



动脉粥样硬化斑块钙化机制的研究进展*

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摘要 心血管疾病中动脉粥样硬化斑块的钙化是动脉粥样硬化的临床标志之一, 主要发生在动脉血管的内膜。动脉粥样硬化斑块核心的钙化不会增加斑块的易损性, 而粥样斑块纤维帽上的微钙化会加强纤维帽的周向应力, 致使斑块的易损性增加。动脉粥样硬化斑块的钙化机制包括被动钙化和主动钙化, 被动钙化受激素和局部信号的调节, 主动钙化机制涉及复杂的细胞生命过程, 基质囊泡、细胞凋亡、外泌体、氧化应激反应和细胞自噬等均参与了钙化过程。本文对动脉粥样硬化斑块的钙化机制的进展进行综述。

关键词 动脉粥样硬化斑块, 被动钙化机制, 主动钙化机制

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近 10 年来, 对冠状动脉粥样硬化斑块钙化的研究, 不再只关注管腔狭窄, 已经转向斑块易损性的机制研究。大量研究发现动脉粥样硬化斑块微钙化的出现与斑块的易损性关系密切, 由此推测微钙化可能是引起斑块破裂的一个重要因素。众多研究表明, 位于内膜-中膜边界附近的大钙化不会增加斑块的易损性^[1-2]。而斑块纤维帽中存在大量的微钙化, 增强了纤维帽的基底周应力, 与斑块的易损性密切相关^[1]。微钙化对基底周应力的增强效果取决于其大小、形状和相互之间的距离。如果基底应力很小, 纤维帽上的微钙化不足以使纤维帽达到破裂阈值, 但是如果稳定斑块中纤维帽的基底应力已经达到其破裂阈值的 1/5~1/2 时, 纤维帽上的微钙化将很可能促使非易损斑块转变为易损斑块。长期以来, 心血管钙化被当成一个被动过程。近年来的研究表明, 心血管钙化还涉及一个主动钙化过程, 主动钙化机制涉及复杂的细胞生命过程, 细胞凋亡、基质囊泡、氧化应激反应、外泌体和自噬等均参与了血管钙化过程。本文综述了动脉粥样硬化斑块的钙化机制进展。

1 动脉粥样硬化组织的钙化

动脉粥样硬化组织钙化 (atherosclerotic calcification) 通常是无定形的矿化, 常见于体循环

的厚壁弹性动脉如外周动脉、冠状动脉和颈动脉血管, 主要发生于损伤内膜的基底部, 与弹性内膜结合, 邻近于血管的中膜层, 晚期也会累及血管中膜。动脉钙化包括软骨细胞和成骨细胞分化, 以及与骨相关的蛋白质, 如骨形态发生蛋白 (bone morphometric protein, BMP) BMP-2 和 BMP-4、骨连接蛋白、骨钙蛋白和骨保护蛋白。在组织形态上首先可见的是微钙化 (μCalcs), 微钙化是指大小 1~100 μm 的钙化颗粒, 可用 von Kossa 对磷酸盐染色或用茜素红对钙成分染色来检测。电子显微镜可见小至 100 nm 的微钙化颗粒。高分辨率 μCT 对钙化组织非常敏感, 可从冠脉中的坏死核心和软组织中分辨出脂质, 近年来用于检测微钙化的数量、位置、形态、聚集特征和矿化程度^[3-4]。微钙化可通过相互融合或者生长而形成大于 1 mm 的钙化颗粒, 之后才可利用常规的临床影像技术如 CT、MRI、SPECT 等进行检测。

1.1 动脉粥样硬化斑块坏死核心的钙化

基于动脉粥样硬化钙化的形态和空间分布, 可分为 4 种钙化模式^[4]: a. 坏死核心边缘微钙化。在

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钙化的初始阶段, 软组织和坏死核心的交界处出现亚微米和微米级别的钙化颗粒, 受弹性纤维层的约束 (图 1a); b. 坏死核心内微钙化. 直径 $<100\text{ }\mu\text{m}$ 的钙化颗粒分散在坏死核心中 (图 1b); c. 坏死核心内大钙化. 微钙化颗粒通过融合或通过结晶过程形成大钙化 (图 1c); d. 晚期大钙化. 大钙化一直生长, 填满整个坏死核心区, 形成晚期钙化 (图 1d). 在临床动脉粥样硬化斑块标本中, 模式 a 最常见. 大多数动脉粥样硬化斑块中存在两种及两种以上的钙化模式. 随着动脉粥样硬化斑块坏死核心厚度的增加, 模式 c 逐渐增多.

大钙化多见于已愈合破裂斑块中, 而在纤维化粥样斑块、薄帽纤维粥样斑块、斑块破裂中很少见, 甚至在病理性内膜增厚时也很少见^[5]. 众多研究表明, 位于内膜-中膜边界附近的大钙化不会增加斑块的易损性, 生物力学研究也证明了大钙化降低了斑块的易损性^[6]. 坏死核心中微钙化颗粒通过融合或生长方式而形成较大的钙化颗粒^[7], 可能促进动脉壁异位骨化 (heterotopic ossification). 因此提示, 动脉粥样硬化斑块核心的大钙化不会增加斑块的易损性. 虽然绝大多数微钙化存在于坏死核心中, 但它们对于斑块易损性的影响可以忽略^[8].

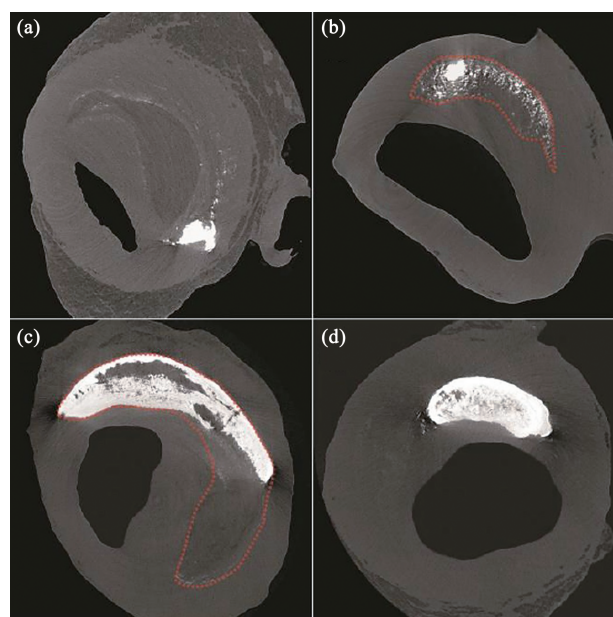


Fig. 1 Calcification patterns of human coronary fibroatheromas under high resolution μCT at $2.1\text{ }\mu\text{m}$ resolution (revised from ref. [4])

图1 分辨率 $2.1\text{ }\mu\text{m}$ 的高分辨率 μCT 成像的钙化模式^[4]

(a) 坏死核心边缘微钙化. (b) 坏死核心内微钙化. (c) 坏死核心内大钙化. (d) 晚期大钙化.

1.2 动脉粥样硬化斑块纤维帽的钙化

Maldonado 等^[4]采用分辨率为 $6.7\text{ }\mu\text{m}$ 的 μCT 检测破裂的动脉粥样斑块, 发现大部分微钙化位于脂质池, 少量位于纤维帽, 且位于纤维帽的微钙化大部分在组织应力集中的地方. 他们对斑块纤维帽的观察显示, 靠近脂质池一侧的微钙化要多于靠近管腔侧, 提示这些微钙化可能来源于坏死核心. 动脉粥样硬化斑块纤维帽肩部和中心均含有微钙化, 大部分在 $5\sim 15\text{ }\mu\text{m}$ 之间, 其中含有 $>5\text{ }\mu\text{m}$ 微钙化的纤维帽更薄. 微钙化也存在于适应性内膜增厚、病理性内膜增厚和纤维化粥样斑块中^[9]. Vengrenyuk 等^[3]利用有限元分析方法分析了微钙化的生物力学机制, 发现微钙化形成会增大纤维帽的局部组织应力, 导致纤维帽破裂, 从而增加斑块的易损性.

2 动脉粥样硬化斑块钙化的机制

2.1 被动钙化

心血管钙化以前被认为是一种被动的再矿化过程, 与血管退变、衰老以及慢性肾功能不全、高磷血症、糖尿病等代谢紊乱疾病有关^[10-12]. 被动钙化与动脉粥样硬化中局部细胞活动、脂质积聚和炎症无关, 其特征是无定形的钙和磷酸盐沉积. 被动钙化被认为是血管钙化的主要形式^[13-14], 发生于整个动脉粥样硬化进程的晚期, 与斑块的破裂无关^[10, 12].

被动钙化受激素和局部信号的调节. 在血液循环中, 钙同时以游离和与蛋白质结合的形式存在. 钙离子可与弹性蛋白和胶原蛋白结合, 带正电荷, 能吸附带负电荷的磷酸盐和碳酸根离子触发矿化过程^[15]. 心血管钙化时, 血浆中钙和磷浓度升高^[16]. 无机磷酸盐可直接诱导细胞成骨分化. 矿化过程中, 细胞中核苷酸焦磷酸酶/磷酸二酯酶 1 (nucleotide pyrophosphatase/phosphodiesterase 1, NPP1) 转变成抑制钙化的胞外无机焦磷酸酶 (extracellular inorganic pyrophosphate, ePPi)^[17]. 组织非特异性碱性磷酸酶 (tissue-nonspecific alkaline phosphatase, TNAP) 可以水解和清除 ePPi. ePPi 是一种钙化抑制分子, 可以防止磷酸钙聚集和羟基磷灰石晶体的生长和聚集^[18-19]. TNAP 过表达可提高磷酸盐水平而导致血管钙化^[20]. 钙能增加血管平滑肌细胞和胞外基质囊泡膜上的 PiT1 表达水平, 使成骨样细胞和磷酸盐聚集, 而提高钙和磷含量, 并促进胞外基质囊泡的释放^[21]. 血清钙和磷受甲状旁腺激素 (parathyroid hormone,

PTH) 和 1,25-二羟基维生素 D 调节^[22]. 慢性肾病和肾功能衰竭患者的钙化与 PTH 和维生素 D 信号途径也有关系^[23-24]. 但是, 钙评分显示 PTH 与冠状动脉钙化无关, 1,25-二羟基维生素 D 似乎与心血管疾病和钙化负相关^[16]. 高磷血症与肾磷分泌障碍和血管钙化相关^[25-26]. 成纤维细胞生长因子 23 (fibroblast growth factor-23, FGF-23) 可调节矿物质代谢, 通过促进肾脏磷酸盐的排泄, 从而限制磷酸盐的促钙化作用^[27]. FGF-23 还能增强血管钙化抑制分子骨保护素 (osteoprotegerin) 的表达, 但 FGF-23 在血管钙化方面的作用仍然存在争议^[28]. Fetuin 是循环血液中的一种糖蛋白, 是磷灰石的主要抑制分子. Fetuin 水平降低会增加血管钙化严重性和心血管死亡率^[29-31].

2.2 主动钙化

最近 10 年的许多研究, 将血管钙化的模式从一个被动的过程转变成了一个被动和主动并存的复杂过程. 在这个主动调节过程中, 部分血管组织细胞会分化为成骨细胞样表型, 包括侵入钙化血管内膜的血管平滑肌细胞、可分泌基质囊泡的钙化血管细胞、内膜层的周细胞和周细胞样细胞, 以及中膜层中的滋养管壁细胞^[13].

2.2.1 基质囊泡与矿物生长

当前研究认为, 血管平滑肌细胞的成骨转化触发了基质囊泡的形成和释放^[32]. 基质囊泡可以扩散至胶原纤维中. 如果没有矿化, 基质囊泡还可能融合并形成更大的囊泡^[33]. 基质囊泡膜含有钙和磷结合蛋白和转运分子, 包括依赖钠盐的磷酸共转运体 piT1 和 piT2, 和促聚蛋白 (annexin) 钙结合蛋白家族成员 A2、A5 和 A6. 当磷酸钙聚集于这些基质囊泡中, 基质囊泡钙化并形成羟基磷灰石晶体, 该过程类似于骨形成^[34]. 基质囊泡内的矿物生长并穿透基质囊泡膜以后, 可以在间隙空间中继续矿化与生长^[35], 后续过程可能遵循被动矿化机制.

2.2.2 细胞凋亡与血管钙化

凋亡细胞普遍见于动脉粥样硬化核、纤维帽和破裂纤维帽中. 血管平滑肌细胞凋亡和巨噬细胞钙化后形成的细胞碎片可诱导羟基磷灰石矿物的成核. 血管平滑肌细胞凋亡使磷脂酰丝氨酸暴露于凋亡细胞表面, 促进了斑块血管钙化. 血管平滑肌细胞凋亡可加速血管钙化, 吞噬细胞对动脉粥样硬化组织中凋亡碎片的不完全清除将促进促炎性细胞因子的释放和钙盐晶体聚集、成核. 受细胞凋亡微

环境的影响, 斑块中残余的血管平滑肌细胞, 可促进斑块内微钙化的形成^[36]. ⁴⁵Ca 标记的平滑肌细胞来源的凋亡小体具有和基质囊泡类似的功能, 可以促进钙聚集^[37]. 但在生理条件下, 血管平滑肌细胞释放的基质囊泡对钙化没有明显作用. 高磷或尿毒症患者的血清可诱导血管平滑肌细胞凋亡、促进钙化^[38]. 抑制胃泌素释放肽可以抑制血管平滑肌细胞表型改变、凋亡和基质囊泡释放, 缓解高磷诱导的血管钙化^[39]. 补锌可以通过锌敏感受体 GPR39 依赖的 ERK1/2 信号途径, 抑制人瓣膜间质细胞的凋亡, 抑制细胞钙化和成骨分化^[40].

氧化型低密度脂蛋白 (oxidized low-density lipoprotein, ox-LDL) 诱导的血管内皮细胞损伤和凋亡被认为是动脉粥样硬化的重要起始事件. ox-LDL 的激活, 可促使单核细胞黏附于血管内皮细胞, 并侵入内皮下间隙, 转化为巨噬细胞, 促进血管钙化^[41].

2.2.3 外泌体与血管钙化

人脐静脉内皮细胞 (human umbilical vein endothelial cells, HUVEC) 可释放外泌体发挥生物学功能^[42]. HUVEC 经 ox-LDL 处理后, 可通过释放富含转移相关肺腺癌转录物 1 (metastasis-associated lung adenocarcinoma transcript 1, MALAT1) 的外泌体, 促进 M2 巨噬细胞的极化^[43]. M2 巨噬细胞可促进钙沉积, 加速钙化^[41]. 同型半胱氨酸诱导血管内皮细胞凋亡, 促进了血管钙化^[44].

与正常 HUVEC 来源的外泌体相比, 高糖 (high glucose, HG) 诱导的 HUVEC 外泌体中多功能蛋白聚糖 Versican 上调^[45]. 该富 Versican 的外泌体被血管平滑肌细胞募集和活化后, Versican 作用于血管平滑肌细胞线粒体, 降低线粒体膜电位和线粒体三功能蛋白 α 亚基 (hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase, alpha subunit, HADHA)、细胞色素氧化酶 4 (Cytochrome oxidase-4, Cox-4) 蛋白的表达水平, 最终导致线粒体功能紊乱和细胞钙化或衰老. Krüppel 样因子 2 (Krüppel-like factor 2, KLF2) 是一种剪切反应的转录因子, 是动脉粥样硬化的保护因子^[46]. HUVECs 中过表达 KLF2, 或在切应力作用下 KLF2 表达增加, 可促进富 miR-143/150 的细胞外囊泡释放, 该囊泡可抑制平滑肌细胞靶基因如 *ELK1*、*KLF4* 的表达, 从而诱导平滑肌细胞的动脉粥样硬化表型^[47-48]. 动物实验同样证明, 过表达

KLF2的血管内皮细胞所生成的细胞外囊泡,可降低 *ApoE*^{-/-}小鼠主动脉粥样硬化的病变程度^[49]. Ox-LDL处理和 *KLF2* 基因过表达,还可分别促进 HUVECs 释放富 miR-155的细胞外囊泡,作用于单核细胞并调控巨噬细胞表型^[50].

2.2.4 氧化应激与血管钙化

起始于早期慢性肾病的氧化性弹性内膜损伤(oxidative elastic lamina injury),与血管平滑肌细胞的再编程、凋亡和最终不可逆的钙化有关^[51].在慢性肾衰病人中,血清磷没有显著增加,但氧化应激水平和血管钙化显著增加,氧化应激可能促进血管钙化^[52]. β -磷酸甘油是无机磷供体,可以活化碱性磷酸酶,提高磷水平. β -磷酸甘油可以促进牛主动脉血管平滑肌细胞的活性氧生成,促进血管平滑肌向成骨细胞转化^[53]. NRF2 (NF-E2-related factor 2) 活化剂叔丁基氢醌(tert-butylhydroquinone, tBHQ)可以显著抑制活性氧水平,抑制血管平滑肌细胞的钙沉积^[54]. 核因子- κ B受体活化因子配体(receptor-activator of nuclear factor- κ B ligand, RANKL)可诱使人主动脉内皮细胞活性氧生成,通过促钙化的旁分泌信号促进血管

平滑肌细胞的钙化^[55]. Fibulin-3可以通过抑制氧化应激而抑制磷酸盐诱导的血管平滑肌细胞钙化^[56].

2.2.5 细胞自噬与血管钙化

使用自噬抑制剂3-甲基腺嘌呤可抑制血管平滑肌细胞自噬,促进血管平滑肌细胞钙化,而使用丙戊酸促进自噬则可以减轻钙化^[57]. TGF- β 1可以诱导血管平滑肌细胞钙化,而促进自噬可以抑制血管平滑肌细胞钙化^[58]. BMP也是TGF- β 超家族的一部分,在促进成骨分化、动脉钙化、糖尿病血管钙化和慢性肾病钙化等病变中起重要作用. BMP4可通过抑制基质Gla蛋白(matrix Gla protein, MGP),促进血管内皮钙化^[59]. BMP-2可通过作用Runx2等促进血管中膜层钙化^[60].但是,微钙化先于BMP-2等钙化蛋白的改变^[9]. MGP可以抑制主动脉内膜的内皮间充质转化和中膜平滑肌细胞向骨软骨原细胞的分化. 人体缺失MGP(如Keutel综合征)会导致异常钙化,而提高MGP水平可减少动脉粥样硬化钙化^[61]. 柠檬酸铁可促进自噬而抑制高磷诱导的平滑肌细胞钙化^[62]. 干扰mTOR2或抑制自噬,可以促进间充质基质细胞(mesenchymal stromal cells)的衰老、凋亡和成骨细胞分化,加

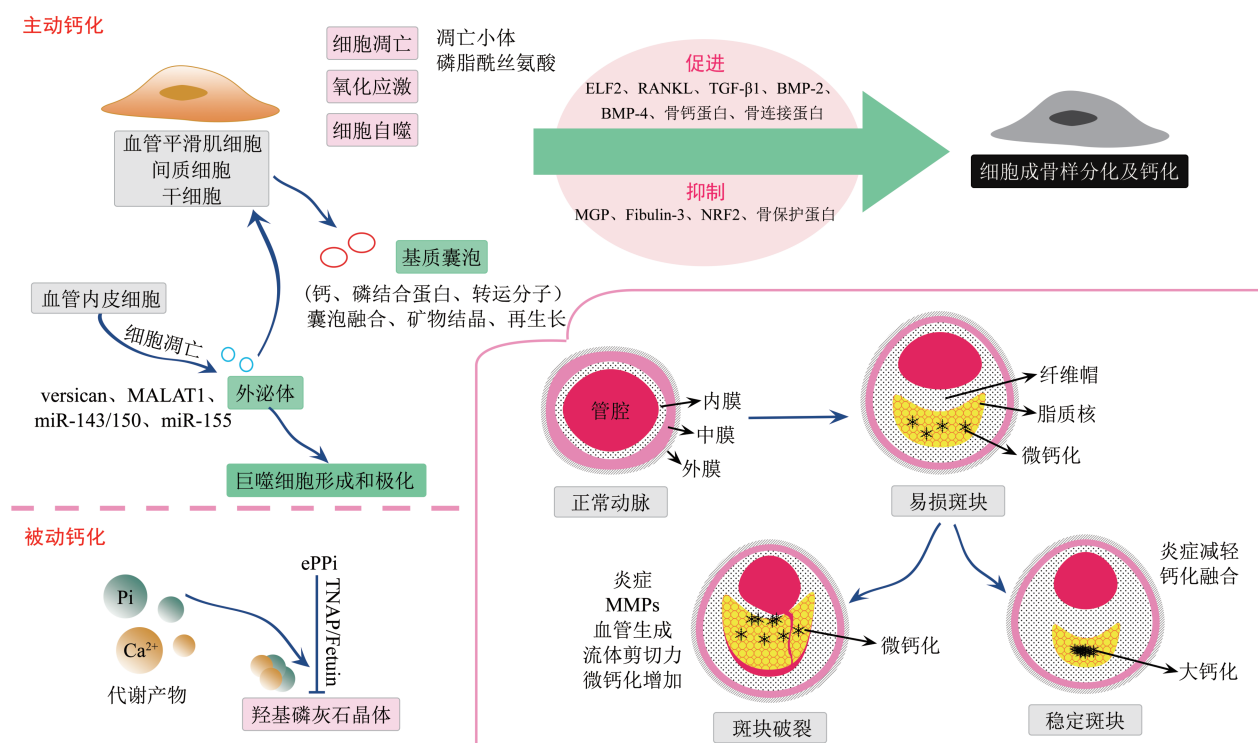


Fig. 2 The role of vascular calcification in progression of atherosclerotic plaque

图2 血管钙化与动脉粥样硬化斑块进展

动脉粥样硬化斑块的钙化机制包括被动钙化和主动钙化,被动钙化受TNAP/Fetuin等的调控,主动钙化机制涉及血管平滑肌细胞、间质细胞、干细胞等复杂的细胞生命过程,基质囊泡、外泌体、细胞凋亡、氧化应激反应和细胞自噬等均参与了细胞成骨样分化及钙化过程.微钙化参与了易损斑块的形成,但后期钙化的发展可能导致斑块破裂或稳定斑块两种不同的结局.

速血管钙化^[63]。

3 展 望

长期以来,钙评分被用于评估冠状斑块负荷。大面积钙化是晚期稳定斑块的一个特征,因此钙化提高了动脉粥样硬化斑块稳定性的观点备受支持。近年来,越来越多的研究关注动脉粥样硬化纤维帽上是否存在微钙化,将高分辨率 μ CT用于检测微钙化是一个重要的突破。现有证据支持微钙化的大小、形状及位置与斑块的易损性直接相关,但是,血管钙化形成的机制,特别是动脉粥样硬化内膜层钙化的机制尚不清楚。血管钙化可能是一个涉及主动钙化与被动钙化的复杂过程。BMPs、炎症细胞因子和巨噬细胞在血管矿化的促进方面起重要作用,而MGP、骨桥蛋白、Fetuin和ePPI则抑制钙化。促进血管钙化与抑制血管钙化的血管因子二者平衡被打破以后,血管开始主动钙化。在一定阶段后,将启动被动矿化过程,提高钙化程度。动脉粥样硬化钙化的主动机制还与基质囊泡、细胞凋亡、细胞氧化应激、外泌体和细胞自噬等有关(图2)。血管钙化过程涉及血管平滑肌细胞、血管内皮细胞、瓣膜间质细胞等的相互作用。在动脉粥样硬化组织钙化过程中,组织内部应力发生改变。分析微钙化与斑块易损性的内在关联,研究血管钙化过程中细胞之间的相互作用,明确血管钙化过程的力学调控新机制,将为心血管疾病的诊断和防治提供新策略。

参 考 文 献

- [1] Scimeca M, Anemona L, Granaglia A, *et al.* Plaque calcification is driven by different mechanisms of mineralization associated with specific cardiovascular risk factors. *Nutrition, Metabolism, and Cardiovascular Diseases: NMCD*, 2019, **29**(12): 1330-1336
- [2] Tesaro M, Mauriello A, Rovella V, *et al.* Arterial ageing: from endothelial dysfunction to vascular calcification. *Journal of Internal Medicine*, 2017, **281**(5): 471-482
- [3] Vengrenyuk Y, Kaplan T J, Cardoso L, *et al.* Computational stress analysis of atherosclerotic plaques in ApoE knockout mice. *Annals of Biomedical Engineering*, 2010, **38**(3): 738-747
- [4] Maldonado N, Kelly-Arnold A, Laudier D, *et al.* Imaging and analysis of microcalcifications and lipid/necrotic core calcification in fibrous cap atheroma. *The International Journal of Cardiovascular Imaging*, 2015, **31**(5): 1079-1087
- [5] Burke A P, Weber D K, Kolodgie F D, *et al.* Pathophysiology of calcium deposition in coronary arteries. *Herz*, 2001, **26**(4): 239-244
- [6] Mauriello A, Servadei F, Zoccai G B, *et al.* Coronary calcification identifies the vulnerable patient rather than the vulnerable Plaque. *Atherosclerosis*, 2013, **229**(1): 124-129
- [7] Kelly-Arnold A, Maldonado N, Laudier D, *et al.* Revised microcalcification hypothesis for fibrous cap rupture in human coronary arteries. *Proc Natl Acad Sci USA*, 2013, **110**(26): 10741-10746
- [8] Cardoso L, Weinbaum S. Microcalcifications, their genesis, growth, and biomechanical stability in fibrous cap rupture. *Adv Exp Med Biol*, 2018, 1097: 129-155
- [9] Roijers R B, Debernardi N, Cleutjens J P, *et al.* Microcalcifications in early intimal lesions of atherosclerotic human coronary arteries. *The American Journal of Pathology*, 2011, **178**(6): 2879-2887
- [10] Otsuka F, Sakakura K, Yahagi K, *et al.* Has our understanding of calcification in human coronary atherosclerosis progressed? *Arterioscler Thromb Vasc Biol*, 2014, **34**(4): 724-736
- [11] Jeevarethinam A, Venuraju S, Dumo A, *et al.* Relationship between carotid atherosclerosis and coronary artery calcification in asymptomatic diabetic patients: a prospective multicenter study. *Clinical Cardiology*, 2017, **40**(9): 752-758
- [12] Yahagi K, Kolodgie F D, Lutter C, *et al.* Pathology of human coronary and carotid artery atherosclerosis and vascular calcification in diabetes mellitus. *Arterioscler Thromb Vasc Biol*, 2017, **37**(2): 191-204
- [13] Cho K I, Sakuma I, Sohn I S, *et al.* Inflammatory and metabolic mechanisms underlying the calcific aortic valve disease. *Atherosclerosis*, 2018, 277: 60-65
- [14] Demer L L, Tintut Y. Inflammatory, metabolic, and genetic mechanisms of vascular calcification. *Arterioscler Thromb Vasc Biol*, 2014, **34**(4): 715-723
- [15] Urry D W. Neutral sites for calcium ion binding to elastin and collagen: a charge neutralization theory for calcification and its relationship to atherosclerosis. *Proc Natl Acad Sci USA*, 1971, **68**(4): 810-814
- [16] Gronhøj M H, Gerke O, Mickley H, *et al.* Associations between calcium-phosphate metabolism and coronary artery calcification; a cross sectional study of a middle-aged general population. *Atherosclerosis*, 2016, 251: 101-108
- [17] Shimokado A, Sun Y, Nakanishi M, *et al.* Smad3 plays an inhibitory role in phosphate-induced vascular smooth muscle cell calcification. *Experimental and molecular pathology*, 2014, **97**(3): 458-464
- [18] Villa-Bellosta R. Synthesis of extracellular pyrophosphate increases in vascular smooth muscle cells during phosphate-induced calcification. *Arterioscler Thromb Vasc Biol*, 2018, **38**(9): 2137-2147
- [19] Villa-Bellosta R, O'Neill W C. Pyrophosphate deficiency in vascular calcification. *Kidney International*, 2018, **93**(6): 1293-1297
- [20] Sheen C R, Kuss P, Narisawa S, *et al.* Pathophysiological role of vascular smooth muscle alkaline phosphatase in medial artery calcification. *J Bone Miner Res*, 2015, **30**(5): 824-836

- [21] Hasegawa T, Yamamoto T, Tsuchiya E, *et al.* Ultrastructural and biochemical aspects of matrix vesicle-mediated mineralization. *The Japanese Dental Science Review*, 2017, **53**(2): 34-45
- [22] Hsu J J, Fong F, Patel R, *et al.* Changes in microarchitecture of atherosclerotic calcification assessed by (18)F-NaF PET and CT after a progressive exercise regimen in hyperlipidemic mice. *J Nucl Cardiol*, 2020, doi: 10.1007/s12350-019-02004-3
- [23] Nizet A, Cavalier E, Stenvinkel P, *et al.* Bone alkaline phosphatase: An important biomarker in chronic kidney disease - mineral and bone disorder. *Clin Chim Acta*, 2020, 501: 198-206
- [24] Wang J, Zhou J J, Robertson G R, *et al.* Vitamin D in vascular calcification: a double-edged sword? *Nutrients*, 2018, **10**(5): 652
- [25] Disthabanchong S, Srisuwarn P. Mechanisms of vascular calcification in kidney disease. *Adv Chronic Kidney Dis*, 2019, **26**(6): 417-426
- [26] Ray M, Jovanovich A. Mineral bone abnormalities and vascular calcifications. *Adv Chronic Kidney Dis*, 2019, **26**(6): 409-416
- [27] Fayed A, Radwan W A, Amin M, *et al.* Prediction of mortality and need for renal replacement therapy in patients of acute kidney injury using fibroblast growth factor 23. *Saudi J Kidney Dis Transpl*, 2019, **30**(5): 1044-1051
- [28] Nakahara T, Kawai-Kowase K, Matsui H, *et al.* Fibroblast growth factor 23 inhibits osteoblastic gene expression and induces osteoprotegerin in vascular smooth muscle cells. *Atherosclerosis*, 2016, 253: 102-110
- [29] Koca N, Ersoy A, Sensoy B, *et al.* The association between cardiac valvular calcification and fetuin-A levels in kidney transplant recipients. *Clin Exp Nephrol*, 2019, **23**(10): 1250-1256
- [30] Mancio J, Barros A S, Conceicao G, *et al.* Epicardial adipose tissue volume and annexin A2/fetuin-A signalling are linked to coronary calcification in advanced coronary artery disease: Computed tomography and proteomic biomarkers from the EPICHEART study. *Atherosclerosis*, 2020, 292: 75-83
- [31] Muzasti R A, Loesnihari R. High fetuin-A Level as a protective factor to abdominal aortic calcification in indonesian regular hemodialysis patients. *Open Access Maced J Med Sci*, 2019, **7**(5): 721-725
- [32] Hutcheson J D, Goettsch C, Bertazzo S, *et al.* Genesis and growth of extracellular-vesicle-derived microcalcification in atherosclerotic plaques. *Nature Materials*, 2016, **15**(3): 335-343
- [33] Ruiz J L, Weinbaum S, Aikawa E, *et al.* Zooming in on the genesis of atherosclerotic plaque microcalcifications. *The Journal of Physiology*, 2016, **594**(11): 2915-2927
- [34] Amizuka N, Hasegawa T, Oda K, *et al.* Histology of epiphyseal cartilage calcification and endochondral ossification. *Frontiers in Bioscience (Elite edition)*, 2012, 4: 2085-2100
- [35] Bottini M, Mebarek S, Anderson K L, *et al.* Matrix vesicles from chondrocytes and osteoblasts: their biogenesis, properties, functions and biomimetic models. *Biochim Biophys Acta Gen Subj*, 2018, **1862**(3): 532-546
- [36] Grootaert M O J, Moulis M, Roth L, *et al.* Vascular smooth muscle cell death, autophagy and senescence in atherosclerosis. *Cardiovascular Research*, 2018, **114**(4): 622-634
- [37] Proudfoot D, Skepper J N, Hegyi L, *et al.* Apoptosis regulates human vascular calcification in vitro: evidence for initiation of vascular calcification by apoptotic bodies. *Circulation Research*, 2000, **87**(11): 1055-1062
- [38] Ciceri P, Galassi A, Alfieri C, *et al.* Uremic patients with increased vascular calcification score have serum with high calcific potential: role of vascular smooth muscle cell osteoblastic differentiation and apoptosis. *Blood Purification*, 2019, **48**(2): 142-149
- [39] Park H J, Kim Y, Kim M K, *et al.* Inhibition of gastrin-releasing peptide attenuates phosphate-induced vascular calcification. *Cells*, 2020, **9**(3): 737
- [40] Chen Z, Gordillo-Martinez F, Jiang L, *et al.* Zinc ameliorates human aortic valve calcification through GPR39 mediated ERK1/2 signaling pathway. *Cardiovascular Research*, 2020, doi: 10.1093/cvr/cvaa1090
- [41] Shioi A, Ikari Y. Plaque calcification during atherosclerosis progression and regression. *J Atheroscler Thromb*, 2018, **25**(4): 294-303
- [42] Zeng Y, Yao X, Liu X, *et al.* Anti-angiogenesis triggers exosomes release from endothelial cells to promote tumor vasculogenesis. *J Extracell Vesicles*, 2019, **8**(1): 1629865
- [43] Huang C, Han J, Wu Y, *et al.* Exosomal MALAT1 derived from oxidized low-density lipoprotein-treated endothelial cells promotes M2 macrophage polarization. *Molecular Medicine Reports*, 2018, **18**(1): 509-515
- [44] Fang K, Chen Z, Liu M, *et al.* Apoptosis and calcification of vascular endothelial cell under hyperhomocysteinemia. *Medical Oncology (Northwood, London, England)*, 2015, **32**(1): 403
- [45] Li S, Zhan J K, Wang Y J, *et al.* Exosomes from hyperglycemia-stimulated vascular endothelial cells contain versican that regulate calcification/senescence in vascular smooth muscle cells. *Cell Biosci*, 2019, **9**: 1
- [46] Boon R A, Horrevoets A J. Key transcriptional regulators of the vasoprotective effects of shear stress. *Hamostaseologie*, 2009, **29**(1): 39-40, 41-33
- [47] Cordes K R, Sheehy N T, White M P, *et al.* miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature*, 2009, **460**(7256): 705-710
- [48] Xin M, Small E M, Sutherland L B, *et al.* MicroRNAs miR-143 and miR-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury. *Genes Dev*, 2009, **23**(18): 2166-2178
- [49] Hergenreider E, Heydt S, Treguer K, *et al.* Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. *Nature Cell Biology*, 2012, **14**(3): 249-256
- [50] He S, Wu C, Xiao J, *et al.* Endothelial extracellular vesicles modulate the macrophage phenotype: potential implications in atherosclerosis. *Scand J Immunol*, 2018, **87**(4): e12648
- [51] Chang J F, Liu S H, Lu K C, *et al.* Uremic vascular calcification is correlated with oxidative elastic lamina injury, contractile smooth

- muscle cell loss, osteogenesis, and apoptosis: The human pathobiological evidence. *Frontiers in Medicine*, 2020, **7**: 78
- [52] Huang M, Zheng L, Xu H, *et al.* Oxidative stress contributes to vascular calcification in patients with chronic kidney disease. *J Mol Cell Cardiol*, 2019, **138**: 256-268
- [53] Zhang W, Li Y, Ding H, *et al.* Hydrogen peroxide prevents vascular calcification induced ROS production by regulating Nrf-2 pathway. *Renal Failure*, 2016, **38**(7): 1099-1106
- [54] Wei R, Enaka M, Muragaki Y. Activation of KEAP1/NRF2/P62 signaling alleviates high phosphate-induced calcification of vascular smooth muscle cells by suppressing reactive oxygen species production. *Scientific Reports*, 2019, **9**(1): 10366
- [55] Harper E, Rochfort K D, Smith D, *et al.* RANKL treatment of vascular endothelial cells leading to paracrine pro-calcific signaling involves ROS production. *Mol Cell Biochem*, 2020, **464**(1-2): 111-117
- [56] Luong T T D, Schelski N, Boehme B, *et al.* Fibulin-3 attenuates phosphate-induced vascular smooth muscle cell calcification by inhibition of oxidative stress. *Cell Physiol Biochem*, 2018, **46**(4): 1305-1316
- [57] Dai X Y, Zhao M M, Cai Y, *et al.* Phosphate-induced autophagy counteracts vascular calcification by reducing matrix vesicle release. *Kidney International*, 2013, **83**(6): 1042-1051
- [58] Liu D, Cui W, Liu B, *et al.* Atorvastatin protects vascular smooth muscle cells from TGF-beta1-stimulated calcification by inducing autophagy *via* suppression of the beta-catenin pathway. *Cell Physiol Biochem*, 2014, **33**(1): 129-141
- [59] Buyukterzi Z, Can U, Alpaydin S, *et al.* Enhanced serum levels of matrix Gla protein and bone morphogenetic protein in acute coronary syndrome patients. *J Clin Lab Anal*, 2018, **32**(3): e22278
- [60] Chen S, Wang J, Cai C, *et al.* N-myc downstream-regulated gene 2 (NDRG2) promotes bone morphogenetic protein 2 (BMP2) - induced osteoblastic differentiation and calcification by janus kinase 3 (JAK3)/signal transducer and activator of transcription 3 (STAT3) signaling pathway. *Med Sci Monit*, 2020, **26**: e918541
- [61] Zwakenberg S R, Van Der Schouw Y T, Vermeer C, *et al.* Matrix Gla protein, plaque stability, and cardiovascular events in patients with severe atherosclerotic disease. *Cardiology*, 2018, **141**(1): 32-36
- [62] Ciceri P, Falleni M, Tosi D, *et al.* Therapeutic effect of iron citrate in blocking calcium deposition in high Pi-calcified VSMC: role of autophagy and apoptosis. *International Journal of Molecular Sciences*, 2019, **20**(23): 5925
- [63] Schaub T, Gürgen D, Maus D, *et al.* mTORC1 and mTORC2 differentially regulate cell fate programs to coordinate osteoblastic differentiation in mesenchymal stromal cells. *Scientific Reports*, 2019, **9**(1): 20071

Recent Advances in Calcification Mechanism of Atherosclerotic Plaque*

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Abstract Calcification of atherosclerotic plaque, which is mainly observed in the intima of arterial vessels, is one of the clinical signs of atherosclerosis in cardiovascular diseases. Calcification in atheroma dose not increase the vulnerability of plaque, while microcalcification (μCalcs) located within the fibrous cap could intensify the background circumferential stress in the cap, resulting in increased vulnerability of plaque. The mechanisms of calcification in atherosclerotic plaques include passive calcification and active calcification. Passive calcification is regulated by hormones and local signals. The mechanisms of active calcification are a cell-mediated process, participated by matrix vesicles, cell apoptosis, exosomes, oxidative stress response and autophagy. This paper reviews the calcification mechanisms of atherosclerotic plaque.

Key words atherosclerotic plaque, passive calcification mechanism, active calcification mechanism

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