

Notch信号通路在骨微环境血管生成中的研究进展

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摘要 骨骼中血管高度分布, 骨血管为骨的发育、再生和修复提供必要的营养物质, 维持骨微环境及骨代谢的平衡。近年的研究表明, 老年化所导致的骨微环境血管形成能力下降是诱发骨质疏松症的关键因素之一。其中Notch信号通路在血管生成的调控中扮演着重要的角色, 但目前关于Notch信号通路调节骨微环境血管生成的报道相对较少。鉴于此, 该文主要综述Notch信号通路在骨微环境血管生成中的作用机制, 为骨血管生成的机制研究及骨质疏松的防治提供理论基础。

关键词 Notch信号通路; 骨性疾病; 骨微环境血管生成

Research Progress of Notch Signaling Pathway in Angiogenesis of Bone Microenvironment

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Abstract The blood vessels in the bone are highly distributed, and the bone vessels provide the necessary nutrients for the development, regeneration and repair of the bone, maintaining the balance of the bone microenvironment and bone metabolism. Recent studies have shown that the decline in bone microenvironmental angiogenesis caused by aging is one of the key factors inducing osteoporosis. Among them, Notch signaling pathway plays an important role in the regulation of angiogenesis, but there are relatively few reports on the regulation of bone microenvironment angiogenesis by Notch signaling pathway. In view of this, this paper mainly reviews the mechanism of Notch signaling pathway in bone microenvironment angiogenesis, providing a theoretical basis for the study of the mechanism of bone angiogenesis.

Keywords Notch signaling pathway; bone disease; bone microenvironment angiogenesis

人体骨骼系统发育过程中, 骨形成与骨微环境血管生成关系密切, 骨组织丰富的骨血管能够提供骨生长所需的营养、激素以及生长因子^[1-3]。在骨折愈合和修复中, 骨微环境的血管生成对骨痂的形成至关重要, 骨折中血管形成受损可导致骨愈合和再生延迟^[4-5]。因此, 骨微环境血管生成在骨形成及

骨折恢复中发挥着重要作用。有报告指出, 60岁以上的人群骨量逐渐减少不仅与骨吸收增强和骨形成衰减有关^[6], 与骨骼血流减少也有关^[7]。骨微环境的血管生成能力的衰退是骨质疏松的主要诱因之一^[8]。一项发表在*Nature*的研究表明, 随着年龄的增加, 机体骨组织中血管内皮细胞(vascular endothelial

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cells, VECs)表达的CD31和血小板生长因子(platelet-derived growth factor, PDGF)以及其受体 β 水平均下降,骨形成能力降低^[9]。这提示,老年化导致骨微环境血管生成能力减弱,骨形成能力减弱,进而导致骨质疏松。

骨微环境血管生成需要不同类型血管内皮细胞和各种信号通路之间相互协调,其中Notch信号通路在骨微环境血管生成中发挥重要作用。Ramasamy团队^[9-11]发现,在老年小鼠中骨血管生成下降的过程中,Notch信号通路呈抑制状态,而激活Notch信号通路可逆转老年化引起的骨血管生成下降,使小鼠恢复正常的骨生成速率,维持机体正常的骨量和骨密度,这意味着Notch信号通路的激活能够促进骨血管生成,进而改善骨代谢,缓解骨质流失,起到防治骨质疏松的作用^[12]。但目前Notch信号通路促进骨微环境血管生成的报道很少,本文主要综述Notch信号通路在骨微环境血管生成中的作用机制,为骨血管生成的机制研究及骨质疏松的防治提供理论依据。

1 骨微环境中的血管生成与骨生成

1.1 血管生成

成人骨髓中的骨髓基质细胞(bone marrow stromal cells, BMSCs)是一种具有多分化潜能的细胞,可分化为成骨细胞、脂肪细胞、软骨细胞、神经元等。BMSCs分化为成骨细胞,成骨细胞分泌相关的促血管生成因子,进而促进骨组织再生^[13]。研究证实,成体哺乳动物(包括人类)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的血管内皮细胞,且聚集于靶器官,参与新血管的形成过程^[14]。

在血管生成的过程中,许多细胞因子发挥着重要作用,其中研究最深入的当数血管内皮细胞生长因子(vascular endothelial growth factor, VEGF)^[15]。VEGF与其受体Flk结合促进BMSCs向血管内皮细胞分化,从而促进血管再生并维持脉管系统的完整性^[16]。也有研究报道,有丝分裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)/细胞外信号调节激酶(extrocellular signal-regulated kinase, ERK)信号通路参与VEGF诱导BMSCs向血管内皮细胞分化的调控^[13]。Tetzlaff等^[17]报道,VEGF能够协同Notch信号通路调节血管生成。

1.2 骨血管生成偶联骨形成

在骨微环境中,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^[18]。在小鼠骨骼系统中存在独特形态、分子功能特性的新亚型毛细血管(H型毛细血管),这些血管在特定位置调控血管内皮细胞生长,同时为毛细血管周围的骨祖细胞生长提供特殊的微环境^[19]。血管生成随着年龄的增加和绝经后骨质疏松的发生而受到破坏,骨质疏松期间H型毛细血管的减少伴随着BMSCs的促血管生成潜力下降^[20]。

骨血管内皮细胞中Notch信号传导的激活促进了H型毛细血管柱中血管内皮细胞增殖,增加了H型毛细血管的丰度,偶联血管生成和骨生成^[11]。成骨细胞及其前体细胞主要分布在H型毛细血管内皮细胞周围,通过分泌VEGF、FGF(fibroblast growth factor)、EGFL(epidermal growth factor-like)家族等细胞因子参与骨血管生成的调控。成骨细胞分泌的VEGF通过结合VEGF受体可以促进血管内皮细胞增殖及血管化,促进骨血管生成^[21]。VEGF受体Np1和Np2作为跨膜受体,在结合Sema3之后参与VEGF家族发挥在骨血管生成上的重要作用,由此调节骨生成以及维持正常骨量,而Np2基因敲除小鼠则出现骨量丢失的症状^[22]。

VEGFA能协同FGF9促进II型糖尿病小鼠长骨再生过程中的血管生成、骨生成及骨重建^[23]。FGF信号通路参与血管生成和骨形成的调节,FGF可诱导VEGFA和VEGFR2(vascular endothelial growth factor receptor 2)表达,刺激骨动脉血管扩张,在小鼠中敲除FGF2将导致骨形成率及骨小梁体积减小^[24]。敲除FGF9和FGF18则导致VEGFA表达减少,血管生成能力下降^[25]。成骨细胞还可以通过EGFL信号通路调控骨血管生成,成骨细胞分泌的EGFL6能够通过激活ERK1/2促进血管内皮细胞的增殖及血管化^[26],EGFL7可通过整合蛋白介导的信号通路调节骨血管的生成^[27]。

2 Notch信号通路

Notch信号通路是参与细胞增殖、分化和凋亡过程的信号系统^[28]。Notch信号通路在哺乳动物中由4个Notch受体(Notch1~4)和5个配体[DLL(Delta-like)1、DLL3、DLL4、Jagged1、Jagged2]构成^[29]。另

外还包括细胞内效应分子(Hes1、Hes5)以及相关的酶^[30]。Notch受体是一种异二聚体跨膜蛋白,其组成包括胞内结构域(Notch intracellular domain, NICD)、跨膜结构域(transmembrane domain, TM)和胞外结构域(Notch extracellular domain, NECD)。NICD主要包含5个部分:1个RAM(RBP2J kappa associated molecular)区,可与DNA结合蛋白(C2 promoter binding protein, CBF)结合;6个锚蛋白重复序列(ankyrin repeats, ANK),是启动Notch的增强子,可介导Notch与其他蛋白质之间的相互作用;2个核定位信号(nuclear localization signal, NLS);1个翻译启动区(translational active domain, TAD);1个PEST(proline-glutamate-serine-threonine)区域,与Notch受体的降解有关^[31]。

3 Notch信号通路调控骨微环境血管生成

研究报告,Notch信号通路与骨微环境血管生成密切相关,能够促进小鼠长骨中的血管内皮细胞增殖和血管生成^[11,19]。在老年小鼠中骨血管生成下降的过程中,Notch信号通路呈抑制状态,而激活Notch信号通路可逆转老年化引起的骨血管生成下降,使小鼠恢复正常的骨生成速率,维持机体正常的骨量和骨密度^[10-11]。条件性敲除小鼠成骨细胞中Presenilin-1和Presenilin-2阻断Notch1信号通路后,小鼠表现出老年性骨质疏松症症状^[12]。Hey1是Notch信号通路的重要下游效应因子^[32],Gurel等^[33]在磷酸盐限制型小鼠实验中发现,Hey1的表达减少与干骺端髓血管减少相关。

Notch信号通路能够通过骨形态发生蛋白(bone morphogenetic protein, BMP)调控骨微环境血管生成。BMP属于转化生长因子 β (transforming growth factor β , TGF β)家族亚家族成员,其功能广泛,能够促进成骨细胞的增殖及分化,在BMSCs分化为成骨细胞的过程中起关键作用,主要通过SMADs依赖性(BMP/SMADs信号通路)和非SMADs依赖性(MAPK信号通路)途径发挥生物学作用^[34]。Mouillesseaux等^[35]报道,Notch1信号通路能够通过SMAD6调控血管内皮细胞的BMP响应性以及新的血管分支形成。Notch1信号通路还能通过BMP9调控骨生成和血管生成,同时激活BMP9和Notch信号通路能够促进骨髓间充质干细胞向成骨和血管分化^[29]。在该过程中,血管内皮细胞分泌的Noggin作为重要的细胞因子,激活了骨形态发生蛋白的表达调控骨生成作用,而

骨血管内皮细胞中Notch信号传导,控制着血管分泌因子Noggin的释放。Noggin刺激骨祖细胞的分化和软骨细胞的成熟,从而影响骨小梁、生长板形态和肥大软骨细胞释放VEGF^[11]。

Notch信号通路参与VEGF调控骨微环境血管生成的过程。血管生成的主要调节因子是VEGFA^[36],在血管生成中它主要通过VEGFR2结合发出血管生成信号^[21],诱导顶端细胞(tip cell)的萌芽和迁移。顶端细胞VEGFR2活化能增加Notch配体DLL4的表达,从而激活相邻血管内皮细胞中的Notch信号传导,进而改变DLL4/Notch信号传导致使顶端细胞失活^[37]。与其他器官血管内皮细胞中的DLL4/Notch信号传导途径相比,血管内皮细胞的Notch信号传导对血管发生具有相反的作用,确切的分子机制尚未完全确定,但可能与骨脉管系统中缺乏真正的顶端细胞有关^[37]。

成骨细胞可以分泌VEGF等细胞因子对血管内皮细胞的增殖和血管形成进行调控,在该过程中,VEGF-Notch-Noggin信号传导途径证明,血管生成和骨生成之间是相互偶联的,通过调节Noggin表达水平能够改变Notch信号传导对骨稳态的影响以及血管内皮细胞与骨细胞的通信^[21,38]。然而,骨组织细胞中VEGF的分泌与血管内皮细胞中Notch信号传导之间的反向联系尚未得到明确证实。

在低氧条件下,Notch信号通路还可以协同低氧诱导因子(hypoxia-inducible factor, HIF)参与H型毛细血管生成的调控,增加H型毛细血管和骨祖细胞调节骨形成。缺氧是VEGF表达的重要调节因素^[39]。在缺氧应激条件下,HIF水平增加,其活性受脯氨酰羟化酶结构域蛋白(prolyl hydroxylase domain proteins, PHD)调节。常氧情况下,PHD利用氧气来羟基化HIF,针对性地降解HIF,所以HIF在缺氧条件下更加稳定^[40]。HIF信号传导VEGF表达,并通过血管内皮细胞中的旁分泌信号传导刺激发芽血管生成,协同Notch信号通路调控H型毛细血管的生成^[39]。

综上所述,Notch信号通路能够协同BMP、VEGF和HIF等信号通路调控骨微环境中血管内皮细胞及骨组织细胞的增殖及分化,进而发挥对骨血管生成的调控作用。

4 NOTCH信号通路参与骨疾病的调控

Notch信号通路广泛参与骨疾病的调控。Lin

等^[41]报道, 中药淫羊藿甙(icariin, ICA)和根瘤菌能够通过Notch信号通路上调成骨细胞相关因子的表达, 下调相关脂肪生成因子的表达。ICA能够抑制过氧化物酶体增殖活化受体 γ (peroxisome proliferator-activated receptor γ , *PPAR γ*)、CCAAT增强子结合蛋白 α (CCAAT/enhancer binding protein α , *C/EBP α*)和脂肪酸结合蛋白4(fatty acid-binding protein 4, *FABP4*)的基因表达, 并且下调骨组织中Notch1胞内结构域(Notch1 intracellular domain, N1ICD)和Jagged1的蛋白表达^[42]。这表明, ICA通过Notch信号通路抑制*PPAR γ* 、*C/EBP α* 和*FABP4* mRNA的表达进而抑制间充质干细胞分化为脂肪细胞, 这是ICA治疗骨质疏松临床疗效的相关机制理论基础^[42]。

雌激素替代疗法治疗骨质疏松症的相关研究中, 人BMSCs培养中加入雌激素, 雌激素通过促进Jagged1的表达来增强hBMSCs的Notch信号通路传导, 进而增强hBMSC增殖和分化, 这是Notch信号通路增强绝经后骨质疏松症患者hBMSCs的增殖和分化的重要机制^[43]。HCS(Hajdu-Cheney syndrome)也被称为遗传性骨发育不良并肢端溶骨症, 是一种常染色体显性遗传骨骼疾病, 特点是明显骨质疏松、身材矮小、面容粗糙畸形、长骨弯曲、脊柱异常^[44], HCS发病机制研究中发现, Notch2信号传导异常与破骨细胞活跃程度密切相关^[45], 其具体机制在于Notch2信号的传导异常导致核因子 κ B配体受体激活剂的表达增强, 进而导致破骨细胞生成增加, 骨吸收上升, 诱发骨量丢失^[44]。另外, 最新的研究显示, Notch信号通路还参与非编码RNA调控骨质疏松的过程。Wang等^[46]报道, 长链非编码RNA LINC00311通过抑制DLL3表达来调节Notch信号通路, 进而促进骨质疏松大鼠成骨细胞的增殖与分化, 同时抑制破骨细胞凋亡。

5 小结

在骨微环境中, 血管生成和骨形成关系密切。其中Notch信号通路在骨微环境血管生成和骨形成中发挥重要作用, 能通过BMP通路、VEGFA/VEGFR2调控骨血管生成及骨形成, 同时还能协同HIF参与骨微环境中H型毛细血管生成的调控。在衰老的过程中, 骨微环境血管生成能力的衰退是导致骨质疏松的主要诱因之一, 而Notch信号的激活能够逆转骨血管生成能力的下降, 促进骨生成, 进而起到预防

骨质疏松症的效果, 但Notch信号通路与骨微环境血管生成的确切机制还尚未明确, 有待进一步探究。

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