·综述·

低氧诱导因子1α调控骨代谢和骨微环境血管生成的研究进展

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摘要:骨微环境血管生成能力的衰退是造成老年性骨质疏松的重要原因之一。老年化导致的骨血管生成能力下降致使骨形成能力减弱,导致骨量流失并诱发骨质疏松。在骨微环境中,骨血管生成能够促进成骨细胞分化,增强骨形成。而成骨细胞则通过分泌VEGF以及FGF2等血管生成因子促进血管内皮细胞的增殖及分化,进而促进骨血管生成。低氧诱导因子1α(HIF-1α)广泛参与骨代谢及血管生成等多个生理过程的调控,且参与骨形成及骨血管生成偶联的调控,在骨血管生成的调控中有着举足轻重的作用。本文主要综述HIF-1α在骨代谢和骨微环境血管生成中的作用机制,为骨血管生成防治骨质疏松的机制研究及骨相关疾病的靶向治疗提供理论基础及研究思路。

关键词:低氧诱导因子1α;骨代谢;血管生成;骨血管

Research progress of hypoxia-inducible factor 1α in angiogenesis of bone microenvironment

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Abstract: Decline in angiogenic capacity of the bone microenvironment is one of the important causes of senile osteoporosis. The decreased bone angiogenesis caused by aging leads to weakened bone formation, leading to loss of bone mass and induction of osteoporosis. In the bone microenvironment, the bone angiogenesis promotes osteoblast differentiation and enhances bone formation. Osteoblasts promote the proliferation and differentiation of vascular endothelial cells by secreting angiogenic factors such as VEGF and FGF2, thereby promoting bone angiogenesis. Hypoxia-inducible factor 1α (HIF-1α) is widely involved in the regulation of multiple physiological processes such as bone metabolism and angiogenesis, and is involved in the regulation of bone formation and angiogenesis, and plays a pivotal role in the regulation of bone angiogenesis. This article mainly reviews the mechanism of HIF-1α in bone metabolism and osseointegration of bone microenvironment, and provides theoretical basis and research ideas for the mechanism of osteoporosis prevention and treatment of bone angiogenesis.

Key words: hypoxia-inducible factor 1α; bone metabolism; angiogenesis; bone vessels

骨血管为骨组织提供氧、营养、激素、细胞因子等物质,在骨生长发育、骨缺损修复以及骨代谢平衡中发挥重要作用。有研究[1]报道,骨组织血流量和骨密度高度相关,骨质疏松患者的骨血流量供给相对要低于正常人群。动物实验[2]也证实,去卵巢骨质疏松小鼠在骨量下降的同时伴随着骨血液供给水平的下降。近年来,《Nature》相继报道了两项研究[3-4],关于骨血管生成与骨形成相互耦联,抑制骨血管生成导致骨形成受阻。此外,老年化导致的骨血管生成能力下降也抑制了骨形成,导致骨量流失。骨血管生成与骨形成是一个相互偶联的过程,骨血管的生成能够促进骨形成,成骨细胞也能够分泌促血管生成细胞因子调控血管内皮细胞的增殖及血管化[5]。低氧诱导因子1α(hypoxia inducible factor

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1α,HIF-1α)在骨代谢过程中发挥着重要作用[6],并且是血管生成的主要调节因子,通过与促血管生成因子如内皮细胞生长因子(vascular endothelial growth factor, VEGF)等协同参与脉管系统形成[7]。血管生成是肿瘤生长的先决条件,自然杀伤细胞(natural killer cells,NK)中 HIF-1α 的缺失抑制了肿瘤生长[8]。关于非小细胞肺癌(non-small cell lung cancer,NSCLC)血管生成分子机制的研究表明,HIF-1α 参与 miR-206 调节的血管生成过程,miR-206 能够抑制 STAT3 / HIF-1α/ VEGF 途径来降低血管生成[9]。

日前,关于 HIF-1α 调控骨微环境血管生成的报道较为鲜见,因此,本文主要综述 HIF-1α 在骨微环境血管生成中的作用机制,为骨血管生成防治骨质疏松的机制研究提供理论基础。

1 HIF1α 的生物学功能

HIF-1α 是 HIF-1 蛋白家族的三个成员之一,由 α 亚基和 β 亚基组成的二聚体,在软骨细胞和其他细胞类型的氧稳态中起关键作用[10-11]。在含氧量正常的条件下,HIF-1α 在氧依赖性降解(oxygen-dependent degradation,ODD)结构域内的关键脯氨酸残基处被脯氨酰羟化酶(prolyl hydroxylases, PHD)羟基化,羟基化时,HIF-1α 与一种 E3 泛素连接酶(Von Hippel-Lindaum, VHL)蛋白结合,随后被蛋白酶体降解[11-13]。在低氧条件下,HIF-1α 的脯氨酰羟基化被抑制,HIF-1α 在细胞核中积累后与 HIF-1 的 β 亚基异二聚体反式激活 HIF 反应基因,包括参与血管生成的基因,如 VEGF[14-16]。迄今为止,已经确定的 HIF 的靶基因有超过 100 个[17],它们在多种生物过程中发挥不同作用,包括调控能量代谢、血管生成、红细胞生成、细胞存活、细胞凋亡和调节 pH[18]。

HIF-1α 能够通过靶向作用于 GLUT1、ADRP、CAXII、VEGF 等细胞因子调控各种生理过程[19]。如通过 GLUT1 调控葡萄糖的转运,通过 ADRP 调控脂代谢,通过 CAXII 调控细胞内 pH 稳态[20]。此外,HIF-1α 还能通过激活 BNIP3 调节细胞自噬和凋亡过程[21]。

2 HIF1α 调控骨代谢

高原鼠兔(ochotona curzoniae)是一种高耐缺氧物种,生活在青藏高原海拔 3 000~5 000 米处,Li 等[22]发现在高原鼠兔的大多数组织中,HIF-1α 蛋白表达水平明显高于生活在海平面的小鼠,并随着栖息地高度的增加而增加。在骨生成过程中,HIF-1α 能够调控成骨细胞的增殖和迁移,促进 BMP 诱导干细胞成软骨分化,促进软骨细胞外基质的分泌。低氧环境下成骨细胞中 HIF-1α 表达上调,但成骨细胞活性降低且凋亡增多,过表达 HIF-1α 能缓解缺氧所引起的凋亡及活性降低,而敲除 HIF-1α 后成骨细胞活性进一步下降[23]。在小鼠中过表达 HIF-1α 能够增加骨形成,提高长骨体积[24]。这些研究提示,低氧应激时成骨细胞 HIF-1α 分泌适应性增多,HIF-1α 能够调控 VEGF 等下游基因促进骨血管生成,同时能够缓解缺氧引起的细胞凋亡,促进成骨细胞的增殖及分化,促进骨形成。

在破骨细胞中,HIF-1α 似乎扮演着另一种角色。破骨细胞位于低氧骨膜区域,当卵巢功能正常时,雌激素抑制破骨细胞 HIF-1α 功能;去卵巢后,雌激素缺乏致使 HIF-1α 功能趋向稳定,破骨细胞活性增强,促进骨吸收。给予外源性雌激素干预后,HIF-1α 受到抑制,骨吸收减弱[25]。此外,将去卵巢小鼠破骨细胞 HIF-1α 基因敲除后,破骨细胞活性减弱,骨量增加。在睾丸切除的雄鼠中也发现类似现象,睾丸切除后 HIF-1α 蛋白表达上调,骨量流失;补充睾酮后 HIF-1α 受到抑制,骨吸收减弱[26]。由此表明,抑制破骨细胞中 HIF-1α 表达能够抑制破骨细胞活性,削弱骨吸收,改善骨代谢。

在软骨内骨形成期间,生长板中心内的软骨细胞增殖并合成无血管细胞外基质,随着软骨细胞的增殖和分化会出现软骨细胞肥大并释放相关分子,包括刺激血管侵入生长板的促血管生成细胞因子 VEGF[27],在骨骼发育和骨修复中,VEGF 依赖性血管侵入无血管软骨是骨形成的关键步骤[28],HIF-1α 信号传导在该过程中参与血管生成和骨生成的耦合。近期,Stegen 等[29]发表在《Nature》的研究报道了软骨细胞中过度的 HIF-1α 信号传导通过干扰细胞的生物能和生物合成致使骨骼发育不良。其具体机制体现在葡萄糖氧化的减少导致能量缺乏,这限制了细胞的增殖,激活了未折叠蛋白质反应同时也减少胶原蛋白的合成,外源性补充谷氨酰胺使之通量增加,进而 α-酮戊二酸水平升高,这又反过来增加了胶原蛋白上的脯氨酸和赖氨酸羟基化。在这种代谢调节方式下的胶原蛋白修饰使得软骨基质能更好的抵抗蛋白酶介导的降解,从而增加骨量。因此,不适当的 HIF-1α 信号传导会导致由胶原蛋白过度修饰引起的骨骼发育不良,这种效应也可能导致与细胞外基质相关的其他疾病,例如癌症和纤

维化[29]。

近期有研究[30]报道,在 microRNAs 调控骨代谢过程中 HIF-1α 也发挥了重要的介导作用。关于 miR-21 在促进骨髓间充质干细胞(bone marrow mesenchymal stem cells, BMSCs)的迁移和成骨分化的实验中发现,miR-21 通过增加 P-Akt 和 HIF-1α 活化程度来促进 BMSCs 的成骨分化能力。Costa V 等[31]采用荧光激活细胞分选术(fluorescence-activated cell sorting, FACS),通过基因表达和蛋白质分析来研究 HIF-1α 和 miR-675-5p 在血管生成和成骨耦合作用的相关研究中发现,miR-675-5p 通过增加 HIF-1α 表达和激活 Wnt /β-catenin 信号通路来促进人骨髓间充质干细胞(human bone marrow mesenchymal stem cells, hMSC)向成骨细胞分化。

3 HIF1α 调控骨微环境血管生成

成体哺乳动物(包括人)外周血、骨髓中的内皮祖细胞(endothelial progenitor cells, EPCs)与骨髓中的多能成体祖细胞(multipotent adult progenitor cells, MAPCS)在体内外均可分化为成熟血管内皮细胞(endothelial cells, ECs),且聚集于靶器官,参与新血管的形成过程[32]。许多细胞因子都具有成血管活性,一项关于 HIF-1α 信号传导在调节血管生成素(angiogenin, ANG)表达和上皮-间质转化(Epithelial-mesenchymal transition, EMT)在缺氧视网膜色素上皮细胞中作用的研究[33]发现,在 ARPe-19 的缺氧小鼠模型中,ANG 的表达水平增加,阻断 HIF-1α 信号传导会抑制 ANG 的高表达。目前已知受 HIF-1α 调控的下游基因有 60 多种,其中最主要的下游基因是 VEGF[34],在 HIF-1α 缺失的情况下,VEGF 表达降低[35]。进一步研究[36]发现,HIF-1α 通过与 VEGF 启动子区域中的缺氧反应元件结合而上调 VEGF 的产生,内皮细胞生长因子受体 1(vascular endothelial growth factor receptor, VEGFR-1)和内皮细胞生长因子受体 2(vascular endothelial growth factor receptor, VEGFR-2)是在内皮细胞上表达的两种均由 HIF-1α 介导的受体,而 VEGF 是通过 VEGFR-1 和 VEGFR-2 实现内皮细胞的趋化和促进有丝分裂,进而引起细胞增殖、迁移和血管生成[37],提示 HIF-1α 在血管生成中有着不可或缺的作用。有研究[38-39]报道,HIF-1α 在诱导血管生成中效果显著,其诱导的新生血管结构正常,无组织水肿、血管平直舒缓少曲折,囊性血管形成率几乎为零。

骨骼是一种高度血管化的组织,但骨腔中的骨微环境则是一个天然的低氧环境。在骨组织中,氧分压约为 1%~6%或小于 1%,其中骨内膜氧分压小于 1.8%,骨腔内血管氧分压小于 1.3%[40-41]。HIF-1α 是低氧或缺氧条件下调控细胞内稳态的核心转录因子。低氧时 HIF-1α 分泌增多并转运至细胞核中与 HIF-1β 形成聚合体,启动 VEGF 及促红细胞生成素(erythropoietin, EPO)等下游基因的转录,引起一系列的耐氧适应性反应[42]。

在血管的发育过程中,HIF-1α 通过调控 VEGF 基因促进血管生成。低表达 HIF-1α 时,即便是在低氧条件下,VEGF 表达也会受到抑制。HIF-1α 还能介导 VEGF 调控血管生成与骨生成间的耦联[43]。在骨生成过程中,软骨细胞分泌 VEGF 并激活血管的生成,促进软骨发育。过表达 HIF-1α 能诱导 VEGF 等血管生成因子过量分泌,刺激长骨血管生成,在血管过度生长的长骨区域也伴随着过度活跃的骨生成现象[44]。近年 Kusumbe 等[45]在 Nature 上报道,在小鼠的骨骼系统中存在 H 型内皮及 L 型内皮两种特殊的毛细血管亚型,其中 H 型内皮是骨血管生成的关键,成骨细胞及其前体细胞主要分布在 H 型内皮细胞周围。而过表达 HIF-1α 能够扩增 H 型内皮及干骺端血管,提高成骨细胞前体细胞数,促进骨生成[24]。

VHL 基因敲出小鼠的 HIF-1α 表达显著上调,血管分布明显增加,并且在牵引成骨过程中骨生成增加,但在成骨细胞中缺乏 HIF-1α 的小鼠血管新生和骨愈合明显受损[46],过表达 HIF-1α 的 MSC 血管生成和成骨活性都有明显增强[47-48]。去铁胺(deferoxamine, DFO)是一种广泛使用的缺氧模仿剂[16, 49]。在一项有关促进骨质疏松性骨缺损愈合的潜在机制研究[50]中,用 DFO 构建缺氧模型,从聚乳酸-羟基乙酸共聚物(poly(lactic-co-glycolic acid), PLGA)释放的 DFO 可以激活 HIF-1α 信号通路后影响几种下游的血管生成因子,包括 VEGF、FGF-2、ANGFT-1。VEGF 和 FGF-2 不仅促进血管生成,而且对 MSC 的成骨分化具有刺激作用[51-53],因此 HIF-1α 在加速骨缺损愈合过程中发挥重要作用。

胫骨软骨发育不全(tibial dyschondroplasia, TD)是一种棘手的家禽疾病,其特征是胫骨生长板(tibial growth plates, TGP)中出现非血管化和非矿化的软骨块[54],其病因是由于血液供应降低或缺乏导致胫骨软骨细胞死亡,进而导致骨骼发育异常。Genin 等[55]的研究表明,脉管系统缺失与 TD 生长

板损伤关系重大，且可能与 HIF-1α 的表达异常有关。一项关于血管生成与 TD 的研究[54]发现，TD 的直接原因是胫骨血管生成受到抑制，抑制 HIF-1α 和 VEGFA / VEGFR 信号传导途径能够阻断软骨细胞的营养供应，导致软骨细胞死亡，胫骨生长板发育受阻。而上调 HIF-1α 能够激活 VEGFA 及其在软骨细胞中的受体，进而刺激血管生成，从而实现在缺氧条件下促进胫骨生长板的正常发育。见图 1。

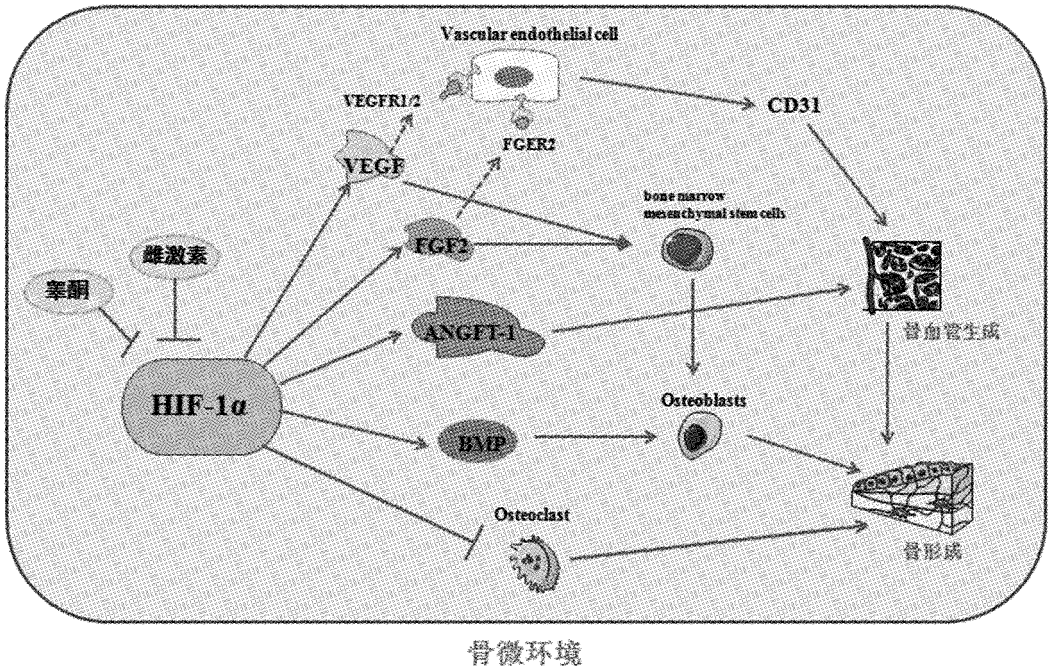

图 1 HIF-1α 调控骨代谢、骨微环境血管生成作用机制图

Fig.1 Mechanism of HIF-1α regulating bone metabolism and angiogenesis in bone microenvironment

4 小结

HIF-1α 在骨代谢中发挥着重要作用，能够调控成骨细胞、破骨细胞以及软骨细胞的增殖、活性及功能。HIF-1α 还广泛参与血管生成过程的调控。在骨微环境中，血管生成能力的减弱将导致骨形成能力衰退。HIF-1α 能够通过调控 VEGF 及 FGF2 等血管生成因子促进血管生成，进而促进骨形成。目前关于 HIF-1α 在骨血管生成中的研究还处于初级阶段，其确切机制尚未清楚，如 HIF-1α 与外泌体、非编码 RNA 之间的关系及其在骨血管生成中的调控作用等仍有待更深入地进一步研究。

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