

综述



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重点项目, 2019 年 9 月和国家自然科学基金委员会联合举办蛋白活性多肽基础与临床高峰论坛, 发表中英文论文 100 余篇, 其中以第一作者和通讯作者在国际肾脏病专业期刊以及 *Atherosclerosis*、*Cell Death & Disease* 等杂志发表 SCI 论文 30 篇。

慢性肾脏病相关的血管钙化发病新机制

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摘要: 血管钙化是导致慢性肾脏病(chronic kidney disease, CKD)患者高心血管疾病发病率和死亡率的关键因素, 造成了极大的医疗卫生及经济负担, 探索其发病机制及干预手段十分迫切。CKD血管钙化是受多种细胞主动调控的异位成骨过程。血管平滑肌细胞(vascular smooth muscle cells, VSMCs)在促钙化环境下发生成骨样转分化, 分泌基质囊泡形成钙磷结晶沉积位点是CKD血管钙化发生和发展的关键事件。本文就CKD血管钙化研究新机制、新技术及肌肉细胞因子鸢尾素(Irisin)在CKD血管钙化中的作用及机制进行综述。

关键词: 慢性肾脏病; 血管钙化; 发病机制; 鸢尾素

New mechanisms of chronic kidney disease-associated vascular calcification

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Abstract: Vascular calcification is the crucial factor of high cardiovascular disease morbidity and mortality in patients with chronic kidney disease (CKD), which causes a huge medical and economic burden. It is urgent to explore its pathogenesis and intervention methods. CKD-associated vascular calcification is an ectopic osteogenesis process actively regulated by multiple cells. Vascular smooth muscle cells (VSMCs) undergo osteogenic differentiation in a pro-calcification environment, and secrete matrix vesicles to form calcium and phosphorus crystal deposition sites, which are key events in the development of CKD-associated vascular calcification. This article reviews the new mechanism and technology of CKD-associated vascular calcification and discusses the role of the myokine Irisin in CKD-associated vascular calcification.

Key words: chronic kidney disease; vascular calcification; pathogenesis; Irisin

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心血管疾病 (cardiovascular disease, CVD) 是慢性肾脏病 (chronic kidney disease, CKD) 患者死亡的主要原因^[1, 2], 也是 CKD 疾病负担的主要来源。血管钙化 (vascular calcification) 是 CKD 患者发生不良心血管事件的关键因素。与动脉粥样硬化背景下的内膜钙化不同, 中膜钙化是 CKD 相关血管钙化的典型特征。矿物质沿着动脉中膜弥漫性沉积, 引起血管僵硬增加、脉压增大、心脏后负荷增大、冠脉灌注不足等病理改变^[3–5], 导致 CKD 患者发生严重的心血管不良事件。

现阶段的研究表明 CKD 血管钙化的发生与骨形成类似, 是在矿物质代谢异常、尿毒症毒素、炎症、氧化应激等促钙化因素影响下, 由多种细胞参与的、主动的、可调控的异位成骨过程^[6, 7]。调节血管钙化的细胞可能包括血管平滑肌细胞 (vascular smooth muscle cells, VSMCs)、内皮细胞 (endothelial cells, ECs)、周细胞、巨噬细胞及循环祖细胞等, 其中 VSMCs 和 ECs 作为动脉壁的主要细胞类型, 在血管钙化的发生和发展中起关键作用, 本文就 VSMCs、ECs、自噬、线粒体稳态失衡、代谢重编程、细胞外基质 (extracellular matrix, ECM) 重塑、肠道菌群失调以及肌肉细胞因子鸢尾素 (Irisin) 在 CKD 相关的血管钙化中的作用及机制研究进行综述。

1 VSMCs

VSMCs 成骨样转化是 CKD 血管钙化的病理生理学基础, 在 CKD 矿物质代谢紊乱、尿毒症毒素累积等环境因素作用下, VSMCs 成骨转录因子 (Runx2、Osterix、Msx2 等) 表达上调, 收缩蛋白表达减少, 而骨形成相关蛋白表达和分泌增加, 如碱性磷酸酶 (alkaline phosphatase, ALP)、骨形态发生蛋白 (bone morphogenetic proteins, BMPs) 以及富含胶原的 ECM^[8], 从收缩表型转变为成骨样表型^[9, 10]。成骨样 VSMCs 分泌的基质囊泡 (matrix vesicles, MVs) 和凋亡小体则作为矿化物沉积和晶体成核的初始位点, 进一步形成钙化灶^[11]。

近期的研究发现 Runx2 除诱导成骨蛋白转录外, 还可以通过组蛋白 H2AX 磷酸化响应 DNA 损伤反应, 成为联系 DNA 损伤信号与成骨基因转录及细胞凋亡的桥梁, 从而加速血管钙化^[12]。成骨相关转录因子 Osterix 是 Runx2 的下游基因^[13], 在醛固酮诱导的小鼠血管钙化模型中表达显著上调, 敲低 Osterix 后可抑制 VSMCs 成骨转化和钙化^[14]。BMPs

是转化生长因子- β (transforming growth factor- β , TGF- β) 超家族成员, 最初由于其促进异位骨形成作用得到重视, 近来发现 BMPs 不仅对血管系统发生和稳态维持发挥重要作用^[15, 16], 并对血管钙化发生和发展、VSMCs 表型转化及增殖等至关重要^[17, 18]。BMP2 成骨信号在去分化 VSMCs 中显著增强^[19], 可通过 ALK/Smad 通路增强成骨转录因子 Runx2、T 细胞因子蛋白 / 淋巴增强因子 (T cell factor/lymphoid enhancer factor, TCF/LEF)、Msx2 等表达, 也可通过 Wnt/ β -catenin 与 Smad 信号协同或平行地促进 ALP 表达和基质矿化^[20]。在载脂蛋白 E (apolipoprotein E, ApoE) 敲除小鼠中, 促进 VSMCs 的 BMP2 基因表达加速了主动脉内膜钙化, 反之, 抑制 BMPs 信号则使钙化形成减少^[19]。缺乏 BMP2/4 抑制基质 γ -羧基谷氨酸蛋白 (matrix γ carboxyglutamic acid protein, MGP) 也可导致 VSMCs 成骨样分化及矿化的发生^[21]。相反, 应用选择性 BMP 抑制剂 DMH1 则可抑制 VSMCs 成骨分化, 抑制动脉中膜钙化^[22, 23]。

除转录水平改变外, 表观遗传学改变也参与 VSMCs 成骨样转分化过程。近来发现微小 RNA (microRNA, miRNA) 在 VSMCs 成骨样转化和钙化中发挥双向调节作用: 抑制内源性 miRNA125b 可通过靶向 Osterix 促进 VSMCs 成骨样分化^[24], 而富含 miRNA-204/miRNA-211 的外泌体可通过靶向 BMP2 减轻 VSMCs 的成骨样转化和钙化^[25]。miRNA26 可负性调节糖原合酶激酶 3 β , 通过 Wnt/ β -catenin/C/EBP α 信号通路调节 VSMCs 成骨样转分化^[26]。除 miRNA 外, DNA 甲基化及组蛋白修饰也参与血管钙化进程, 如高磷可改变 DNA 甲基转移酶 (DNA methyltransferase, DNMT) 的活性, 调节 SMA22 α 启动子甲基化进而诱导钙化^[27], 而组蛋白赖氨酸去甲基化酶 JMJD2B 则可通过靶向 Runx2 启动子区域的转录抑制子和增强子调节 Runx2 的表达^[28]。

钙离子是维持细胞正常生理功能的关键信号分子, VSMCs 存在复杂的钙信号调节机制, 涉及钙感受受体 (CaR)、钙泵、钙通道及钠钙转换体等, 参与维持细胞内钙稳态。异常升高的钙离子可以单独或协同磷酸盐刺激 VSMCs 成骨样转化, 钙离子不仅诱导氧化应激, 抑制 MGP 表达, 还可直接沉积于血管壁成为羟基磷灰石的主要成核物, 加剧血管钙化的发生和发展。收缩型 VSMCs 高表达电压门控 L 型钙通道以及肌浆网 / 内质网 RyR 受体, 成骨样 VSMCs 则高表达 T 型钙通道且低表达 RyR 受

体^[29],由此可见细胞内钙离子浓度变化是影响VSMCs表型的关键因素。CKD患者血管壁处于高钙负荷,过度钙摄取和储存钙释放导致VSMCs细胞内钙超载,促进线粒体功能障碍,氧化应激损伤,细胞表型转化,衰老和死亡^[30-33]。此外,感知细胞外钙离子浓度的CaR表达下调可以加剧体内外血管钙化,而增加CaR敏感性的拟钙剂R-586可抑制CKD血管钙化进展^[34,35],考虑到高钙可通过CaR增加MGP的转录^[36],推测通过CaR感知细胞外钙离子浓度可能是VSMCs对高钙的抵御反应。

近来研究表明,衰老与VSMCs钙化关系密切。衰老的VSMCs不仅衰老标志物表达上调,还伴随着Runx2、ALP、BMP2等成骨标志物表达增加,更具有成骨样转化倾向^[37]。CKD背景下多种病理性因素如尿毒症毒素、氧化应激损伤、炎症等可诱导VSMCs过早衰老,加重血管钙化的发生。研究表明,CKD患者冠脉中膜衰老标志物p16和 β -半乳糖苷酶阳性细胞增多,且伴随Runx2表达增加,表现出与实际年龄无关的血管早衰表型,与中膜钙化显著相关^[38]。硫酸吲哚酚(indoxyl sulfate, IS)可上调高血压大鼠主动脉弓VSMCs衰老相关标志物p53及核纤层蛋白lamin A前体(prelamin A)表达,促进 β -半乳糖苷酶活性,加重VSMCs成骨样转化及主动脉钙化^[39]。研究表明,prelamin A在钙化的人VSMCs中积累,可激活DNA损伤反应,促进VSMCs成骨分化与衰老,加速血管钙化^[37]。

VSMCs表型转化在分子水平上涉及基因表达、表观遗传学修饰、蛋白水平、钙离子以及能量代谢转变等;在空间上涉及内质网、线粒体等多种细胞器,还涉及细胞衰老、自噬、死亡等生命活动,是多时空共同协调的复杂过程,涉及多种信号通路,是血管钙化至关重要的一环,因此,明确血管钙化中细胞的生物学行为及其确切机制,寻求抑制细胞成骨样转化的方法是防治血管钙化的重要思路。

2 ECs

ECs是血管内膜的主要组成,是血管壁抵御血清刺激(如炎症因子、尿毒症毒素、磷等)的第一道屏障,其完整性的维持对于血管壁稳态至关重要。研究表明,ECs在血管钙化中发挥重要作用:首先,ECs是血管钙化细胞的来源之一。在炎症等刺激因素下,ECs经历内皮-间充质转化(endothelial-mesenchymal transition, EndMT),进而获得多种分化潜

能,迁移和侵袭能力增加,黏附功能下降^[40,41]。EndMT是ECs获得骨/软骨表型的重要机制^[42,43]。TNF α /IL-6可诱导人主动脉ECs发生EndMT,下调BMPR2,通过JNK信号增强BMP9诱导的成骨^[44]。甲状旁腺激素(parathyroid hormone, PTH)可诱导尿毒症大鼠ECs发生EndMT,促进血管钙化的发生^[45]。高糖可诱导人主动脉ECs通过EndMT转化为软骨细胞^[46]。在机械应激刺激下,ECs通过上调BMP2表达,调节血管间质细胞中成骨因子的表达,促进血管钙化^[47]。其次,ECs还可以合成和分泌多种生物活性物质以自分泌/旁分泌的形式参与血管钙化。硫化氢(H₂S)是由ECs内胱硫醚裂解酶(cystathionine- γ -lyase, CSE)催化产生的气体信号分子,可减轻磷酸盐诱导的VSMCs钙化^[48]。由ECs合成和分泌的甲状旁腺激素相关肽(parathyroid hormone-related protein, PTHrP)可通过激活BMP2信号参与血管钙化^[49]。内皮素(endothelin, ET)可通过ET受体激活成骨细胞前体细胞钠依赖性磷转运体,诱导其成骨样转化^[50]。重要的是,ECs来源的胞外囊泡(extracellular vesicles, EVs)可介导细胞间通信参与血管钙化的发展。高糖刺激脐静脉ECs释放大量的Notch3的EVs,通过mTOR通路促进VSMCs钙化和衰老^[51]。衰老的ECs可分泌大量富含钙离子和BMP2的EVs^[52],不仅作为钙化的成核位点,还可促进VSMCs成骨样转化和钙化,介导毗邻细胞早衰。

ECs通过分泌多种生物活性物质和EVs,以及机械传导血流动力学变化等在血管钙化中发挥重要作用,深入阐明ECs在不同病理刺激下功能变化,进一步揭示ECs和VSMCs之间的联系有助于血管钙化的防治。

3 自噬

自噬是一种细胞自我消化过程,通过自噬溶酶体隔离并降解损伤细胞器和蛋白质等成分,从而优化细胞能量供应和细胞器的自我更新^[53]。自噬是血管钙化的内源性保护机制之一,在磷酸盐诱导的VSMCs钙化模型和CKD大鼠模型中,高磷诱导活性氧(reactive oxygen species, ROS)产生,促进自噬发生,进而通过减少MV的释放抑制钙化^[27]。在高磷饲料喂养的DBA/2小鼠中,也观察到自噬水平增加,抑制自噬则加剧VSMCs钙化,而使用雷帕霉素进一步诱导自噬则抑制VSMCs成骨转化,

缓解血管钙化^[54]。此外, 阻断自噬可显著促进高磷诱导的 ECs 凋亡^[28]。上述结果表明, 自噬实际上是高磷诱导钙化背景下机体的适应性反应, 通过调节 VSMCs 成骨样转化、凋亡和矿化 MVs 释放等活动影响钙化进程。CKD 血管内自噬诱导或维持存在缺陷是明确的, 但不同条件下自噬反应的结果是自适应、促凋亡还是促进 MVs 释放, 仍需进一步研究。

线粒体自噬是自噬的特殊形式, 受损线粒体被运送到溶酶体进行降解。依赖 PINK1/Parkin 信号或线粒体外膜受体 (如 FUNDC1、BNIP3、BCL2-L13 等) 介导的线粒体自噬可通过特异性吞噬降解去极化的损伤线粒体, 参与线粒体稳态维持, 可防止线粒体 DNA 损伤积累、ROS 蓄积和细胞死亡, 在血管钙化中发挥重要作用^[55, 56]。糖酵解产物乳酸可通过 BNIP3 介导的线粒体自噬缺陷诱导氧化应激和凋亡, 促进 VSMCs 成骨样转化和钙化^[57]。由此可见, 线粒体自噬有助于清除钙化 VSMCs 中功能失调的线粒体, 抑制血管钙化的发生。进一步利用 PINK1 敲除小鼠进行研究将有助于明确线粒体自噬在血管钙化中的直接作用。

4 线粒体稳态失衡

线粒体作为细胞能量代谢的中心, 除供能外还广泛参与脂质代谢、钙调控、ROS 生成以及细胞自噬与凋亡, 线粒体稳态的维持对于细胞稳态至关重要。

线粒体生物发生可通过调节线粒体功能和 ATP 水平影响 VSMCs 表型转化。有研究表明: 二甲双胍可通过减轻 β -甘油磷酸盐诱导的线粒体生物发生受损, 抑制丙酮酸脱氢酶激酶 (pyruvate dehydrogenase kinase, PDK) 介导的 VSMCs 氧化应激和凋亡, 进而降低 VSMCs 成骨样转化和钙化^[58]。过氧化物酶体增殖物激活受体 γ 共激活因子 1 α (peroxisome proliferator-activated receptor γ coactivator 1 α , PGC1 α) 在线粒体生物发生中发挥关键作用, 可通过激活 Sirt3 抑制线粒体 ROS 水平, 减轻血管钙化的发生^[59]。上述研究表明, 线粒体生物发生障碍可以影响 VSMCs 表型转化及钙化。

线粒体分裂/融合是线粒体稳态调节最重要的方式, 裂变过程有助于清除损伤线粒体, 融合过程则有助于线粒体修复、信息传递和细胞内能量重分配^[60]。然而, 过度分裂则使线粒体碎片化, 引起氧

化应激, 破坏线粒体基因组、呼吸功能和 ATP 的产生, 促进疾病的发生。动力相关蛋白 1 (Dynamin-related protein 1, Drp1) 是调控线粒体分裂的关键蛋白, Drp1 在线粒体分裂蛋白 1 (fission 1, FIS1) 的招募下从胞质转位至线粒体外膜并组装成环是诱导线粒体分裂的关键事件^[61]。在高磷诱导的钙化过程中, Drp1 表达增加, 线粒体碎片化, ROS 水平升高, 导致凋亡事件和钙化的发生, 而阻断 Drp1 则有助于减少线粒体分裂和氧化应激损伤, 抑制钙化^[62]。在人颈动脉钙化组织中 Drp1 高表达, 抑制 Drp1 可减弱人 VSMCs 线粒体功能障碍, 细胞骨架重排, 并降低 ALP 活性, 抑制基质矿化和血管钙化; 在前蛋白转化酶枯草杆菌蛋白酶/kexin 9 (proprotein convertase subtilisin/kexin type 9, PCSK9) 功能获得性动脉粥样硬化模型中, Drp1 缺失对小鼠血管钙化无影响, 然而, 当氧化应激诱导钙化时, 抑制 Drp1 则抑制了钙化的发生^[63], 提示抑制 Drp1 减轻血管钙化或需要氧化应激的参与。此外, Drp1 依赖的线粒体过度分裂往往伴随着细胞凋亡的发生, 促进细胞色素 C 和凋亡诱导因子的释放, 而细胞凋亡调控因子 Bax 也可以与裂变相关蛋白共同调控线粒体分裂^[64]。

由此可见, 线粒体稳态失衡是血管钙化的重要机制之一, 线粒体稳态调节的诸多方式之间是深入交叉的, 涉及氧化应激、自噬、凋亡、糖脂代谢紊乱等多个生物学过程, 这些过程之间的交互对话与调控机制及干预靶点尚不明确, 仍需进一步探索。

5 代谢重编程

几十年前, Warburg 发现即使在氧气充足的条件下, 肿瘤细胞内的糖酵解速率仍在增加^[65], 这种所谓的 Warburg 效应最初被认为是为有氧呼吸损伤引起的, 但现阶段研究认为它是细胞为了满足快速增殖的需求而主动选择的代谢程序。除肿瘤细胞外, 有氧条件下糖酵解速率增加的糖代谢重编程在非恶性细胞中也普遍存在, 如活化的巨噬细胞、骨骼肌细胞或淋巴细胞^[66–68]。越来越多的研究表明, 代谢重编程是决定细胞表型和信号转导的关键因素。

葡萄糖分解代谢 (线粒体氧化磷酸化和糖酵解) 是细胞产能的主要途径, VSMCs 也可以通过调节细胞代谢途径适应转分化过程中的能量需求转变 (图 1), 而且越来越多的证据表明, 线粒体呼吸和糖酵解的代谢转变与调控 VSMCs 成骨样转分化和

血管钙化信号有关^[69]。值得注意的是,即使在氧气充足的情况下,VSMCs也依赖糖酵解供能,VSMCs中乳酸产量等于甚至大于摩尔耗氧量。有两个假说解释了这一现象^[70]:(1)糖酵解和氧化磷酸化为VSMCs不同生物学行为提供能量,氧化代谢与平滑肌细胞等长收缩密切相关,而糖酵解与钠钾转运过程相关^[71, 72]。(2)糖酵解可提供大量L-乳酸,L-乳酸是增强线粒体呼吸储备能力的底物,可以加强细胞对能量危机的抵抗能力,并延长存活时间。此外,有氧酵解可以更高效地代谢葡萄糖,葡萄糖产生乳酸的速度比线粒体中葡萄糖完全氧化的速度快10~100倍,当ATP危机时细胞可以快速激活有氧酵解提供能量^[73]。

大多数研究表明,有氧糖酵解是VSMCs成骨样转分化的特征之一。高磷诱导下VSMCs线粒体功能障碍,主要表现为线粒体膜电位降低,电子传递链效率下降致使ROS累积^[74],ATP相关呼吸、

备用呼吸及最大呼吸能力降低,ATP产量下降^[75],促进高磷诱导下VSMCs成骨样转化,细胞凋亡增加,产生富含促钙化物质的MVFs,进而促进血管钙化。研究表明,钙化VSMCs葡萄糖摄取增加,糖酵解相关限速酶水平增高^[75],更倾向于无氧糖酵解供能,导致乳酸堆积。丙酮酸脱氢酶激酶4(pyruvate dehydrogenase kinase 4, PDK4)是调节丙酮酸氧化的关键酶,在高磷诱导的钙化VSMCs中PDK4表达增加,糖酵解相关基因表达升高,乳酸生成增多,而敲除PDK4后糖酵解相关基因表达减少,乳酸生成降低,钙化减轻^[76]。在维生素D₃诱导的钙化模型中,骨钙素可以通过低氧诱导因子1 α (hypoxia-inducible factor 1 α , HIF1 α)依赖的方式增加葡萄糖转运和糖酵解水平,并刺激VSMCs成骨分化和钙化^[77]。乳酸是糖酵解的主要代谢产物,可通过Drp1介导的线粒体过度分裂和BNIP3介导的线粒体吞噬缺陷,促进细胞凋亡,最终加速VSMCs成

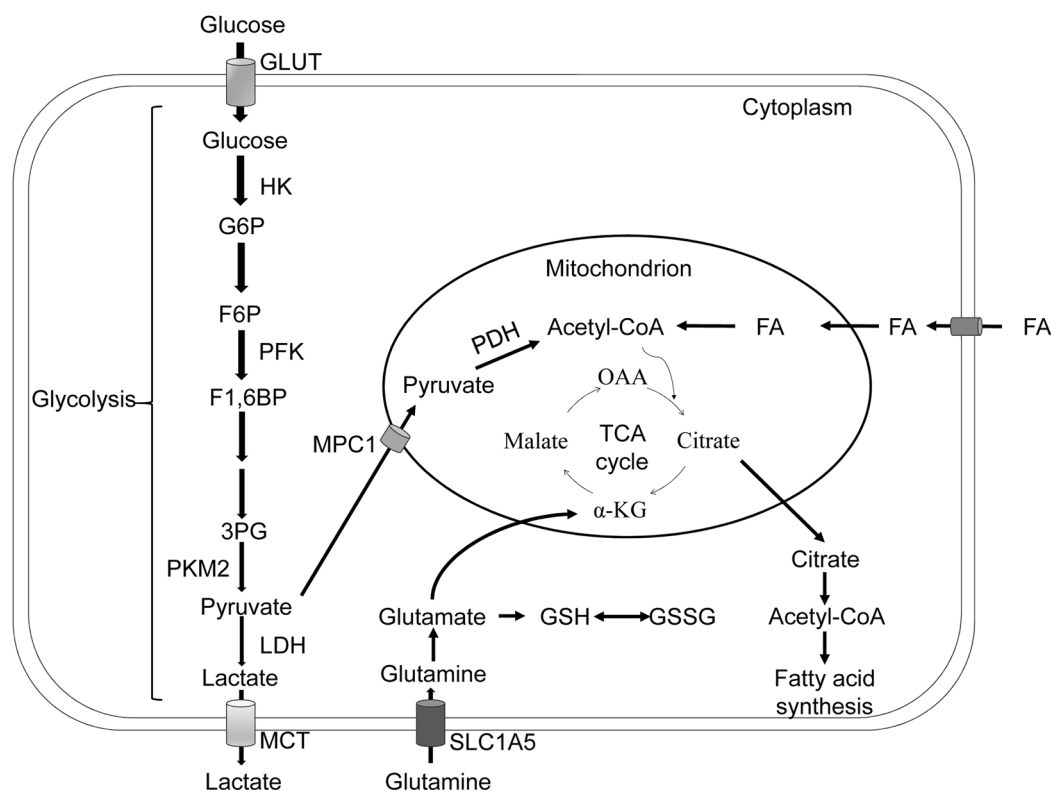


图 1. 血管平滑肌细胞代谢途径

Fig. 1. Metabolic pathways of vascular smooth muscle cells (VSMCs). GLUT, glucose transporter; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; F1,6BP, fructose-1,6-bisphosphate; 3PG, 3-phosphoglycerate; HK, hexokinase; PFK, phosphofructokinase; PKM2, pyruvate kinase isoform M2; LDH, lactate dehydrogenase; MPC1, mitochondrial pyruvate carrier 1; MCT, monocarboxylate transporter; PDH, pyruvate dehydrogenase; OAA, oxaloacetate; FA, fatty acid; GSH, glutathione; SLC1A5, solute carrier family 1, member 5; TCA, tricarboxylic acid; α -KG, α -ketoglutarate.

骨样转化和钙化^[40]。与前述研究结果相反, Alesutan 等的研究表明, 无机磷处理的 VSMCs 线粒体呼吸增加而糖酵解受损, VSMCs 利用长链脂肪酸等氧化底物供能, 线粒体呼吸储备和电子耦合效率降低, 氧化应激增加, 通过线粒体呼吸对应激条件的抵御能力降低, 靶向线粒体呼吸的化合物则可调节 β -甘油磷酸诱导成骨样转化和钙化^[69, 78]。线粒体有氧呼吸不仅提供 ATP 供能, 且三羧酸循环产生的代谢产物可以用于各种生物分子的合成, 电子传递过程中释放的 ROS 还可以调节细胞功能。ROS 水平是线粒体呼吸参与血管钙化的重要媒介。在无机磷诱导的血管钙化过程中, 对线粒体呼吸功能检测显示最大呼吸和备用呼吸水平均下降^[75]。

代谢重编程在血管钙化中的作用是当前研究的热点。葡萄糖代谢在 VSMCs 成骨样转化和钙化过程中的作用是复杂的, 糖酵解 / 有氧氧化的比率变化与线粒体稳态和乳酸积累密切相关, 是 VSMCs 转分化的开关。明确两种代谢状态的差别, 寻找转换靶点有助于进一步理解 VSMCs 转分化和钙化的发生。目前研究主要集中在代谢过程中限速酶的作用, 而代谢产物的研究较少。此外, 脂肪酸和氨基酸代谢重编程在 VSMCs 表型转化中的作用也不应被忽视。

6 ECM 重塑

ECM 由胶原蛋白、弹性蛋白和蛋白多糖等组分构成, 是维持血管壁功能和结构稳定的基础。在血管钙化的过程中, ECM 组成改变并参与血管钙化的调节。弹性蛋白是 ECM 的主要成分, 对于维持动脉壁动力学功能至关重要。弹性蛋白不足、弹性纤维断裂、无序或生化修饰致其完整性受损时, 不溶性胶原蛋白分泌增加并沉积。除此之外, 弹性纤维降解产生的蛋白片段还可以激活下游细胞因子, 促使其进一步降解, 加重 ECM 重构^[79]。胶原蛋白可以结合羟基磷灰石结晶并促进其沉积, 参与 VSMCs 表型调节, I 型胶原和纤维连接蛋白诱导合成型 VSMCs^[80], IV 型胶原和层粘连蛋白则促进收缩型 VSMCs 表型。除组分改变外, VSMCs 对 ECM 的空间结构变化 (力学状态) 也高度敏感。三维培养可维持 VSMCs 收缩表型和组织形态^[81]。

基质金属蛋白酶 (matrix metalloproteinases, MMPs) 属于锌依赖性内肽酶家族, 负责 ECM 的降解或吸收, 在 ECM 重构中发挥重要作用, 与血管钙化关

系密切。研究表明在 CKD 主动脉中 MMP2/9 表达增加, 可通过 Wnt 通路促进 VSMCs 成骨分化和血管钙化^[82], 抑制 MMPs 则可减轻 CKD 大鼠主动脉钙化^[83]。MMPs 介导弹性蛋白降解产生弹性蛋白源性肽, 促进细胞凋亡, 还可刺激成骨转录因子 Cbfa1 和 ALP 表达, 促进 VSMCs 成骨样转化^[84]。除了降解基质作用外, MMPs 还能够与多种细胞因子相互作用促进血管钙化, 如 TGF- β 、TN-C 等。研究发现 MMP-2 可通过上调 TGF- β /BMP-2 促进 VSMCs 钙化^[76, 84]。在弹性蛋白植入钙化大鼠模型中, MMP2 和 TN-C 在钙化部位高表达, MMP 抑制剂 BB-1101 在下调 MMP2 的同时抑制 TN-C 的表达并减轻钙化^[85]。

作为细胞赖以生存的微环境, ECM 各组分在 VSMCs 生存、转分化及转录调控等生物学过程中的作用不应被忽视, ECM 成分及力学特性的研究可能为血管钙化防治提供新的思路。

7 肠道菌群失衡

肠道菌群是影响人类健康的关键因素, 大量研究提出肠道菌群失衡是 CKD 患者发生不良心血管事件的危险因素^[86]。尿毒症毒素进入肠道改变肠道微环境, 造成肠道菌群失衡, 使其对氨基酸及胆碱等物质代谢异常, 引起肠源性尿毒症毒素生成增多, 同时随着肾功能恶化, 肾脏清除受损, 共同加剧了尿毒症毒素的累积和肠道屏障损伤, 形成恶性循环。目前已证实诸多肠道菌群代谢产物与 CKD 血管钙化相关: 氧化三甲胺 (trimethylamine oxide, TMAO) 可激活 NLRP3 和 NF- κ B 信号, 促进 VSMCs 成骨分化和 CKD 大鼠血管钙化^[59, 87]。IS 和对甲酚硫酸盐 (p-cresyl sulfate, PCS) 可抑制 ECs 增殖和损伤修复^[88], 促进 ECs 释放促钙化囊泡^[89]。IS 还可通过 Notch 信号诱导 VSMCs 成骨样分化和凋亡^[90]。动物实验表明, IS 促进高血压大鼠主动脉成骨标志物上调及钙化^[91]。使用限制肠道对菌群代谢物吸收的口服活性炭吸附剂 AST-120 可减少 CKD 患者主动脉钙化^[92]。另外, 肠道屏障功能障碍、肠道菌群失调及易位与 CKD 系统性炎症状态、氧化应激和肾功能恶化相关^[93–95], 加速了血管钙化的发展。

上述结果表明, 这些肠源性尿毒症毒素很可能是潜在的血管毒素, 调节肠道菌群组成和功能有望为 CKD 患者尿毒症毒素、炎症及血管钙化管理提供新的策略。然而, 由于菌群组成的复杂性以及功

能的多样性，对于 CKD 患者应审慎地补充特定益生菌，尤其应充分评估其对骨代谢的影响。

总之，CKD 状态下钙化抑制剂的丢失、钙磷超负荷、炎症反应、肠道菌群紊乱及尿毒症毒素积累构成 CKD 患者促钙化环境，引起细胞自噬功能缺陷、线粒体功能障碍、代谢重编程、氧化应激增强、表观遗传学变化，成骨样转化导致血管钙化；而且，ECs 损伤、钙化 MVs 释放、ECM 重塑和细胞衰老也参与了 CKD 相关的血管钙化的发生和进展 (图 2)。

8 组学研究和血管钙化

需要注意的是，CKD 相关血管钙化过程中所发生的生物学事件在时间和空间上是相互交叉串联的，涉及基因表达、转录调控、蛋白质及代谢等多个层面的变化，寻找不同路径的共同调控靶点是十分必要的。传统组学研究如基因组学、转录组学、蛋白质组学和代谢组学，以及近期的单细胞组学和时空组学利用算法对大数据进行分析与整合以获得对生物体不同生理病理状态下的全面理解。

基因组学是对生物体的所有 DNA 序列进行系

统研究，通过识别基因组变异有助于筛选与疾病相关的候选基因，包括单核苷酸多态性 (single nucleotide polymorphism, SNP) 以及结构变异。全基因组关联分析 (genome-wide association study, GWAS) 在人类基因组中识别了多个与血管钙化相关的 SNP 位点，如慢性肾脏病人群中与 ABCA4 基因毗邻的 rs3789422^[96]。与冠状动脉疾病及心肌梗死相关的 SNP 风险位点也与血管钙化高度相关，如 rs4977574, rs3825807 及 rs12526453 等^[97]。外显子测序在基因和表型的关联性方面具有更强的优势，研究已证实 SMAD6 与 PCSK9 等与血管钙化相关^[98, 99]。表观基因组学旨在描绘染色质组装机动力学以及更高级的染色质结构，这些改变在不影响 DNA 序列的情况下产生可遗传且可逆的遗传信息表达变化，如 DNA 甲基化，非编码 RNA 以及组蛋白修饰等。尿毒症环境干扰了机体基因调控机制，驱动了基因组甲基化改变，接受肾移植或透析的患者与健康人群比较在血管钙化、炎症以及细胞衰老相关功能基因出现显著的 CpG 甲基化差异^[100]。IS 则可通过促进 Klotho 基因 CpG 高甲基化抑制 Klotho 表达进而促进大鼠

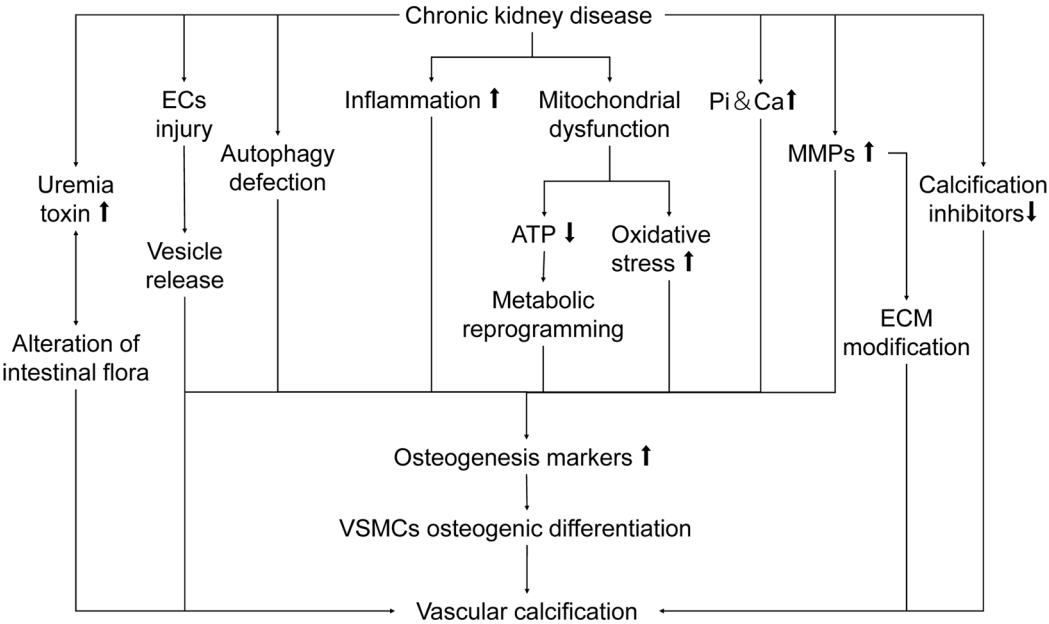


图 2. 慢性肾脏病血管钙化新机制

Fig. 2. New mechanism of vascular calcification in chronic kidney disease (CKD). The loss of calcification inhibitors, calcium and phosphorus (Pi) overload, oxidative stress, inflammation, and intestinal flora alteration constitute a pro-calcifying environment in patients with CKD, leading to defective autophagy, mitochondrial dysfunction, metabolic reprogramming, and epigenetic changes in calcified vascular smooth muscle cells (VSMCs). Additionally, endothelial cells (ECs) injury, calcified matrix vesicles release, extra-cellular matrix (ECM) remodeling and senescence are also involved in the occurrence and development of vascular calcification in CKD. MMPs, matrix metalloproteinases.

主动脉钙化^[101]。

转录组学通过对组织 / 细胞转录出的所有 RNA (mRNA 和非编码 RNA) 进行分析获得全面的转录本信息, 从而进行基因表达水平、转录本结构变异以及新转录本研究, 揭示疾病遗传网络, 寻找生物标志物及干预手段。对人类不同部位粥样硬化动脉的转录组分析显示, 在颈动脉中免疫相关过程优先参与, 而在股动脉中骨发育相关基因被富集使其更易发生骨样钙化^[102]。对维生素 D₃ 联合尼古丁诱导的血管钙化大鼠基因表达谱改变的研究发现, 在钙化早期促钙化基因表达上调, 包括成骨、氧化以及炎症相关基因; 而在钙化后期抑制钙化基因高表达, 包括焦磷酸合成基因、还原和凋亡调节基因等^[103]。高磷诱导下 VSMCs 有 379 个 mRNA 和 728 个 lncRNA 与对照组存在差异表达, 可能与 VSMCs 钙化和成骨分化相关^[104]。有研究通过 RNA-Seq 和生物信息学分析预测了 *circSamd4a* 抑制高磷诱导的大鼠 VSMCs 钙化, 并通过实验验证了 *circSamd4a*-miRNA-mRNA 轴参与血管钙化, 为血管钙化的干预提供了新的思路^[105]。

作为基因和转录组学的补充, 蛋白质组学和代谢组学对于确定某种蛋白质 / 代谢物结构、功能及其与疾病变化的关系提供了更为直接的证据。对于体液的组学研究筛选出了许多与血管钙化相关的蛋白和代谢物, 如纤维蛋白原、载脂蛋白 C-II、抗凝血酶 III 以及代谢物琥珀酸和丙氨酸等, 有助于血管钙化的筛查和管理^[106]。对 IS 和 PCS 处理的 CKD 大鼠钙化动脉的蛋白质组学研究发现, IS 和 PCS 暴露激活主动脉炎症和凝血通路, 直接促进了血管钙化, 并与糖稳态受损密切相关^[107]。检测高糖诱导下钙化人脐静脉 ECs 外泌体蛋白谱发现 *versican* 通过调节线粒体功能参与高糖诱导的 VSMCs 钙化和衰老^[108]。对血液样本中的代谢物检测发现, 循环 3-羟基丁酸脂水平升高与颅内颈动脉钙化相关^[109]。一项联合体内外脂质组学分析和 shRNA 文库筛选的研究发现, 饱和脂肪酸 1,2-二硬脂酰-PA 介导了钙化 VSMCs 饱和脂肪酸的脂毒性和血管钙化的发展^[110]。对 VSMCs 蛋白组学和代谢组学的研究发现 PKC δ 在调节糖脂代谢、细胞氧化还原状态和维持 VSMCs 分化中发挥重要作用^[111]。代谢组学与原发疾病高度相关, 目前在血管钙化中的研究较少, 联合多组学分析或能提供更多的信息。

组学研究在血管钙化中的应用仍处于起步阶

段, 组学研究有助于鉴别血管钙化过程中钙化细胞的来源和组织类型, 绘制疾病分期。利用组学数据分析方法, 有助于收集和整合不同时空下的生物信息, 促进治疗靶点的发现^[112]。但目前主要运用算法对组学数据加以分析, 尚缺乏强有力的实验证据; 且在不同种族、疾病背景等情况下机体基因表达、蛋白质修饰以及代谢改变存在巨大差异, 使各组学分析数据复现性不佳。探讨如何提高组学复现性以及大数据整合与分析算法, 深入研究重复性好的生物分子与血管钙化的联系可能为干预血管钙化提供有效的手段。

9 肌肉细胞因子 Irisin 与 CKD 相关的血管钙化

肌肉细胞因子 Irisin 是 2012 年被发现的具有 112 个氨基酸残基的多肽, 主要由骨骼肌细胞中的前体分子 III 型纤连蛋白结构域 5 (fibronectin type III domain-containing protein 5, FNDC5) 剪切释放入血液到达靶器官发挥生理作用。研究发现, 运动过程中肌肉的收缩运动可诱导骨骼肌细胞分泌 PG-C1 α , 激活的 PGC-1 α 促进其下游分子 FNDC5 的表达, 从而增加血液中 Irisin 的含量^[113]。

近年来, 本课题组对 Irisin 在肾脏疾病及其并发症中的作用进行了较系统研究: 我们发现慢性肾衰竭血液透析患者血清 Irisin 水平显著下降, 与增加的心脑血管疾病死亡风险独立相关^[114]; 在慢性肾衰竭小鼠的骨骼肌组织中 Irisin 前体基因 FNDC5 表达显著下调, 尾加压素 II 介导慢性肾衰竭骨骼肌萎缩, 与其促进骨骼肌细胞自噬、抑制 Irisin 表达有关^[115]; 在 Irisin 与 CKD 相关血管钙化方面我们做了深入研究, 临床研究发现低水平的 Irisin 与血液透析及腹膜透析患者主动脉钙化独立相关^[50, 116]。动物及细胞实验证实: Irisin 显著减轻 CKD 小鼠主动脉钙化; 拮抗高磷介导的小鼠及人 VSMCs 钙化和成骨样转化, 减轻高磷介导的 VSMCs 线粒体结构和功能损伤, 维持细胞 ATP 水平, 抑制 VSMCs 凋亡, 以上作用依赖 AMPK/Drp1 信号通路的激活^[117]。此外, 我们还发现, 高磷抑制 VSMCs 自噬并促进焦亡, Irisin 通过活化 VSMCs 自噬和抑制 NLRP3-caspase 1 途径介导的细胞焦亡而改善 CKD 相关的血管钙化^[118]。

10 总结

血管钙化与 CKD 患者高心血管疾病发病率和

死亡率密切相关,深入研究血管钙化调控机制,寻找合适的干预措施对于减轻CKD患者血管钙化负担是十分必要的。CKD促钙化环境(尿毒症毒素,钙化抑制剂减少,氧化应激,炎症状态,肠道菌群失调等)介导VSMCs成骨样转分化、ECs损伤、线粒体功能障碍、代谢重编程、ECM重塑等新机制在CKD相关血管钙化发生和发展中发挥了重要作用。组学研究在筛选研究目标、评估干预手段以及在不同层次完整理解血管钙化方面具有独特优势,是一种高效的研究技术。肌肉细胞因子Irisin通过激活VSMCs细胞AMPK信号抑制线粒体分裂,活化VSMCs自噬和抑制NLRP3-caspase 1途径介导的细胞焦亡从而改善高磷介导的VSMCs钙化,抑制CKD血管钙化,是极具潜力的治疗CKD相关血管钙化有效生物分子。期望这些血管钙化发生的新机制,为CKD相关的血管钙化防治带来新的曙光。

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