77] YU X, WAN Q, CHENG G, et al. CoCl2, a mimic of hypoxia, enhances bone marrow mesenchymal stem cells migration and osteogenic differentiation via STAT3 signaling pathway [J]. Cell Biol Int, 2018, 42(10): 1321-9.

[78] LIN W C, CHUANG C C, YAO C, et al. Effect of cobalt precursors on cobalt-hydroxyapatite used in bone regeneration and MRI [J]. J Dent Res, 2020, 99(3): 277-84.