DOI: 10.11844/cjcb.2025.01.0002
中国细胞生物学学报 Chinese Journal of Cell Biology 2025, 47(1): 8–17 CSTR: 32200.14.cjcb.2025.01.0002

研究论文

BMP9通过Wnt/β-catenin和VEGFa信号诱导前脂肪细胞成骨分化

王景江 刘洋*
(重庆医科大学附属第二医院骨科, 重庆 400010)

摘要 该文旨在研究Wnt/β-catenin信号通路和血管内皮细胞生长因子a(VEGFa)在骨形态发生蛋白9(BMP9)诱导前脂肪细胞成骨分化中的作用。通过BMP9重组腺病毒(AdBMP9)感染前脂肪细胞, 利用Western blot检测β-catenin和VEGFa的蛋白表达水平, 荧光素酶报告质粒检测Wnt/β-catenin信号活化程度。在过表达或沉默β-catenin及VEGFa后, 用AdBMP9感染细胞, 通过活性测定和染色检测成骨早期标志物碱性磷酸酶(ALP)的活性, 利用Western blot检测成骨晚期标志物骨桥素(OPN)、骨钙素(OC)以及成骨转录因子Runt相关转录因子2(Runx2)的蛋白表达情况, 茜素红染色检测钙盐沉积情况, Micro-CT和H&E染色检测前脂肪细胞在裸鼠皮下异位成骨情况。结果发现BMP9能上调前脂肪细胞中β-catenin和VEGFa的蛋白水平, 并增加β-catenin/Tcf4转录活性; 激活Wnt/β-catenin信号或过表达VEGFa能促进BMP9介导的ALP活性、OPN及OC蛋白表达和钙盐沉积; 过表达β-catenin和VEGFa能促进BMP9诱导的前脂肪细胞裸鼠皮下异位成骨; 过表达β-catenin和VEGFa均增加了BMP9诱导的成骨转录因子Runx2的活性, 而沉默VEGFa抑制了BMP9上调β-catenin/Tcf4转录活性的作用。这些结果表明Wnt/β-catenin和VEGFa信号在BMP9诱导前脂肪细胞成骨分化的过程中起着至关重要的调控作用。

关键词 骨形态发生蛋白9; 前脂肪细胞; Wnt/β-catenin信号; 血管内皮细胞生长因子a; 成骨分化

BMP9-Induced Osteogenic Differentiation of Preadipocytes Through Wnt/β-Catenin and VEGFa Signaling Pathways

WANG Jingjiang, LIU Yang*
(*Department of Orthopaedics, Second Affiliated Hospital, Chongqing Medical University, Chongqing 400010, China*)

Abstract The aim of this study is to examine how Wnt/β-catenin and VEGFa (vascular endothelial growth factor a) signaling pathways influence the osteogenic differentiation of preadipocytes induced by BMP9 (bone morphogenetic protein 9). Preadipocytes were infected with AdBMP9 (BMP9 recombinant adenovirus). Western blot

收稿日期: 2024-10-16 接受日期: 2024-12-09
重庆市教委科学技术研究项目青年项目(批准号: KJQN202100413)、重庆市自然科学基金面上项目(批准号: CSTB2022NSCQ-MSX0052)和重庆医科大学附属第二医院宽仁英才项目(批准号:〔2022〕28)资助的课题
*通信作者。Tel: 13996482275, E-mail: liuy@hospital.cqmu.edu.cn
Received: October 16, 2024 Accepted: December 9, 2024
This work was supported by the Scientific and Technological Research Program of Chongqing Municipal Education Commission (Grant No.KJQN202100413), the Natural Science Foundation of Chongqing (Grant No.CSTB2022NSCQ-MSX0052) and the Kuanren Talents Program of the Second Affiliated Hospital of Chongqing Medical University (Grant No.〔2022〕28)
*Corresponding author. Tel: +86-13996482275, E-mail: liuy@hospital.cqmu.edu.cn

analysis was conducted to assess the protein expression levels of β-catenin and VEGFa, while a luciferase reporter assay was utilized to evaluate the activation of the Wnt/β-catenin signaling pathway. After overexpressing or silencing β-catenin and VEGFa, the cells were infected with AdBMP9. ALP (alkaline phosphatase) activity, an early osteogenic marker, was assessed through enzyme activity assays and staining. Western blot was used to detect the protein expression of late osteogenic markers, including OPN (osteopontin), OC (osteocalcin), and the osteogenic transcription factor Runx2 (Runt-related transcription factor 2). Alizarin red staining was used to assess calcium deposition. Ectopic bone formation of preadipocytes in nude mice was examined using Micro-CT and H&E staining. It was found that BMP9 upregulated the protein levels of β-catenin and VEGFa in preadipocytes and increased β-catenin/Tcf4 transcriptional activity. Activation of the Wnt/β-catenin signaling or overexpression of VEGFa promoted BMP9-mediated ALP activity, OPN and OC protein expression, and calcium deposition. Overexpression of β-catenin and VEGFa enhanced BMP9-induced ectopic bone formation in preadipocytes in nude mice. Additionally, overexpression of β-catenin and VEGFa promoted BMP9-induced Runx2 expression, while silencing VEGFa inhibited the BMP9-induced increase in β-catenin/Tcf4 transcriptional activity. These results imply that the Wnt/β-catenin and VEGFa signaling pathways are crucial regulators in the osteogenic differentiation of preadipocytes induced by BMP9.

Keywords bone morphogenic protein 9; preadipocytes; Wnt/β-catenin signaling; vascular endothelial growth factor a; osteogenic differentiation

随着我国老龄化进程加剧, 骨质疏松症(osteoporosis, OP)的防治已成为当前迫切需要解决的医疗和社会问题。骨髓间充质干细胞(mechenchymal stem cells, MSCs)能分化为骨和脂肪等多种组织, MSCs在正常生理状况下的成骨和成脂分化程度在特定转录因子的调控下处于相对平衡状态。但研究表明, MSCs在OP发生时, 会以减少成骨分化为代价, 生成更多脂肪组织, 因此MSCs成骨和成脂的分化失衡是OP的重要病因之一[1-6]。MSCs在成脂分化过程中, 首先分化为前脂肪细胞, 随后进一步发育为成熟脂肪细胞。如果能够通过细胞因子诱导前脂肪细胞进行成骨分化, 就可以通过减少脂肪生成来促进骨形成, 从而有望逆转MSCs的成骨与成脂分化失衡, 为OP提供有效的治疗方案。本课题组通过研究已经发现骨形态发生蛋白9(bone morphogenetic protein 9, BMP9)能有效诱导前脂肪细胞成骨转分化, 并初步证明Wnt/β-catenin信号参与了该过程[8], 但具体作用尚需进一步阐明。

血管内皮细胞生长因子(vascular endothelial growth factor, VEGF)家族包括VEGFa、b、c、d、e和胎盘生长因子, 哺乳动物主要表达VEGFa。VEGFa不仅对MSCs成骨和成脂分化有重要的调控作用[9], 还与BMP2、4、6存在促骨形成的协同作用[10-12], 但VEGFa与不同亚型BMP产生的成骨协同作用并不相同[13]。VEGFa近期被报道能通过PI3K/Akt信号促进BMP9所介导的MSCs的体内外成骨[14], 提示VEGFa和BMP9亦有成骨协同作用, 但VEGFa在BMP9促进前脂肪细胞成骨分化中的作用尚不明确。因此, 本研究进一步探讨了Wnt/β-catenin和VEGFa信号对BMP9诱导前脂肪细胞成骨分化的影响, 从而为OP的治疗提供新的理论依据。

1 材料与方法

1.1 材料

表达BMP9(AdBMP9)、VEGFa(AdVEGFa)、绿色荧光蛋白(green fluorescent protein, GFP)(AdGFP)、Wnt3a(AdWnt3a)、Wnt10b(AdWnt10b)、β-catenin(AdBC)以及针对β-catenin和VEGFa的小干扰RNA(AdsimBC、AdsimVEGFa)的腺病毒由AdEasy系统构建[8]; β-catenin的荧光素酶报告质粒pTOP-Luc由重庆医科大学何百成教授惠赠; 小鼠3T3-L1前脂肪细胞购自美国典型菌种保藏中心(American Type Culture Collection, ATCC); 碱性磷酸酶(alkaline phosphatase, ALP)化学发光检测试剂盒、Promega光度计(GloMax20/20)购自BD公司; ALP染色试剂盒购自上海碧云天生物有限公司; 茜素红S染料购自Sigma公司; 实验抗体均购自Santa Cruz公司; DMEM高糖培养基和胎牛血清购自Hyclone公司; Lipofectamine购自Invitrogen公司。

1.2 细胞处理分组

将细胞分为空白对照(Control组), AdBMP9(BMP9组)、AdGFP(GFP组)、AdWnt3a(Wnt3a组)、AdWnt10b(Wnt10b组)、AdBC(BC组)、AdVEGFa(VEGFa组)、AdsimBC(simBC组)和AdsimVEGFa(simVEGFa组)单独感染组, 以及AdBMP9与以上不同病毒共同感染组(包括BMP9+BC组、BMP9+Wnt3a组、BMP9+Wnt10b组、BMP9+VEGFa组、BMP9+simBC组、BMP9+simVEGFa组)。

1.3 前脂肪细胞培养和ALP活性检测及染色

3T3-L1前脂肪细胞采用DMEM培养基(含10%胎牛血清、100 kU/L青霉素和100 mg/L链霉素)在5% CO_2及37 ℃环境下培养。按照1.2中的方案处理细胞5和7天后, 测定ALP活性。处理8天后, 利用BCIP/NBT显色试剂盒进行ALP染色。

1.4 钙盐沉积实验

将前脂肪细胞接种于24孔板中, 待融合至30%时, 首先用AdBMP9感染细胞, 然后再分别用AdBC、AdSimBC、AdVEGFa或AdsimVEGFa共感染细胞, 加入成骨诱导培养基培养14天后用茜素红S染色: 弃去培养基, PBS清洗1次, 加入200 μL/孔、0.05%的戊二醛室温下固定10 min, 去离子水洗1次后加入250 μL/孔、0.4%的茜素红S溶液, 观察有红色物质堆积时弃去染液, 最后用去离子水终止染色反应。随后在显微镜下成像并拍照记录[8]。

1.5 Western blot

将细胞接种于6孔板中, 待其贴壁后添加不同处理因素。在相应的时间点提取细胞裂解液, 然后室温下经SDS-PAGE、电转膜、BSA封闭、4 ℃孵育1:1 000稀释的一抗过夜以及37 ℃孵育1:5 000稀释的二抗1 h等步骤后, 最后进行显影和成像保存。

1.6 荧光素酶报告基因的检测

将细胞接种于T25培养瓶中, 待其贴壁后用Lipofectamine转染荧光素酶报告质粒pTOP-Luc, 4 h后更换培养液。12 h后, 将细胞消化并重新种于24孔板, 贴壁后添加不同处理因素。24 h后, 裂解细胞并根据试剂盒说明进行荧光素酶活性测定。

1.7 裸鼠皮下注射成骨模型建立及H&E染色

将3T3-L1前脂肪细胞以30%~50%的细胞密度接种到100 mm^2培养皿中, 分别使用AdBMP9独自和与AdBC或AdVEGFa共同感染细胞24 h。收集细胞并以PBS重悬, 调整细胞的浓度至5×10^7个/mL。随机将动物分为BMP9、BMP9+BC和BMP9+VEGFa组, 每组5只。以裸鼠的四肢根部为异位成骨注射点, 分别将100 μL的PBS细胞重悬液(约5×10^6个细胞)注射至动物皮下。饲养5周后将动物处死, 分离并收集皮下移植细胞形成的包块, 然后以10%福尔马林溶液固定, 经脱钙, 石蜡包埋切片, 进行H&E染色, 在显微镜下进行组织学观察并拍照。本研究动物实验已经获得重庆医科大学附属第二医院伦理委员会的批准(〔2021〕623号)。

1.8 Micro-CT

裸鼠皮下骨组织包块用Micro-CT(Explore Locus SP, GE Healthcare, 美国)扫描, 并用相应软件(Micview V2.1.2)进行三维重建分析数据。

1.9 统计学分析

所有计量资料以$\bar{x}\pm s$表示, 用Excel软件进行数据整理和统计。所有实验均进行3次重复, 两组之间比较采用t检验, 利用SPSS 17.0统计软件进行统计分析。以$P<0.05$认定为有统计学差异。

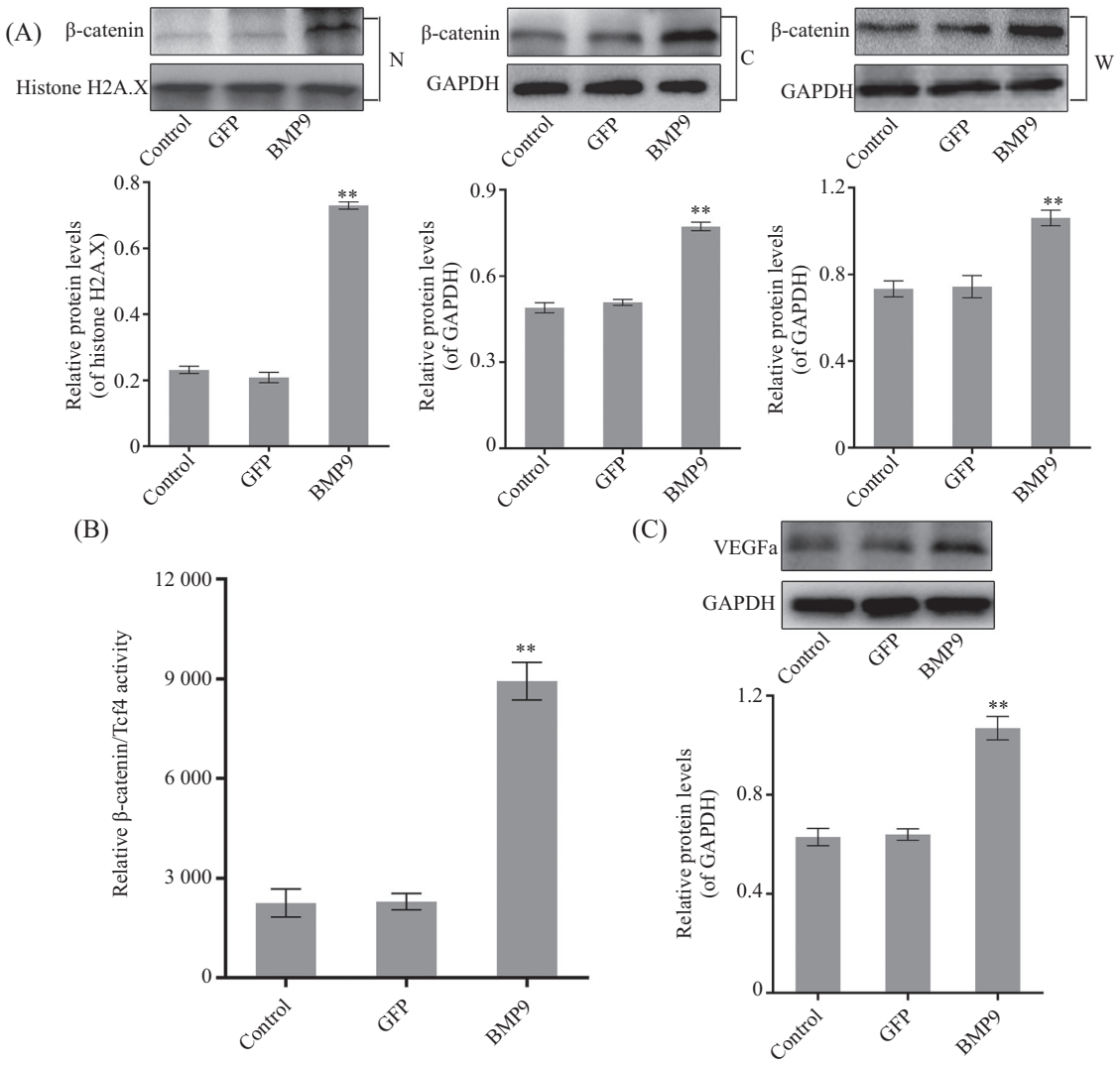
2 结果

2.1 BMP9能增加前脂肪细胞中Wnt/β-catenin和VEGFa信号的活性

为明确BMP9对前脂肪细胞中Wnt/β-catenin和VEGFa信号活性的影响, 我们提取了在被AdBMP9感染1天后的3T3-L1细胞的总蛋白、胞质蛋白和胞核蛋白, 通过Western blot检测了β-catenin和VEGFa的表达情况。结果显示BMP9在显著增加细胞总蛋白和胞质蛋白中β-catenin蛋白水平的同时, 也促进了β-catenin的细胞核转位(图1A)。此外, 荧光素酶报告基因检测*β-catenin/Tcf4*转录活性的结果也显示BMP9能明显提高pTOP-Luc报告质粒的荧光素酶活性($P<0.01$)(图1B)。细胞中VEGFa的蛋白表达量在BMP9的刺激下也显著增加(图1C)。以上结果表明, BMP9能够激活前脂肪细胞中的Wnt/β-catenin和VEGFa信号。

2.2 Wnt/β-catenin和VEGFa信号调控BMP9诱导的前脂肪细胞ALP活性

ALP测定结果显示, 过表达β-catenin或Wnt/β-catenin信号通路配体Wnt3a和Wnt10b均能增强BMP9诱导的ALP活性(图2A~图2C), 但沉默β-catenin却抑制BMP9诱导ALP活性的作用(图2D)。过表达或沉默VEGFa则分别促进和阻碍了BMP9诱导ALP活性



A: β-catenin的蛋白表达情况(N: 细胞核; C: 细胞质; W: 总细胞); B: 荧光素酶报告基因pTOP-Luc活性; C: VEGFa的蛋白表达情况。**P<0.01, 与对照组比较。

A: the protein expression of β-catenin (N: nucleus; C: cytoplasm; W: whole cell); B: luciferase activities of pTOP-Luc reporter; C: the protein expression of VEGFa. **P<0.01 *vs* control group.

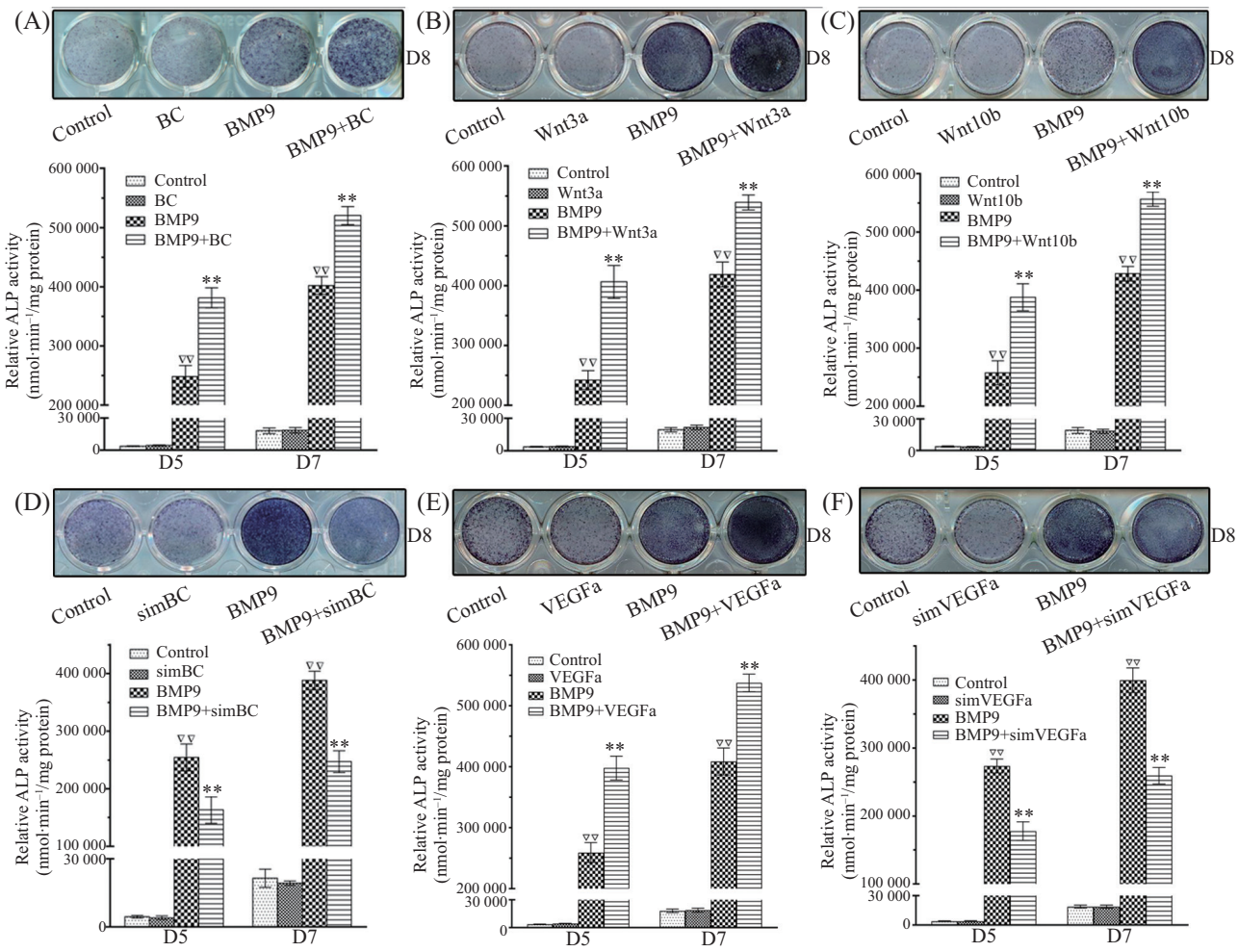
图1 BMP9激活前脂肪细胞中Wnt/β-catenin和VEGFa信号

Fig.1 BMP9 activated Wnt/β-catenin and VEGFa signaling in preadipocytes

的作用(图2E和图2F)。该结果提示, Wnt/β-catenin和VEGFa信号在BMP9刺激前脂肪细胞表达ALP的过程中起到了正向调控作用。

2.3 β-catenin和VEGFa对BMP9在前脂肪细胞中诱导的晚期成骨分化标志物OPN和OC的表达及钙盐沉积的影响

分别在过表达或沉默β-catenin或VEGFa的情况下用AdBMP9感染细胞, 12天后利用Western blot检测成骨晚期标志物骨桥素(osteopontin, OPN)和骨钙素(osteocalcin, OC)的表达水平。结果如图3A和图3B所示: BMP9能够上调OPN和OC在前脂肪细胞中的表达水平; 过表达β-catenin或VEGFa后, BMP9诱导OPN和OC表达的能力进一步增强; 沉默β-catenin或VEGFa后, BMP9诱导的OPN和OC表达水平明显降低。感染14天后用茜素红S染色检测钙盐沉积情况, 结果(图3C和图3D)显示: 和对照组比较, BMP9组有明显矿化结节形成; AdBC或AdVEGFa与AdBMP9共感染组的矿化结节形成明显且较BMP9单独作用组增多, 矿化面积也增大; 而沉默β-catenin或VEGFa后, BMP9诱导的钙盐结节明显变少, 矿化程度亦减弱。这表明Wnt/β-catenin和VEGFa信号对BMP9诱导的前脂肪细胞晚期成骨分化具有促进作用。



A~C: 过表达β-catenin、Wnt3a和Wnt10b对BMP9诱导ALP活性的影响; D: 抑制β-catenin对BMP9诱导ALP活性的影响; E: 过表达VEGFa对BMP9诱导ALP活性的影响; F: 抑制VEGFa对BMP9诱导ALP活性的影响。▽▽P<0.01, 与对照组比较; **P<0.01, 与BMP9组比较。

A-C: effect of β-catenin, Wnt3a and Wnt10b overexpression on BMP9-induced ALP activity; D: effect of β-catenin inhibition on BMP9-induced ALP activity; E: effect of VEGFa overexpression on BMP9-induced ALP activity; F: effect of VEGFa inhibition on BMP9-induced ALP activity. ▽▽P<0.01 *vs* control group; **P<0.01 *vs* BMP9 group.

图2 Wnt/β-catenin和VEGFa信号在前脂肪细胞中对BMP9诱导ALP的影响

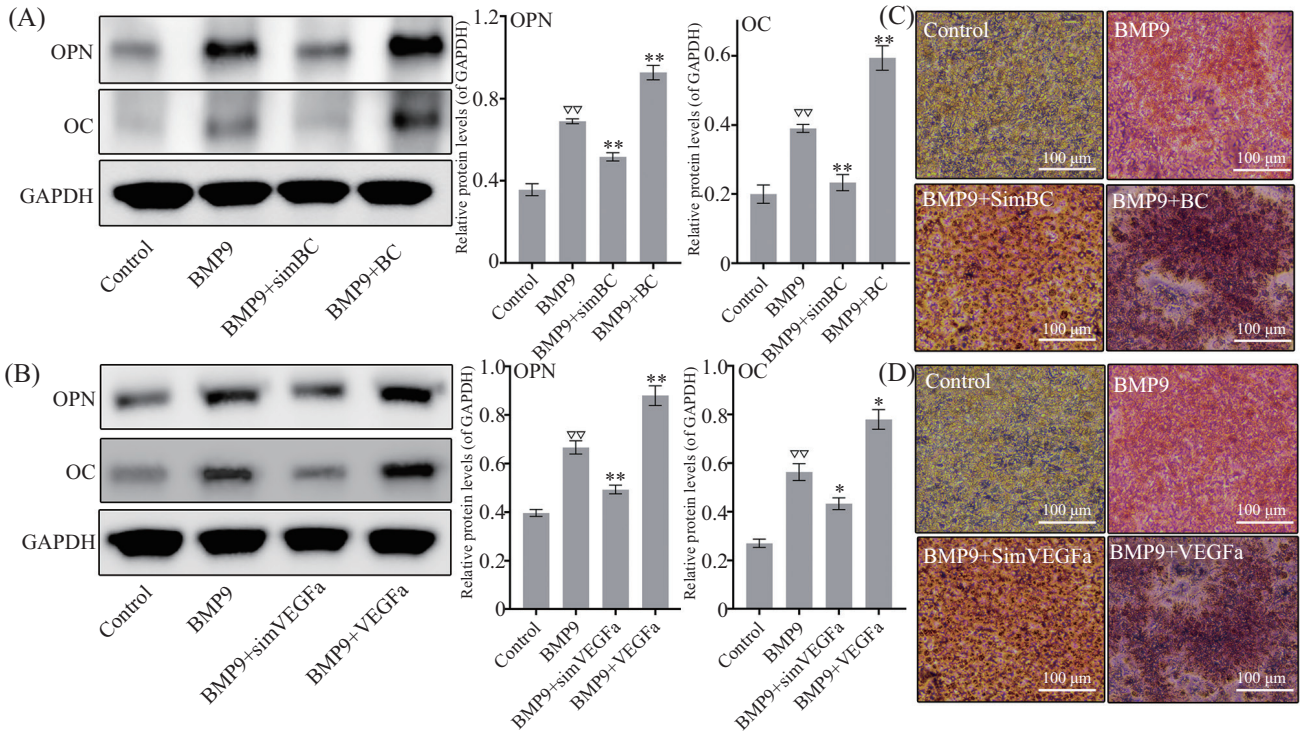
Fig.2 The effect of Wnt/β-catenin and VEGFa signaling on BMP9-induced ALP activity in preadipocytes

2.4 β-catenin和VEGFa对BMP9诱导的前脂肪细胞裸鼠皮下异位成骨的影响

将异位成骨形成的包块进行大体观察发现, AdBMP9+AdBC组和AdBMP9+AdVEGFa组的皮下包块体积均大于AdBMP9组(图4A)。经Micro-CT扫描进行重建和检测后, 发现AdBMP9+AdBC组和AdBMP9+AdVEGFa组包块的总体积以及骨体积明显大于AdBMP9组(图4B~图4D)。H&E染色结果显示, AdBMP9+AdBC组和AdBMP9+AdVEGFa组的骨小梁明显比AdBMP9组多, 厚度也增加(图4E)。体内也实验表明, Wnt/β-catenin和VEGFa信号能促进BMP9诱导的前脂肪细胞裸鼠皮下异位成骨。

2.5 β-catenin和VEGFa对BMP9诱导成骨转录因子Runx2活性的影响

我们在使用AdBMP9、AdBMP9+AdBC以及AdBMP9+AdVEGFa感染细胞48 h后, 通过Western blot检测了Runt相关转录因子2(runt-related transcription factor 2, Runx2)的表达情况。结果如图5A显示, 过表达β-catenin和VEGFa能明显增强BMP9刺激前脂肪细胞表达Runx2的作用。此外, 我们采用荧光素酶报告基因pTOP-Luc检测β-catenin/Tcf4转录活性的结果表明, BMP9能激活前脂肪细胞中的Wnt/β-catenin信号, 但沉默VEGFa会减弱该作用, 而过表达VEGFa则可增强该作用(图5B)。



A、B: OPN和OC的蛋白表达情况; C、D: 茜素红S染色。*P<0.05, **P<0.01, 与BMP9组比较; $^{\nabla\nabla}P$<0.01, 与对照组比较。

A,B: the protein expression of OPN and OC; C,D: Alizarin red S staining. *P<0.05, **P<0.01 *vs* BMP9 group; $^{\nabla\nabla}P$<0.01 *vs* control group.

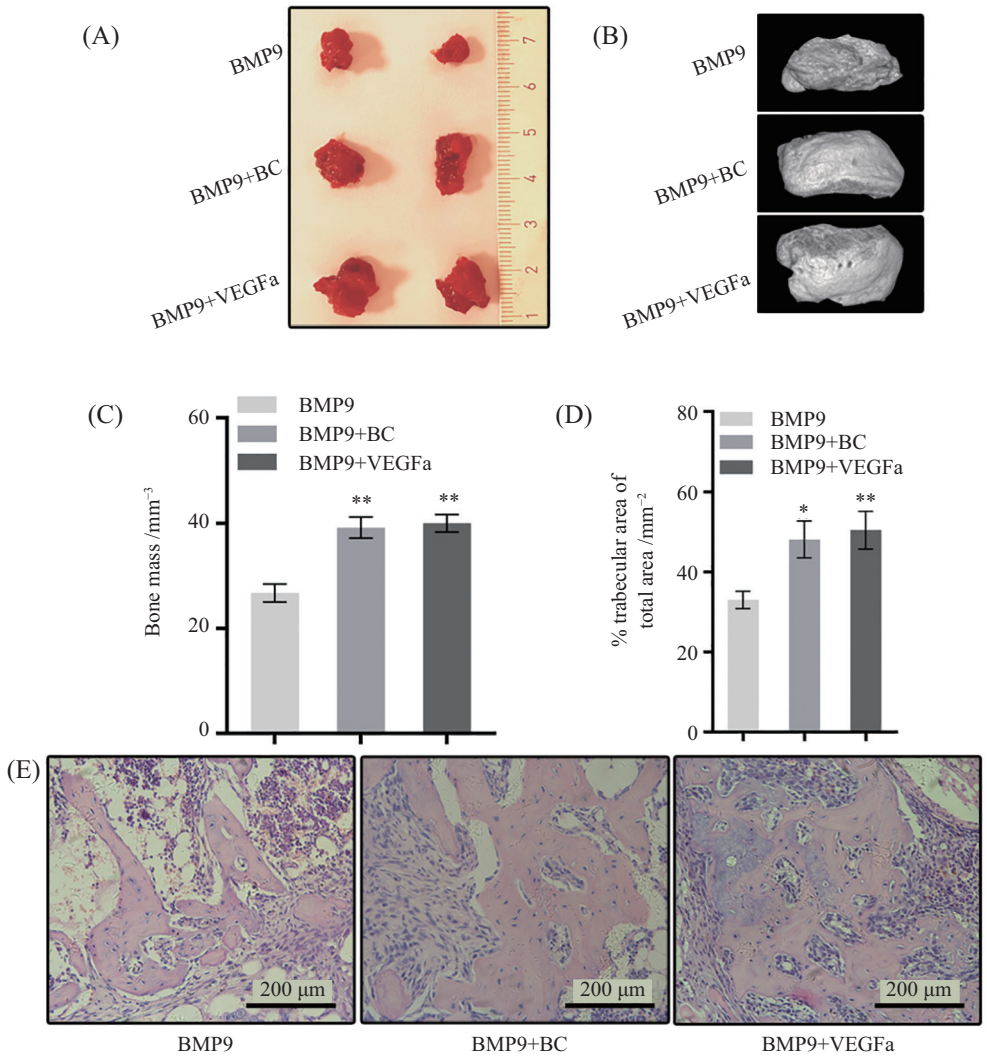
图3 β-catenin和VEGFa促进在前脂肪细胞中BMP9诱导的晚期成骨分化

Fig.3 β-catenin and VEGFa promoted BMP9-mediated late stage of osteogenesis in preadipocytes

3 讨论

尽管Wnt/β-catenin和VEGFa信号已经被证实在骨形成和骨修复过程中起着重要作用[15-16], 但二者在BMP9诱导前脂肪细胞成骨分化中的作用尚未完全明确。有研究发现, Wnt/β-catenin和VEGFa信号均能被BMP9激活, 并且二者在BMP信号诱导的骨形成中发挥了重要的作用[17-24]。基于此, 我们推测Wnt/β-catenin和VEGFa信号可能也参与了BMP9诱导前脂肪细胞成骨分化的过程。为了对此进行验证, 我们首先检测了在前脂肪细胞中BMP9对Wnt/β-catenin信号活性和VEGFa表达的影响。结果发现, BMP9能够在前脂肪细胞中显著激活经典Wnt/β-catenin信号并上调VEGFa的表达, 这表明二者确实与BMP9调控前脂肪细胞成骨分化的生物学作用相关。实际上, 目前已有许多研究也发现BMPs能在多种细胞中激活Wnt/β-catenin和VEGFa信号[19,25-30]。但是, 也有研究表明, BMPs对Wnt/β-catenin和VEGFa信号的影响为抑制作用[31-33]。这些研究结果均提示BMPs能影响Wnt/β-catenin和VEGFa信号的活性, 但具体的作用则可能与细胞种类以及检测时细胞所处的状态和微环境相关, 而我们的结果则证实了在前脂肪细胞中BMP9能增强Wnt/β-catenin和VEGFa信号的活性。

我们前期的研究结果提示BMP9能以抑制糖原合成酶激酶3β(glycogen synthase kinase-3β, GSK3β)活性的方式促进β-catenin的核内转移[8], 但BMP9是否还能通过其他方式激活经典Wnt信号, 尚不明确。有研究表明, BMP9能够对Wnt信号的配体进行调控[34]。与此相似, 还有研究发现BMP2也有调控Wnt信号受体和配体的作用。例如, ZHANG等[26]发现BMP2能通过刺激Wnt受体LRP5的表达来激活成骨细胞中的Wnt/β-catenin信号。在角质形成细胞中, BMP2也同样可以促进Wnt2b、Wnt5b、Wnt7b、Wnt13、Fz6、Fz8和Fz10等Wnt信号受体和配体的表达[27]。由于Wnt/β-catenin通路的转导是由Wnt配体和受体的结合所开启的[35], 所以BMP9在前脂肪细胞中是否也能通过调控Wnt受体和配体的方式激活Wnt/β-catenin信号尚需进一步验证。BMP9自身发挥生物学作用主要通过BMPR-Smad信号通路, 有研究发现BMP4和BMP7分别能够通过Smad1/5和Smad5上调VEGFa的表达[29], 而BMP9在前脂肪细胞



A: 皮下异位成骨包块; B~D: Micro-CT检测异位成骨包块; E: H&E染色结果。*P<0.05, **P<0.01, 与BMP9组比较。

A: macrographic images of ectopic bone mass; B-D: detection of bony masses by Micro-CT scanning analysis; E: H&E staining of retrieved samples. *P<0.05, **P<0.01 *vs* BMP9 group.

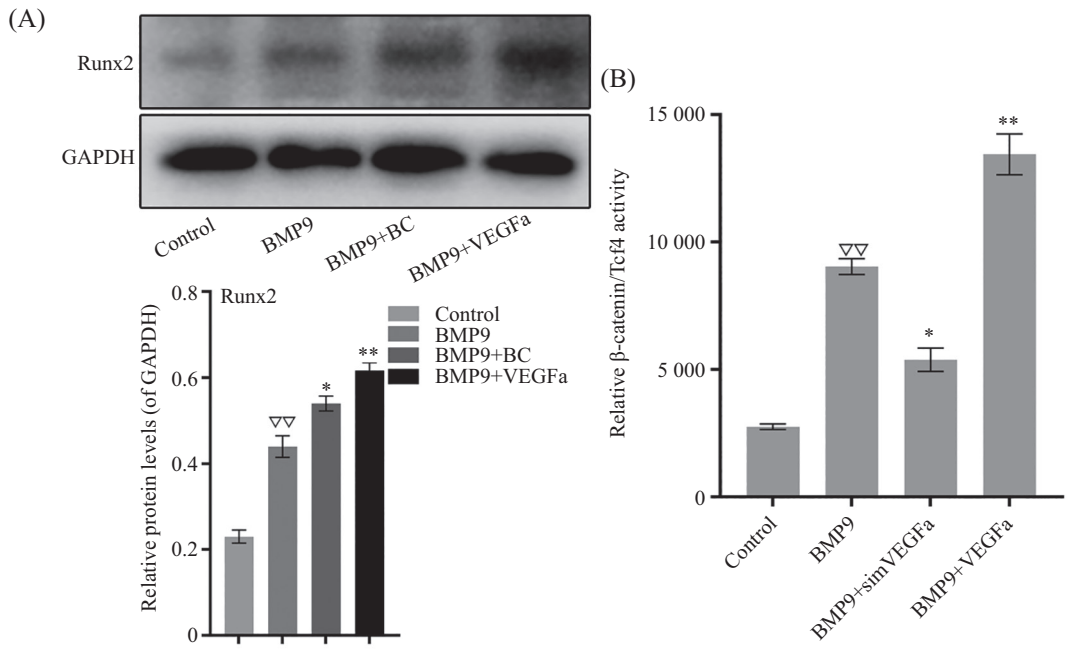
图4 β-catenin和VEGFa对BMP9诱导的前脂肪细胞体内成骨的影响

Fig.4 The effect of β-catenin and VEGFa on BMP9-induced ectopic bone formation of preadipocytes

中调控VEGFa是否与BMPR-Smad或其他途径相关，则尚需进一步研究证实。

在证实了BMP9能在前脂肪细胞中上调Wnt/β-catenin和VEGFa信号活性后，我们随后检测了二者在BMP9诱导前脂肪细胞成骨分化中所起的作用。而结果显示，不管是在体外还是体内环境下，Wnt/β-catenin和VEGFa信号在BMP9诱导前脂肪细胞成骨分化的过程中均发挥了重要的促进作用。这一结果与许多之前的研究发现一致，这些研究都证实了BMPs在多种细胞类型中都具有和Wnt/β-catenin或VEGFa信号协同促进骨形成的生物学作用[17,19,36-40]。然而，我们同时也发现单纯激活Wnt/β-catenin或VEGFa信号本身对前脂肪细胞的成骨分化并无明显作用，提示二者促进BMP9诱导前脂肪细胞成骨分化的效应并不是简单的和BMP9的叠加作用，而是可能通过特定的成骨相关因子进行调节的。

Runx2作为重要的成骨调控因子，不仅被证实能在前脂肪细胞中表达并促进前脂肪细胞成骨分化[41-42]，并且与Wnt/β-catenin和VEGFa信号发挥成骨作用密切相关[10,43-47]。由于我们前期研究还发现BMP9能增加前脂肪细胞中Runx2的活性[8]，所以我们推测Wnt/β-catenin和VEGFa信号增强BMP9诱导前脂肪细胞成骨分化的作用可能与Runx2相关。结果发现，过表达β-catenin或VEGFa均能显著增加



A: 成骨转录因子Runx2的蛋白表达情况; B: 荧光素酶报告基因 pTOP-Luc活性。▽▽P<0.01, 与对照组比较; *P<0.05, **P<0.01, 与BMP9组比较。
A: the protein expression of Runx2; B: luciferase activities of pTOP-Luc reporter. ▽▽P<0.01 *vs* control group; *P<0.05, **P<0.01 *vs* BMP9 group.

图5 β-catenin和VEGFa对BMP9诱导成骨转录因子活性的影响以及VEGFa对BMP9激活Wnt/β-catenin信号的影响

Fig.5 The effect of β-catenin and VEGFa on BMP9-induced osteogenic transcriptional factor and the effect of VEGFa on BMP9-induced Wnt/β-catenin signaling

BMP9在前脂肪细胞中诱导的Runx2的表达水平, 从而对该可能性进行了验证。由于VEGFa被证实能通过特定的方式在不同种类细胞中上调Wnt/β-catenin信号的活性[48-49], 因此我们也检测了VEGFa对BMP9在前脂肪细胞中激活Wnt/β-catenin信号的影响, 结果发现VEGFa对BMP9激活的Wnt/β-catenin信号活性有正向调控作用。以上的这些发现提示了Wnt/β-catenin和VEGFa信号调控BMP9诱导前脂肪细胞成骨分化的可能分子机制。

综上, 本研究通过实验证实在BMP9诱导前脂肪细胞分化的过程中, Wnt/β-catenin和VEGFa信号起着重要的调控作用, 这一结果进一步阐明了BMP9发挥成骨生物学活性的分子机制, 为BMP9在骨量减少相关疾病临床应用提供了理论基础。

参考文献 (References)

[1] MEUNIER P, AARON J, EDOUARD C, et al. Osteoporosis and the replacement of cell populations of the marrow by adipose tissue. A quantitative study of 84 iliac bone biopsies [J]. Clin Orthop Relat Res, 1971, 80(80): 147-54.

[2] WRONSKI T J, WALSH C C, IGNASZEWSKI L A. Histologic evidence for osteopenia and increased bone turnover in ovariectomized rats [J]. Bone, 1986, 7(2): 119-23.

[3] MARTIN R B, CHOW B D, LUCAS P A. Bone marrow fat content in relation to bone remodeling and serum chemistry in intact and ovariectomized dogs [J]. Calcif Tissue Int, 1990, 46(3): 189-94.

[4] JUSTESEN J, STENDERUP K, EBBESEN E N, et al. Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis [J]. Biogerontology, 2001, 2(3): 165-71.

[5] VERMA S, RAJARATNAM J H, DENTON J, et al. Adipocytic proportion of bone marrow is inversely related to bone formation in osteoporosis [J]. J Clin Pathol, 2002, 55(9): 693-8.

[6] SOTTILE V, SEUWEN K, KNEISSEL M. Enhanced marrow adipogenesis and bone resorption in estrogen-deprived rats treated with the PPARgamma agonist BRL49653 (rosiglitazone) [J]. Calcif Tissue Int, 2004, 75(4): 329-37.

[7] GESTA S, TSENG Y H, KAHN C R. Developmental origin of fat: tracking obesity to its source [J]. Cell, 2007, 131(2): 242-56.

[8] LIU Y, LIU Y, ZHANG R, et al. All-trans retinoic acid modulates bone morphogenic protein 9-induced osteogenesis and adipogenesis of preadipocytes through BMP/Smad and Wnt/β-catenin signaling pathways [J]. Int J Biochem Cell Biol, 2014, 47: 47-56.

[9] BERENDSEN A D, OLSEN B R. Regulation of adipogenesis and osteogenesis in mesenchymal stem cells by vascular endothelial growth factor A [J]. J Intern Med, 2015, 277(6): 674-80.

[10] PENG H, WRIGHT V, USAS A, et al. Synergistic enhancement of bone formation and healing by stem cell: expressed VEGF and bone morphogenetic protein-4 [J]. J Clin Invest, 2002, 110(6): 751-9.

[11] KANCZLER J M, GINTY P J, WHITE L, et al. The effect of the delivery of vascular endothelial growth factor and bone morphogenic protein-2 to osteoprogenitor cell populations on bone

formation [J]. Biomaterials, 2010, 31(6): 1242-50.

[12] CUI F, WANG X, LIU X, et al. VEGF and BMP-6 enhance bone formation mediated by cloned mouse osteoprogenitor cells [J]. Growth Factors, 2010, 28(5): 306-17.

[13] PENG H, USAS A, OLSHANSKI A, et al. VEGF improves, whereas sFlt1 inhibits, BMP2-induced bone formation and bone healing through modulation of angiogenesis [J]J Bone Miner Res, 2005, 20(11): 2017-27.

[14] PI C J, LIANG K L, KE Z Y, et al. Adenovirus-mediated expression of vascular endothelial growth factor-a potentiates bone morphogenetic protein9-induced osteogenic differentiation and bone formation [J]. Biol Chem, 2016, 397(8): 765-75.

[15] HU K, OLSEN B R. Osteoblast-derived VEGF regulates osteoblast differentiation and bone formation during bone repair [J]. J Clin Invest, 2016, 126(2): 509-26.

[16] HU L, CHEN W, QIAN A, et al. Wnt/β-catenin signaling components and mechanisms in bone formation, homeostasis, and disease [J]. Bone Res, 2024, 12(1): 39.

[17] NI T, SONG W X, LUO J, et al. BMP-9-induced osteogenic differentiation of mesenchymal progenitors requires functional canonical Wnt/β-catenin signaling [J]. J Cell Mol Med, 2009, 13(8b): 2448-64.

[18] LIN L, QIU Q, ZHOU N, et al. Dickkopf-1 is involved in BMP9-induced osteoblast differentiation of C3H10T1/2 mesenchymal stem cells [J]. BMB Rep, 2016, 49(3): 179-84.

[19] CHEN Y, WHETSTONE H C, YOUN A, et al. Beta-catenin signaling pathway is crucial for bone morphogenetic protein 2 to induce new bone formation [J]. J Biol Chem, 2007, 282(1): 526-33.

[20] XIAO H, WANG X, WANG C, et al. BMP9 exhibits dual and coupled roles in inducing osteogenic and angiogenic differentiation of mesenchymal stem cells [J]. Biosci Rep, 2020, 40(6): BSR20201262.

[21] LIU Z, LI Y, YANG J, et al. Bone morphogenetic protein 9 enhances osteogenic and angiogenic responses of human amniotic mesenchymal stem cells cocultured with umbilical vein endothelial cells through the PI3K/AKT/m-TOR signaling pathway [J]. Aging, 2021, 13(22): 24829-49.

[22] GENG Y, DUAN H, XU L, et al. BMP-2 and VEGF-A modRNAs in collagen scaffold synergistically drive bone repair through osteogenic and angiogenic pathways [J]. Commun Biol, 2021, 4(1): 82.

[23] LI CJ, MADHU V, BALIAN G, et al. Cross-talk between VEGF and BMP-6 pathways accelerates osteogenic differentiation of human adipose-derived stem cells [J]. J Cell Physiol, 2015, 230(11): 2671-82.

[24] ZHANG Y, MADHU V, DIGHE A S, et al. Osteogenic response of human adipose-derived stem cells to BMP-6, VEGF, and combined VEGF plus BMP-6 *in vitro* [J]. Growth Factors, 2012, 30(5): 333-43.

[25] PAPATHANASIOU I, MALIZOS K N, TSEZOU A. Bone morphogenetic protein-2-induced Wnt/β-catenin signaling pathway activation through enhanced low-density-lipoprotein receptor-related protein 5 catabolic activity contributes to hypertrophy in osteoarthritic chondrocytes [J]. Arthritis Res Ther, 2012, 14(2): 1-14.

[26] ZHANG M, YAN Y, LIM Y B, et al. BMP-2 modulates β-catenin signaling through stimulation of Lrp5 expression and inhibition of β-TrCP expression in osteoblasts [J]. J Cell Biochem, 2009, 108(4): 896-905.

[27] YANG L, YAMASAKI K, SHIRAKATA Y, et al. Bone morphogenetic protein-2 modulates Wnt and frizzled expression and enhances the canonical pathway of Wnt signaling in normal keratinocytes [J]. J Dermatol Sci, 2006, 42(2): 111-9.

[28] HADUONG J H, BLAVIER L, BANIWAL S K, et al. Interaction between bone marrow stromal cells and neuroblastoma cells leads to a VEGFA-mediated osteoblastogenesis [J]. Int J Cancer, 2015, 137(4): 797-809.

[29] SHIMIZU T, MAGATA F, ABE Y, et al. Bone morphogenetic protein 4 (BMP-4) and BMP-7 induce vascular endothelial growth factor expression in bovine granulosa cells [J]. Anim Sci J, 2012, 83(9): 663-7.

[30] DECKERS M M, VAN BEZOOIJEN R L, VAN DER HORST G, et al. Bone morphogenetic proteins stimulate angiogenesis through osteoblast-derived vascular endothelial growth factor A [J]. Endocrinology, 2002, 143(4): 1545-53.

[31] KIM H K W, OXENDINE I, KAMIYA N. High-concentration of BMP2 reduces cell proliferation and increases apoptosis via DKK1 and SOST in human primary periosteal cells [J]. Bone, 2013, 54(1): 141-50.

[32] KAMIYA N, KOBAYASHI T, MOCHIDA Y, et al. Wnt inhibitors Dkk1 and Sost are downstream targets of BMP signaling through the type IA receptor (BMPRIA) in osteoblasts [J]. J Bone Miner Res, 2010, 25(2): 200-10.

[33] BAI Y, WANG J, MORIKAWA Y, et al. Bmp signaling represses Vegfa to promote outflow tract cushion development [J]. Development, 2013, 140(16): 3395-402.

[34] ZHU J H, LIAO Y P, LI F S, et al. Wnt11 promotes BMP9-induced osteogenic differentiation through BMPs/Smads and p38 MAPK in mesenchymal stem cells [J]. J Cell Biochem, 2018, 119(11): 9462-73.

[35] CLEVERS H. Wnt/beta-catenin signaling in development and disease [J]. Cell, 2006, 127(3): 469-80.

[36] GEORGES R, BÉATRICE V, FRED D, et al. BMP-2 controls alkaline phosphatase expression and osteoblast mineralization by a Wnt autocrine loop [J]. J Bone Miner Res, 2010, 18(10): 1842-53.

[37] ZHANG H, WANG J, DENG F, et al. Canonical Wnt signaling acts synergistically on BMP9-induced osteo/odontoblastic differentiation of stem cells of dental apical papilla (SCAPs) [J]. Biomaterials, 2015, 39(5): 145-54.

[38] SHEN J, JAMES A W, ZHANG X, et al. Novel Wnt regulator NEL-like molecule-1 antagonizes adipogenesis and augments osteogenesis induced by bone morphogenetic protein 2 [J]. Am J Pathol, 2016, 186(2): 419-34.

[39] SAMEE M, KASUGAI S, KONDO H, et al. Bone morphogenetic protein-2 (BMP-2) and vascular endothelial growth factor (VEGF) transfection to human periosteal cells enhances osteoblast differentiation and bone formation [J]. J Pharmacol Sci, 2008, 108(1): 18-31.

[40] SHARMA S, SAPKOTA D, XUE Y, et al. Delivery of VEGFA in bone marrow stromal cells seeded in copolymer scaffold enhances angiogenesis, but is inadequate for osteogenesis as compared with the dual delivery of VEGFA and BMP2 in a subcutaneous

mouse model [J]. Stem Cell Res Ther, 2018, 9(1): 23.

[41] TAKAHASHI T. Overexpression of Runx2 and MKP-1 stimulates transdifferentiation of 3T3-L1 preadipocytes into bone-forming osteoblasts *in vitro* [J]. Calcif Tissue Int, 2011, 88(4): 336-47.

[42] ZHANG Y, LI X, QIAN S, et al. Down-regulation of type I Runx2 nediated by dexamethasone is required for 3T3-L1 adipogenesis [J]. Mol Endocrinol, 2012, 26(5): 798-808.

[43] DUCY P, ZHANG R, GEOFFROY V, et al. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation [J]. Cell, 1997, 89(5): 747-54.

[44] TRIPTI G, LENGNER C J, HAYK H, et al. Canonical WNT signaling promotes osteogenesis by directly stimulating Runx2 gene expression [J]. J Biol Chem, 2005, 280(39): 33132-40.

[45] KOMORI T. Whole aspect of Runx2 functions in skeletal development [J]. Int J Mol Sci, 2022, 23(10): 5776.

[46] ZHANG Z M, HUANG C X, HUO J Z. Circ_C4orf36 promotes the proliferation and osteogenic differentiation of BMSCs by regulating VEGFA [J]. Biochem Genet, 2023, 61(3): 931-44.

[47] DA SILVA SASSO G R, FLORENCIO-SILVA R, SASSO-CERRI E, et al. Spatio-temporal immunolocalization of VEGF-A, Runx2, and osterix during the early steps of intramembranous ossification of the alveolar process in rat embryos [J]. Dev Biol, 2021, 478: 133-43.

[48] WANG L, ZHANG L, ZHAO L, et al. VEGFA/NRP-1/GAPVD1 axis promotes progression and cancer stemness of triple-negative breast cancer by enhancing tumor cell-macrophage crosstalk [J]. Int J Biol Sci, 2024, 20(2): 446-63.

[49] WU X, YANG X, DAI X, et al. 5-Aza-2'-deoxycytidine ameliorates choroidal neovascularization by inhibiting the Wnt/β-catenin signaling pathway [J]. Invest Ophthalmol Vis Sci, 2024, 65(2): 23.