[文章编号] 1007-3949(2018)26-11-1111-05 ·血管钙化专栏·

血管钙化参与细胞相关研究的新进展

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[关键词] 血管钙化； 血管平滑肌细胞； 内皮细胞； 周细胞； 巨噬细胞； 祖细胞

[摘 要] 血管钙化是一个受细胞和基因主动调节的过程，涉及血管钙化促进因子和抑制因子的平衡失调。血管钙化常见于动脉粥样硬化晚期，与糖尿病、慢性肾病及衰老等疾病密切相关。血管细胞的成骨样分化是血管钙化的关键环节，但参与血管钙化调节的细胞来源尚不十分明确。目前认为调节血管钙化的细胞来源可能包括：血管平滑肌细胞、内皮细胞、周细胞、巨噬细胞及祖细胞等。本文现将相关进展作一简要综述。

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Recent progresses on related research of cells involved in vascular calcification

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[**KEY WORDS**] vascular calcification; vascular smooth muscle cell; endothelial cell; pericyte; macrophage; progenitor cell

[**ABSTRACT**] Vascular calcification is an active cell- and gene-regulated process, involving imbalance between promoters and inhibitors of vascular calcification. It commonly occurs in patients with advanced atherosclerosis and is highly related with diabetes, chronic kidney diseases and aging. Osteogenic differentiation of vascular cells is the key contributor to vascular calcification, but the cell sources of vascular calcification remain unclear. It has been thought that the origins of cells regulating vascular calcification may include vascular smooth muscle cell, endothelial cell, pericyte, macrophage and progenitor cell. This review focuses on the regulatory role of cells in vascular calcification.

血管钙化是指钙磷矿物质在血管壁的异常沉积，可导致血管壁僵硬、顺应性降低，常见于动脉粥样硬化、高血压、慢性肾脏病、糖尿病等疾病[1-2]。血管钙化曾经被认为是钙磷矿物质被动沉积在血管壁的过程，然而，近年来大量的研究发现血管钙化过程涉及大量的骨相关蛋白表达上调，此外敲除血管钙化促进因子能明显抑制小鼠血管钙化，而敲除血管钙化抑制因子可导致小鼠血管钙化[3-5]，说明血管钙化是与骨生成过程类似、受细胞和基因主动调控的生物学过程。因此，血管钙化的防治成为可能，阐明参与调控血管钙化的细胞来源可为血管钙化的治疗提供新的策略。目前认为可能参与调控血管钙化的细胞来源主要包括：血管平滑肌细胞(vascular smooth muscle cell，VSMC)、内皮细胞、周细胞、巨噬细胞、祖细胞等。现将这些细胞参与血管钙化的调节作用及相关研究进行综述。

1 血管平滑肌细胞

血管平滑肌细胞是构成动脉壁中膜的主要细胞类型。在生理状态下，成人的血管壁中 VSMC 呈现收缩表型，主要表达大量的收缩蛋白，如 α 平滑肌肌动蛋白(alpha smooth muscle actin，α-SMA)、平滑肌球蛋白重链(smooth muscle myosin heavy chain，

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SMM-HC)、平滑肌 22α(smooth muscle 22 alpha, SM22α)等,维持其收缩功能。在病理因素刺激下,如钙磷代谢紊乱、细胞损伤或凋亡、氧化应激、机械损伤等,VSMC 可出现成骨样分化,分泌大量的骨相关蛋白包括碱性磷酸酶(alkaline phosphatase, ALP)、Runt 相关转录因子 2(Runt-related transcription factor 2, Runx2)、骨形态发生蛋白(bone morphogenetic protein, BMP),最终形成血管钙化[6-9]。Speer 等[10]和 Naik 等[11]先后采用细胞谱系示踪技术发现 VSMC 在动脉钙化过程中可分化为成骨样细胞。

VSMC 的成骨样分化是血管钙化的关键环节。诱导 VSMC 的成骨样分化和钙化的因子和因素有:转化生长因子 β(transforming growth factor β, TGF-β)超家族信号分子、VSMC 释放的细胞外囊泡(extracellular vesicle, EV)、基质小泡(matrix vesicle, MV)、细胞衰老、氧化应激、钙磷代谢紊乱、炎症等。TGF-β 超家族成员 BMP-2 可诱导 VSMC 成骨转录因子 Runx2 的表达,促进钙化[12],而 Wnt16 可抑制 TGF-β 诱导的 VSMC 软骨样分化[13]。EV 在 VSMC 表型转换和钙化中具有重要作用,VSMC 来源的 EV 可作为外泌体诱导血管钙化,在人的钙化动脉组织可检测到外泌体[14],盘状结构域受体 1(discoidin domain receptor-1, DDR-1)/TGF-β 信号可通过调节 EV 的分泌影响血管钙化的发生[15]。MV 通过诱导细胞信号变化促进 VSMC 钙化[16]。与较年轻的 VSMC 比较,衰老的细胞容易钙化和表达成骨标志物如 ALP、胶原蛋白 1 和 Runx2 等,衰老的 VSMC 可通过启动细胞成骨样分化促进钙化[17]。氧化应激可加速 VSMC 的成骨样分化和细胞钙化[18-19]。高磷高钙是诱导血管平滑肌成骨样分化和钙化的重要因素[20]。

2 内皮细胞

内皮细胞(endothelial cell)具有软骨和成骨细胞分化的潜能。BMP-6 和氧化低密度脂蛋白协同促进内皮细胞的成骨样分化和钙化[21]。Peeters 等[22]发现 SPARC 相关模块化钙结合蛋白 2(SPARC-related modular calcium-binding protein 2, SMOC2)能够抑制人脐静脉内皮细胞的成骨样分化和钙化。缺乏初级纤毛的内皮细胞在 BMP-6 诱导下更易发生钙化[23]。另外,内皮细胞可通过释放信号分子、分泌微泡促进 VSMC 成骨样分化和钙化。损伤的内皮细胞通过分泌 BMP-2 和内皮细胞微粒(endothelial microparticle, EMP),上调 Runx2 和 ALP,诱导 VSMC 成骨样分化和钙化[24]。新近研究表明衰老的内皮细胞分泌的微泡可促进 VSMC 成骨样分化[25]。

Yao 等[26]人采用细胞谱系示踪技术发现内皮细胞是糖尿病小鼠模型钙化血管中成骨样细胞的来源。内皮间充质转化(endothelial-mesenchymal transition, EndMT)是内皮细胞丧失细胞极性、获得迁移和侵袭特性、分化为间充质干细胞(mesenchymal stem cell, MSC)的过程。EndMT 使得内皮细胞具有可塑性,具备成骨样分化的潜能,是调节血管钙化的重要机制[27]。Bostrom 等[28]采用 $ApoE^{-/-}$ 敲除小鼠进一步证实 EndMT 可调控动脉粥样硬化的钙化。

3 周细胞

周细胞(pericyte)是包绕在毛细血管和静脉内皮细胞的细胞,具有收缩、支持、调节血管生长等功能。周细胞具有分化成多种细胞类型的潜能,可作为祖细胞的"储库"。常用于标记周细胞的蛋白包括:血小板源性生长因子受体 β(platelet-derived growth factor receptor beta, PDGFRβ)、硫酸软骨素蛋白聚糖 4(chondroitin sulfate proteoglycan 4, CSPG4)、CD13、CD34、α-SMA、结蛋白(desmin)和 CD146[29]。体外细胞和动物实验均证明周细胞可发生成骨和成软骨样分化[30-32]。例如将周细胞植入无胸腺小鼠后可检测到骨、矿化软骨和非矿化软骨样区[30]。受体酪氨酸激酶 Axl 活化可调节周细胞的成骨样分化和钙化[31]。糖皮质激素地塞米松可通过减少周细胞中血管钙化抑制因子基质 Gla 蛋白(matrix Gla protein, MGP)、骨桥蛋白和血管钙化相关因子的表达,致 ALP 活性增加,从而诱导周细胞成骨样分化和钙化[33]。在人的动脉粥样斑块中,动脉钙化程度与周细胞浸润呈正相关,骨样化生的斑块组织可检测到较多的周细胞和高水平的骨保护素(osteoprotegerin, OPG)[34]。

4 巨噬细胞

巨噬细胞(macrophage)具有吞噬、主动/被动免疫功能和促炎作用,也可降解细胞外基质(extracellular matrix, ECM),参与细胞分化和钙化的调节。体外细胞培养实验表明单核/巨噬细胞通过 2 种独立的机制,即细胞之间的相互作用和分泌促炎因子如肿瘤坏死因子 α(tumor necrosis factor-α,

TNF-α)促进细胞钙化[35]。巨噬细胞的条件培养基可增强 VSMC 中的 BMP-2 表达,以及降低 MGP 的表达,促进 VSMC 成骨样分化[36]。Fu 等[37]通过基因敲除小鼠实验证明软骨寡聚基质蛋白(cartilage oligomeric matrix protein,COMP)的缺乏导致巨噬细胞发生动脉粥样硬化/成骨样表型的转化,促进小鼠血管钙化。最近的研究显示,RAC2 可通过抑制巨噬细胞白细胞介素 1β(interleukin-1β,IL-1β)的表达而抑制钙化,这有可能成为新的干预血管钙化靶点[38]。

5 祖细胞

循环或组织祖细胞(progenitor cell)在一定条件下通过产生内皮细胞和平滑肌细胞参与动脉粥样硬化、损伤血管的内膜新生形成,但其也可能影响晚期动脉粥样硬化斑块的稳定性[39]。血管干细胞(vessel-derived stem cell,VSC)和 MSC 是导致动脉粥样硬化小鼠钙化的重要因素[40]。炎症因子 IL-1β 和 TNF-α 等可促进 MSC 成骨样分化和钙化[41]。Kramann 等[42]采用细胞谱系示踪实验表明,位于动脉外膜的 $Gli1^+$ 间充质干细胞样细胞在动脉损伤后可以迁移至内膜中,参与新生内膜的形成和修复;另外,在 $ApoE^{-/-}$ 小鼠慢性肾脏病(chronic kidney disease,CKD)的损伤过程中,$Gli1^+$ 细胞可发生成骨样分化,参与钙化的形成。敲除 $Gli1^+$ 细胞可显著减弱 CKD 小鼠血管钙化,这表明 $Gli1^+$ 间充质干细胞是驱动血管钙化的关键细胞。Wang 等[43]采用高脂饮食诱导的低密度脂蛋白受体基因敲除小鼠血管钙化模型,发现 TGF-β 信号可招募 MSC 至血管损伤处,参与动脉钙化的发生。尿激酶受体可促进 MSC 向成骨细胞分化和血管钙化[44]。此外,MSC 可通过分泌生物活性物质调节血管钙化,如 MSC 的条件培养基(MSC-CM)可明显抑制 VSMC 的成骨样分化和钙化[45];另有类似的研究报道指出,骨髓来源的 MSC 条件培养基通过阻断 BMP-2 信号通路,抑制 VSMC 的成骨样分化和钙化[46]。内皮祖细胞(endothelial progenitor cell,EPC)也可能参与血管钙化的调控过程。例如临床研究表明:在急性冠状动脉综合征患者,表达骨钙素(osteocalcin,OCN)的未成熟 EPC 与血管钙化的发生呈正相关关系,提示 OCN 阳性的 EPC 可能介导冠状动脉血管钙化[47]。与非钙化患者比较,CKD 钙化患者具有更多的 OCN 阳性的 EPC,表明 EPC 可以通过表达 OCN 直接参与血管钙化的过程[48]。

6 结 语

综上所述,血管钙化是受细胞和基因调控的生物学过程,涉及细胞的成骨样分化,其病理机制十分复杂。尽管近些年来研究人员加大了对血管钙化的研究力度,但是血管钙化的细胞来源及细胞成骨样分化的分子调控机制尚未完全明确,临床上缺乏有效防治血管钙化的药物。因此,进一步阐明调控血管钙化的细胞来源,不同细胞之间的相互作用以及细胞成骨样分化信号机制等,对于理解血管钙化的机制十分必要,这可为血管钙化的防治提供可靠的实验依据和指导意义。

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