VEGF和EGFL在骨血管生成中的作用

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摘要 骨血管分布密集且广泛, 在骨的生长发育中, 骨血管不仅提供必需的氧和营养物质, 而且通过调节各种骨细胞和血管细胞间的相互作用, 为骨形成提供必要的刺激信号。大量研究表明, 局部血管的变化与许多骨疾病的发展密不可分。骨血管生成过程中受大量因子的调节, 该文主要对血管内皮生长因子(vascular endothelial growth factor, VEGF)和表皮生长因子样家族成员[epidermal growth factor (EGF)-like family members, EGFL]在调节骨血管生成中的作用作一综述, 旨在为相关骨疾病的治疗提供理论基础。

关键词 骨血管生成; VEGF; EGFL

The Role of VEGF and EGFL in Bone Angiogenesis

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Abstract Bone is a highly vascularized tissue containing an extensive vascular network of large vessels and capillaries, which provide oxygen and nutrients for bone formation and development through regulation of different signaling pathways between bone cells and endothelial cells. Numbers of studies have proved that the variation of vasculature are closely related to the development of many bone diseases. Many factors play essential roles in the regulation of bone angiogenesis. This study mainly reviewed the functions of vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF)-like family members (EGFL) in the regulation of bone angiogenesis, providing more understanding of the treatment of relevant bone diseases.

Keywords bone angiogenesis; VEGF; EGFL

1 前言

在骨的发育、再生和修复过程中, 血管形成和骨形成密切相关[1]。血管不仅为骨的发育、再生和修复输送必需的氧和营养物质, 而且通过调节各种骨细胞和血管细胞间的相互作用, 为骨形成提供必要的刺激信号[1-2]。大量研究表明, 骨血管与骨形成相伴发生, 骨细胞可分泌促血管生成因子来触发信号引起不同细胞(包括形成血管的内皮细胞以及软骨细胞、成骨细胞、破骨细胞等)释放相关细胞因子进一步促进血管生成[3-4]。与此同时, 骨血管内皮

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细胞释放的因子对软骨细胞及成骨细胞系也起到一定的作用。血管生成在骨折的愈合及修复中也有着重要的作用。局部血管的变化也与许多骨疾病的发展密切相关, 如骨质疏松、骨坏死、骨关节炎、佝偻病、缺血性坏死、骨肿瘤的发生与转移等[5-6]。在调节血管生成及骨生成的因子中, 血管内皮生长因子(vascular endothelial growth factor, VEGF)家族有着举足轻重的作用, 而表皮生长因子样家族成员[epidermal growth factor (EGF)-like family members, EGFL]则是相对新发现的因子, 也参与调控骨血管的生成。因此, 本文主要介绍了VEGF及EGFL在骨血管生成中的作用, 旨在为相关骨疾病的治疗提供理论依据。

2 VEGF在骨血管生成中的作用

2.1 VEGF简介

VEGF属于二联体蛋白质家族, 包括6种成员: VEGF-A(VEGF)、VEGF-B、VEGF-C、VEGF-D、VEGF-E以及胎盘生长因子(placental growth factor, PlGF)[7]。这些成员中VEGF表达丰富, 在内皮细胞的增殖、迁徙及激活中有着重要的作用, 同时对血管生成也意义巨大[8-9]。根据编码的剪接方式不同, *VEGF* mRNA在人体被翻译成4个亚型: VEGF121、VEGF165、VEGF189和VEGF206; 在小鼠则有3个亚型: VEGF120、VEGF164及VEGF188[10]。其中VEGF164和VEGF165表达最为丰富, 通常用于离体实验及动物模型的研究[11]。VEGF受体包括VEGFR1、VEGFR2、VEGFR3、神经纤毛蛋白1(neuropilin1, Npr1)及Npr2[10]。其中, VEGFR1主要在造血干细胞表达, VEGFR2主要表达在血管内皮细胞上, VEGFR3主要在淋巴内皮细胞表达[12]。VEGFR2是VEGF信号主要的受体, 相关信号通过受体VEGFR2触发包括内皮细胞(endothelial cells, ECs)的增殖、分化、生存等一系列过程[13], VEGFR2对血管的渗透性也有一定的促进作用[9]。

2.2 VEGF调节骨血管生成

作为一种强有力的有丝分裂原和血管生成因子, VEGF在骨组织血管生成中也有重要的作用[14]。在小鼠建立股骨骨折模型中, 外源性VEGF能促进血管的形成、骨化以及新骨成熟[15]。Wallner等[16]的研究也指出, VEGFA和成纤维生长因子-9(fibroblast growth factors 9, FGF-9)的联合应用可促进2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。在巩膜小骨膜内成骨的过程中, VEGF不但为相关血管生成提供重要的信号, 也调节骨生成[17]。VEGF/VEGFR2信号可调节Ihh(Indian hedgehog)的释放, 并上调β-联蛋白(β-catenin)及抑制Notch2的表达, 进而在骨生长的早期调节膜内血管生成及成骨细胞的分化[18]。Wang等[19]的研究发现, 特定的ECs灭活编码*VEGFR2*基因导致邻近生长板的骨骺端血管的生成严重下降。不同亚型的VEGF其生物学作用也有所不同, Zelzer等[20]的研究发现, 小鼠只表达VEGF120表现出软骨内血管生成下降、矿化降低及初级骨化中心(the future primary ossification center, POC)中成骨细胞标志物表达的下降。而Maes等[21]指出, VEGF164或者VEGF188的表达使生长中的POC的血管生成丰富。然而, 在基因敲入小鼠中, VEGF188亚型的表达仅导致次级骨化中心(the secondary ossification center, SOC)血管生成受损, 说明VEGF的扩散对血管的募集及血管生成是必需的[21]。Maes等[22]的另一研究也指出, 成骨细胞系中VEGF164过表达引起骨血管生成增加, 并通过激活β-联蛋白信号通路促进骨生成。

2.3 VEGF受其他因素调节

在调节骨血管生成的过程中, VEGF也受各种因素的调节。例如, VEGF在胚胎骨低度肥厚区和矿化区域的软骨细胞中高度表达, 并且这些区域的血管生成是走向成骨的重要一步, VEGF在成骨分化中也有表达并且其表达水平受成骨分化刺激物影响, 例如, 胰岛素样生长因子(insulin-like growth factor, IGF)、维生素D3(VD3)等[23]。有研究指出, 在骨质疏松造模的大鼠中, 柚皮苷可通过VEGF/VEGFR2信号通路调节血管生成, 进一步促进骨折的愈合[24]。在成骨细胞中表达的低氧诱导因子α(hypoxia inducible factor α, HIFα)可以调节VEGF的释放, 敲除成骨细胞中*HIF1α*基因可导致骨血管及骨生成降低; 相反, 成骨细胞过表达*HIF1α*基因可以促进骨血管及骨生成增加[25]。Percival等[26]指出, 新生小鼠成骨细胞中HIF1α表达的增加可促进VEGF的释放, 进一步提高长骨中的血管密度。Ozdel等[27]的研究也发现, 在牵张成骨期间, 通过HIF通路的激活使VEGF的水平升高, 促进血管生成及骨生成。FGFs也参与VEGF的调节。Sivaraj等[28]发现, 在*FGF2*缺失的突变小鼠中, 骨小梁体积及骨形成速率均显著下降, 而*FGF9*和

*FGF18*基因突变的小鼠表现出VEGF表达的下降, 导致POC中血管生成的延迟。此外, VEGF还与*BMP*、*AKT*以及转录因子4(activating transcription factor 4, *ATF4*)等基因关系密切。Yang等[29]指出, 成骨细胞的BMP2间接调控骨膜及骨髓的血管生成, 将成骨细胞*BMP2*基因敲除不但引起VEGF表达下降, 而且导致MSCs生长缺陷, 而这些均与骨膜及骨小梁的血管生成降低有关。Ulici等[30]认为, PI3K-AKT是触发激活VEGF受体的主要信号通路之一, 相对AKT2和AKT3而言, AKT1激酶在ECs中高度表达, 并且*AKT1*基因敲除小鼠表现出长骨变短及血管形成下降。Zhu等[31]的研究发现, 在骨微环境中, 激活ATF4可通过增加VEGF的表达与释放来促进血管生成。此外, 血管生成相关的*NOS3*、*CD14*、*MMP3*及*IL4R*等基因也均与VEGF的表达量及血清浓度相关[32], VEGF的表达还受生长因子、P53、雌激素、TSH、肿瘤和NO等因素的调节[33]。

3 EGFL在骨血管生成中的作用

3.1 EGFL简介

EGF是包含53个氨基酸残基的单一多肽链, 空间上通过半胱氨酸残基之间的相互作用形成3对二硫键, 这些二硫键对EGF的生物活性十分重要, 并且二硫键的破坏会导致EGF的失活[23]。EGF配体家族包括: EGF、HB-EGF、BTC、TGFa、EPGN、AREG、EREG、NRG1-4等[34-35]。其中, EGF、TGFa、HB-EGF以及BTC表现出高度的亲和力, 而AREG、EREG和EPGN则亲和力较低[35]。与EGF有着同源性的EGFL包含单个或多个表皮生长因子样结构域, 为膜结合型或分泌型蛋白质, 根据是否含有跨膜结构域通过自分泌或旁分泌的调节方式发挥其生物作用[36]。EGFL因子通过相关受体(包括EGFR、ErbB2、ErbB3和ErbB4)发挥其生物功能, 其受体的激活可触发细胞内部众多的信号通路, 包括磷脂酰肌醇3激酶(PI3-kinase)、促细胞分裂剂激酶(MAP kinase)、STAT信号通路等[34]。EGFL家族成员的在成骨细胞、破骨细胞、内皮细胞中均有表达。有研究表明, 成骨细胞可释放EGF、HB-EGF、AREG、BTC、EREG及TGFα等, 而HB-EGF、AREG、EREG和NRG在破骨细胞分化中均有表达[37-38]。此外, EGFL在骨重建单位中的表达有差异性, 如EGFL3、EGFL5、EGFL6及EGFL9择优地在成骨细胞中表达, 而EGFL2、EGFL7和EGFL8则在成骨细胞与破骨细胞中均有表达[39]。

3.2 EGFL调节血管生成

EGF及EGFL家族成员都是血管生成因子。在体外实验中, EGF可通过激活PI3K和MAPK信号通路诱导内皮细胞的迁徙与促进血管形成[40]。Mehta等[41]也指出, 在细胞不进行诱导增殖的情况下, HB-EGF和EGF可调节划痕实验中人脐静脉内皮细胞的转移以及促进2D-血管生成实验中的血管生成, 并且HB-EGF和EGF以不依赖VEGF的形式通过PI3K、MAPK及eNOS信号通路的激活来诱导血管生成。Schneider等[42]在全层皮肤切口愈合实验中发现, 转基因表达BTC小鼠切口处血管覆盖面积明显增加, 并且血管的大小及密度也明显提高, 提示BTC可促进伤口部位的血管生成。Riese等[43]指出, ERGN在血管生成及重构过程中有着重要的作用, 尤其是在炎症反应时更为重要。Leker等[44]则发现, TGFa可促进脑卒中后的血管生成及神经新生。Wang等[45]研究发现, 在人体软骨肉瘤细胞中, ARGE可促进VEGF的产生, 并通过FAK/c-Src/PKCδ信号通路抑制miR-206的表达进而促进血管生成。Odiete等[46]指出, NRG-1在许多器官血管的结构及功能上均有着重要的作用, NRG-1和ErbB的受体在血管内皮细胞中都有表达, 并且NRG-1可通过提高VEGF的释放来促进血管生成。近来研究发现, EGFL7特异性表达于内皮细胞, 在血管生成中也发挥着重要作用, 与VEGF的作用不同的是, EGFL7只促进内皮细胞的迁徙和血管结构的形态发生, 而不刺激内皮细胞的增生[47]。有研究指出, EGFL7可通过Notch信号通路发挥调节血管生成的作用, 并且过表达EGFL7可导致非正常的血管形成及重建, 并抑制Notch信号通路的活动和下调Notch靶基因的表达[48-49]。Nikolic等[50]的研究发现, EGFL7在调节血管生成的过程中除了受Notch信号通路的调节外, EGFL7可联合miR-126通过促进VEGF信号的转导、血管生成及血管的完整性, 进一步调节血管的生长。EGFL7可通过Notch信号通路调节生理及病理上的血管生成, 并可能成为理想的治疗癌症的新靶点[49]。此外, 国内也有研究指出, 适度的运动可显著激活心梗心脏EGFL7/miR126-PIK3R2/SPRED1通路, 抑制其下游靶蛋白PIK3R2/SPRED1表达, 促进心脏梗死边缘区血管新生, 且间歇运动的保护效应优于持续运动[51]。这提示, 运动或机械应力可能调节EGFL7的表达。

3.3 EGFL在骨血管生成中的作用

越来越多的研究表明, EGFL家族成员可促进破骨细胞、成骨细胞及内皮细胞之间的交流, 这对骨血管的生成及骨重建过程是必不可少的。如Liu等[52]研究发现, EGF可诱导MSCs中BMP9的表达, 并且BMP9也可通过Smad1/5/8信号通路上调EGFR的表达, EGF和BMP9的信号串话(cross-talk)提示其在血管生成及成骨分化中有着重要的作用。由成骨样细胞分泌的EGFL6通过旁分泌机制诱导血管生成, 成骨分化时EGFL6的释放可上调, 并且EGFL6通过ERK1/2的激活诱导内皮细胞的迁徙与管状结构的形成[39]。Kuek等[53]研究发现, 由成骨细胞表达EGFL6的同源异构体–肾连蛋白(nephronectin, NPNT)可通过p-38和ERK信号通路的激活来调节血管生成, 并且NPNT在病理性骨质疏松中也有着重要的作用。有研究指出, 整合蛋白αvβ3介导的血管生成对骨折的预后十分重要[54]。Nikolic等[55]也发现, EGFL7可作为整合蛋白αvβ3的配体调节生理及病理上的血管生成。此外, Chim等[56]的研究也证实, 在骨微环境中表达的EGFL7可通过整合蛋白介导的信号通路调节骨血管的生成, 并且, 离体实验中EGFL7可诱导SVEC(simian virus 40-transformed mouse microvascular endothelial cell line)细胞中ERK1/2、STAT3、FAK等信号通路的激活进一步促进其细胞的迁徙和管状结构的形成。

4 小结与展望

血管生成与骨生成相伴而生, 骨细胞可分泌促血管生成的因子, 进而促进血管生成。而血管内皮细胞也能释放一些成骨因子, 进一步促进骨生成。这个相互作用的过程中受多种因子的调节, 除VEGF及EGFL家族成员外, FGFs、结缔组织生长因子(CTGF或CCN2)、激酶c-RAF(RAF1)、HIF、基质金属蛋白酶、Notch及BMPs等信号通路等也参与骨血管生成的调控。其中, VEGF作为调节骨血管生成的中心因子, 其在骨血管生成中的枢纽作用已逐渐为人所知。EGFL调控骨血管生成的功能是在近几年才被确定, 其调控骨血管生成的确切机制还有待进一步深入研究。此外, EGFL在血管生成与骨生物学中的重要作用值得更深入地探讨, 这可能为骨折的康复及骨代谢相关疾病的治疗提供新思路。

参考文献 (References)

1 Kusumbe AP, Ramasamy SK, Adams RH. Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. Nature 2014; 507(7492): 323-8.

2 Fan L, Li J, Yu Z, Dang X, Wang K. The hypoxia-inducible factor pathway, prolyl hydroxylase domain protein inhibitors, and their roles in bone repair and regeneration. Biomed Res Int 2014; 2014: 239356.

3 Dai J, Rabie AB. VEGF: an essential mediator of both angiogenesis and endochondral ossification. J Dent Res 2007; 86(10): 937-50.

4 Eshkar-Oren I, Viukov SV, Salameh S, Krief S, Oh CD, Akiyama H, *et al*. The forming limb skeleton serves as a signaling center for limb vasculature patterning via regulation of Vegf. Development 2009; 136(8): 1263-72.

5 Beamer B, Hettrich C, Lane J. Vascular endothelial growth factor: an essential component of angiogenesis and fracture healing. HSS J 2010; 6(1): 85-94.

6 Carulli C, Innocenti M, Brandi ML. Bone vascularization in normal and disease conditions. Front Endocrinol (Lausanne) 2013; 4: 106.

7 Claesson-Welsh L. VEGF receptor signal transduction–a brief update. Vascul Pharmacol 2016; 86: 14-7.

8 Cross MJ, Dixelius J, Matsumoto T, Claesson-Welsh L. VEGF-receptor signal transduction. Trends Biochem Sci 2003; 28(9): 488-94.

9 Ferrara N, Gerber HP, Lecouter J. The biology of VEGF and its receptors. Nat Med 2003; 9(6): 669-76.

10 Hu K, Olsen BR. The roles of vascular endothelial growth factor in bone repair and regeneration. Bone 2016; 91: 30-8.

11 Phillips GD, Stone AM, Jones BD, Schultz JC, Whitehead RA, Knighton DR. Vascular endothelial growth factor (rhVEGF165) stimulates direct angiogenesis in the rabbit cornea. *In Vivo* 1994; 8(6): 961-5.

12 邹文萍, 唐国强, 李光明. VEGF及其受体的生物学特性. 四川生理科学杂志(Zou Wenping, Tang Guoqiang, Li Guangming. Biological characteristics of VEGF and it's recepters. Sichuan Journal of Physiological Sciences) 2012; 34(3): 123-6.

13 Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling–in control of vascular function. Nat Rev Mol Cell Biol 2006, 7(5): 359-71.

14 Heo SH, Choi YJ, Ryoo HM, Cho JY. Expression profiling of ETS and MMP factors in VEGF-activated endothelial cells: role of MMP-10 in VEGF-induced angiogenesis. J Cell Physiol 2010; 224(3): 734-42.

15 Street J, Bao M, Deguzman L, Bunting S, Peale FV, Ferrara, N, *et al*. Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. Proc Natl Acad Sci USA 2002; 99(15): 9656-61.

16 Wallner C, Schira J, Wagner JM, Schulte M, Fischer S, Hirsch T, *et al*. Application of VEGFA and FGF-9 enhances angiogenesis, osteogenesis and bone remodeling in type 2 diabetic long bone regeneration. PLoS One 2015; 10(3): e0118823.

17 Jabalee J, Franz-Odendaal TA. Vascular endothelial growth factor signaling affects both angiogenesis and osteogenesis during the development of scleral ossicles. Dev Biol 2015; 406(1): 52-62.

18 Duan X, Murata Y, Liu Y, Nicolae C, Olsen BR. Berendsen

AD. Vegfa regulates perichondrial vascularity and osteoblast differentiation in bone development. Development 2015; 142(11): 1984-91.
19 Wang L, Benedito R, Bixel MG, Zeuschner D, Stehling M, Sävendahl L, *et al*. Identification of a clonally expanding haematopoietic compartment in bone marrow. EMBO J 2013; 32(2): 219-30.
20 Zelzer E, Mclean W, Ng YS, Fukai N, Reginato AM, Lovejoy S, *et al*. Skeletal defects in VEGF(120/120) mice reveal multiple roles for VEGF in skeletogenesis. Development 2002; 129(8): 1893-904.
21 Maes C, Stockmans I, Moermans K, Van Looveren R, Smets N, Carmeliet P, *et al*. Soluble VEGF isoforms are essential for establishing epiphyseal vascularization and regulating chondrocyte development and survival. J Clin Invest 2004; 113(2): 188-99.
22 Maes C, Goossens S, Bartunkova S, Drogat B, Coenegrachts L, Stockmans I, *et al*. Increased skeletal VEGF enhances beta-catenin activity and results in excessively ossified bones. EMBO J 2010; 29(2): 424-41.
23 Chim SM, Tickner J, Chow ST, Kuek V, Guo B, Zhang G, *et al*. Angiogenic factors in bone local environment. Cytokine Growth Factor Rev 2013; 24(3): 297-310.
24 Song N, Zhao Z, Ma X, Sun X, Ma J, Li F, *et al*. Naringin promotes fracture healing through stimulation of angiogenesis by regulating the VEGF/VEGFR-2 signaling pathway in osteoporotic rats. Chem Biol Interact 2017; 261: 11-7.
25 Wang Y, Wan C, Deng L, Liu X, Cao X, Gilbert SR, *et al*. The hypoxia-inducible factor alpha pathway couples angiogenesis to osteogenesis during skeletal development. J Clin Invest 2007; 117(6): 1616-26.
26 Percival CJ, Richtsmeier JT. Angiogenesis and intramembranous osteogenesis. Dev Dyn 2013; 242(8): 909-22.
27 Ozdel A, Sarisozen B, Yalcinkaya U, Demirağ B. The effect of HIF stabilizer on distraction osteogenesis. Acta Orthop Traumatol Turc 2015; 49(1): 80-4.
28 Sivaraj KK, Adams RH. Blood vessel formation and function in bone. Development 2016; 143(15): 2706-15.
29 Yang W, Guo D, Harris MA, Cui Y, Gluhak-Heinrich J, Wu J, *et al*. Bmp2 in osteoblasts of periosteum and trabecular bone links bone formation to vascularization and mesenchymal stem cells. J Cell Sci 2013; 126(Pt18): 4085-98.
30 Ulici V, Hoenselaar KD, Agoston H, McErlain DD, Umoh J, Chakrabarti S, *et al*. The role of Akt1 in terminal stages of endochondral bone formation: angiogenesis and ossification. Bone 2009; 45(6): 1133-45.
31 Zhu K, Jiao H, Li S, Cao H, Galson DL, Zhao Z, *et al*. ATF4 promotes bone angiogenesis by increasing VEGF expression and release in the bone environment. J Bone Miner Res 2013; 28(9): 1870-84.
32 Saleh A, Stathopoulou MG, Dade S, Ndiaye NC, Azimi-Nezhad M, Murray H, *et al*. Angiogenesis related genes NOS3, CD14, MMP3 and IL4R are associated to VEGF gene expression and circulating levels in healthy adults. BMC Med Genet 2015; 16: 90.
33 马 莉. VEGF及其受体的生物学特性及在肿瘤血管生成中的作用. 中国优生与遗传杂志(Ma Li. The role of vascular endothelial growth factor and its receptor in tumor angiogenesis. Chinese Journal of Birth Health & Heredity) 2016; 24(5): 146-8.
34 Sanderson MP, Dempsey PJ, Dunbar AJ. Control of ErbB signaling through metalloprotease mediated ectodomain shedding of EGF-like factors. Growth factors 2006; 24(2): 121-36.
35 Singh B, Carpenter G, Coffey RJ. EGF receptor ligands: recent advances. F1000Res 2016; 8: 5.
36 Gomez-Gaviro MV, Scott CE, Sesay AK, Matheu A, Booth S, Galichet C, *et al*. Betacellulin promotes cell proliferation in the neural stem cell niche and stimulates neurogenesis. Proc Natl Acad Sci USA 2012; 109(4): 1317-22.
37 Yi T, Lee HL, Cha JH, Ko SI, Kim HJ, Shin HI, *et al*. Epidermal growth factor receptor regulates osteoclast differentiation and survival through cross-talking with RANK signaling. J Cell Physiol 2008; 217(2): 409-22.
38 Nakamura T, Toita H, Yoshimoto A, Nishimura D, Takagi T, Ogawa T, *et al*. Potential involvement of Twist2 and Erk in the regulation of osteoblastogenesis by HB-EGF-EGFR signaling. Cell Struct Funct 2010; 35(1): 53-61.
39 Chim SM, Qin A, Tickner J, Pavlos N, Davey T, Wang H, *et al*. EGFL6 promotes endothelial cell migration and angiogenesis through the activation of extracellular signal-regulated kinase. J Biol Chem 2011; 286(25): 22035-46.
40 Bertrand-Duchesne MP, Grenier D, Gagnon G. Epidermal growth factor released from platelet-rich plasma promotes endothelial cell proliferation *in vitro*. J Periodontal Res 2010: 45(1): 87-93.
41 Mehta VB, Besner GE. HB-EGF promotes angiogenesis in endothelial cells via PI3-kinase and MAPK signaling pathway. Growth factors 2007: 25(4): 253-63.
42 Schneider MR, Antsiferova M, Feldmeyer L, Dahlhoff M, Bugnon P, Hasse S, *et al*. Betacellulin regulates hair follicle development and hair cycle induction and enhances angiogenesis in wounded skin. J Invest Dermatol 2008; 128(5): 1256-65.
43 Riese DJ 2nd, Cullum RL. Epiregulin: roles in normal physiology and cancer. Semin Cell Dev Biol 2014; 28: 49-56.
44 Leker RR, Toth ZE, Shahar T, Cassiani-Ingoni R, Szalayova I, Key S, *et al*. Transforming growth factor alpha induces angiogenesis and neurogenesis following stroke. Neuroscience 2009; 163(1): 233-43.
45 Wang CQ, Huang YW, Wang SW, Huang YL, Tsai CH, Zhao YM, *et al*. Amphiregulin enhances VEGF-A production in human chondrosarcoma cells and promotes angiogenesis by inhibiting miR-206 via FAK/c-Src/PKCdelta pathway. Cancer Lett 2017; 385: 261-70.
46 Odiete O, Hill MF, Sawyer DB. Neuregulin in cardiovascular development and disease. Circ Res 2012; 111(10): 1376-85.
47 王运良, 李 智. EGFL7在肿瘤血管生成及侵袭转移中的研究进展. 中华介入放射学电子杂志[Wang Yunliang, Li Zhi. Research & development of EGFL7 in tumor angiogenesis, invasion and metastasis. Chinese Journal of Interventional Radiology (Electronic Edition)] 2017; 5(2): 112-4.
48 Nichol D, Shawber C, Fitch MJ, Bambino K, Sharma A, Kitajewski J, *et al*. Impaired angiogenesis and altered Notch signaling in mice overexpressing endothelial Egfl7. Blood 2010; 116(26): 6133-43.
49 Nichol D, Stuhlmann H. EGFL7: a unique angiogenic signaling factor in vascular development and disease. Blood 2012; 119(6): 1345-52.

50 Nikolic I, Plate KH, Schmidt MH. EGFL7 meets miRNA-126: an angiogenesis alliance. J Angiogenes Res 2010; 2(1): 9.

51 宋 伟, 田振军, Shao-jun Du. 心梗大鼠持续和间歇运动干预的心肌血管新生相关miRNAs表征与EGFL7/miR126-PIK3R2/SPRED1通路激活的心脏保护效应. 体育科学(Song Wei, Tian Zhenjun, Shao-jun Du. Continuous and interval exercise intervention on the expression of myocardial miRNAs related to angiogenesis and activitation of EGFL7/miR126-PIK3R2 / SPRED1 on protecting the hearts of MI rats. China Sport Science) 2017; 37(2): 57-65.

52 Liu X, Qin J, Luo Q, Bi Y, Zhu G, Jiang W, *et al*. Cross-talk between EGF and BMP9 signalling pathways regulates the osteogenic differentiation of mesenchymal stem cells. J Cell Mol Med 2013; 17(9): 1160-72.

53 Kuek V, Yang Z, Chim SM, Zhu S, Xu H, Chow ST, *et al*. NPNT is expressed by osteoblasts and mediates angiogenesis via the activation of extracellular signal-regulated kinase. Sci Rep 2016; 6: 36210.

54 Tomlinson RE, Schmieder AH, Quirk JD, Lanza GM, Silva MJ. Antagonizing the alphav beta3 integrin inhibits angiogenesis and impairs woven but not lamellar bone formation induced by mechanical loading. J Bone Miner Res 2014; 29(9): 1970-80.

55 Nikolic I, Stankovic ND, Bicker F, Meister J, Braun H, Awwad K, *et al*. EGFL7 ligates alphavbeta3 integrin to enhance vessel formation. Blood 2013; 121(15): 3041-50.

56 Chim SM, Kuek V, Chow ST, Lim BS, Tickner J, Zhao J, *et al*. EGFL7 is expressed in bone microenvironment and promotes angiogenesis via ERK, STAT3, and integrin signaling cascades. J Cell Physiol 2015; 230(1): 82-94.