

自噬在低剪切力诱导动脉粥样硬化发生发展中的作用

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摘 要

动脉粥样硬化(AS)是动脉壁的一种慢性炎症性疾病, 发病机制复杂。在动脉血管腔中, 动脉粥样硬化斑块的分布与血管壁局部剪切力有很大关系, 剪切力低的区域易于产生动脉粥样硬化斑块。自噬是真核细胞的一种高度保守、普遍存在的溶酶体运输途径, 通过清除细胞内受损物质来维持细胞稳态, 自噬功能障碍也会导致动脉粥样硬化。低剪切力可诱导内皮细胞自噬功能受损, 从而导致动脉粥样硬化病变。现就低剪切力通过自噬促进动脉粥样硬化发生发展的研究现状作一综述。

关键词

动脉粥样硬化, 剪切力, 自噬, 内皮细胞

Role of Autophagy in the Occurrence and Development of Atherosclerosis Induced by Low Shear Stress

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Abstract

Atherosclerosis (AS) is a chronic inflammatory disease of the arterial wall, its pathogenesis is complex. In the vascular lumen, atherosclerotic plaques are not randomly distributed, which has a

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lot to do with the shear stress generated by the blood flow in the artery, and atherosclerotic plaques develop preferentially in areas where shear is low. Autophagy is a highly conserved and ubiquitous lysosomal transport pathway in eukaryotic cells, which maintains cellular homeostasis by removing damaged substances in cells. Dysfunction of autophagy can also lead to atherosclerosis. Low shear stress might induce endothelial cell autophagy impairment, therefore contributing to atherosclerotic lesions. This article reviews the research status of low shear stress promoting the occurrence and development of atherosclerosis through autophagy.

Keywords

Atherosclerosis, Shear Stress, Autophagy, Endothelial Cell

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1. 引言

动脉粥样硬化是脉管系统的多因素疾病, 其中, 剪切力(Shear stress, SS)是这一病理过程的关键调节因素之一[1]。越来越多的证据表明, 剪切力对于调节动脉粥样硬化的发生和发展以及维持血管的正常生理功能至关重要[2] [3]。基础自噬对于维持细胞稳态至关重要, 自噬功能障碍被证明会导致多种心血管疾病, 其中包括动脉粥样硬化[4] [5]。在某些情况下, 自噬的激活已被确定为具有心脏保护作用[6]。自噬在低剪切力(Low shear stress, LSS)诱导的内皮细胞损伤和动脉粥样硬化斑块的形成中起着关键作用[7] [8], 其分子机制研究也取得了一些进展。本文将总结现有文献, 梳理自噬在低剪切力诱导动脉粥样硬化形成中分子机制的研究进展, 以期对动脉粥样硬化的自噬靶向治疗提供更多的理论依据。

2. 剪切力与动脉粥样硬化

剪切力(Shear stress)是由血流在内皮表面施加的摩擦力[9], 在血管稳态的维持和紊乱中起着至关重要的作用。作用在内皮细胞上的剪切应力的大小和模式随血流、血液粘度和血管形状等因素而变化[10]。1 和 2.5 Pa (10 和 25 dyn/cm²)之间的剪切力称为层流剪切力或脉动剪切力(Pulsatile shear stress, PSS), 高于此水平的剪切力, 称为高剪切力(High shear stress, HSS), 通常位于血管分叉的内侧壁即分叉脊部位。低于此水平的剪切力称为低剪切力(Low shear stress, LSS)或振荡剪切力(Oscillating shear stress, OSS) [8], 多位于血管分叉的外侧壁及弯曲动脉的内侧壁。其中稳定的层流剪切力, 可促进内皮细胞(Endothelial cells, ECs)有序紧密排列, 抑制凝血、白细胞渗出和平滑肌细胞增殖, 被认为具有动脉粥样硬化保护作用[11], HSS 通过防止内皮炎症, 衰老和凋亡以及促进内皮在流动方向上的对齐来防止动脉粥样硬化[12], 而 LSS 则通过诱导内皮细胞表型变化(例如, 炎症、氧化应激、内质网应激、细胞凋亡、自噬、内皮-间充质转化、内皮通透性、表观遗传调节和内皮代谢适应)在动脉粥样硬化的发生和进展中起着重要作用[13]。因此, 高剪切力和层流剪切力被称为生理剪切力, 具有动脉粥样硬化保护作用[14], 低剪切力则起相反作用[15]。有临床研究表明[16], 冠状动脉粥样斑块主要位于血管分叉的外侧壁即 LSS 部位, 而相当于分流器的具有较高剪切力的分叉脊部位极少有斑块形成, 提示 LSS 区域是粥样斑块的“发源地”和重灾区。除了粥样硬化斑块的形成与 LSS 有关, 甚至斑块本身的 LSS 也是冠心病患者临床不良事件的独立预测因子

[17]。另一项临床研究表明[18], 矫正了药物因素后, LSS 血管段依然保持着和冠状动脉粥样硬化斑块进展与管腔丢失显著的相关性。总得来说, LSS 具有促进脉粥样硬化发生发展的作用。

3. 自噬与动脉粥样硬化

自噬是一种分解代谢过程, 维持细胞正常的生理循环, 并错综复杂地参与饥饿期间营养物质的代谢, 细胞内物质的更新及细胞的生存[19]。目前已经确定的自噬类型有三种, 分别为宏观自噬、微自噬和分子伴侣介导的自噬[20]。本文主要阐述的宏观自噬, 即传统意义上的自噬, 也是最普遍和研究最深入的自噬形式。它大致分为 4 个过程, 即自噬启动、自噬体延伸、自噬体成熟及自噬溶酶体形成[21]。自噬通量(也称自噬流)包括两个步骤, 分别为自噬体和溶酶体融合形成自噬溶酶体, 以及自噬溶酶体中的成分被溶酶体水解酶降解[22]。自噬主要由自噬相关基因(Autophagy related gene, Atg)介导调控, 其中 Atg8 蛋白在自噬膜上经历一种独特的类泛素化修饰与磷脂酰乙醇胺偶联, 这是自噬小体形成必不可少的过程, 对自噬膜的延伸也至关重要[23]。LC3s (包括 LC3A、LC3B、LC3C) 是 Atg8 家族中的一个亚家族, 是自噬小体和自噬的主要标记物[24], 在细胞内有两种存在形式, LC3-I 和 LC3-II。生理情况下, LC3 以 LC3-I 的形式存在, 在自噬被激活的情况下, LC3-I 与磷脂酰乙醇胺偶联形 LC3-II, 自噬通量通常是根据 LC3-II 水平来推断的[21]。p62/SQSTM1 (以下简称 p62) 是哺乳动物中最具特征的自噬底物, 它通过 LC3 相互作用区(LIR)直接与 Atg8 同源物相互作用, 将特定的细胞器和蛋白质聚集体递送到自噬体以进行降解[25]。因此 LC3 水平与自噬的进程相关, p62 水平的积累与自噬通量受损有关[26]。然而, 过度或不足水平的自噬通量会导致心血管疾病的病理变化[27] [28]。在动脉粥样硬化进展过程中, 自噬标志物 LC3II 水平在这一过程中升高, p62 则表达上调, 表明自噬通量在动脉粥样硬化病变的形成和进展过程中受损[22]。来自老年小鼠和人类受试者的数据显示, EC 自噬受损与内皮依赖性血管舒张反应明显减弱有关。与这种效应相吻合的是, 已经证明自噬缺陷会促进内皮 ROS 和炎性细胞因子的增加, 提示自噬可能部分通过 NO 依赖性途径调节血管稳态[29]。此外, ECs 自噬缺陷会导致低密度脂蛋白(LDL)积累[30], miR-216a 的过表达可以通过 ox-LDL 积累增加动脉粥样硬化的早期斑块形成[31], miR-126 可通过抑制 PI3K/Akt/mTOR 通路恢复自噬通量来减轻 ox-LDL 诱导的 HUVECs 损伤, 起抗动脉粥样硬化作用[32]。而 caveolin 1 (Cav-1) 则可通过调节脂筏中 ATG5-ATG12 复合物分布影响自噬, 对动脉粥样硬化起保护作用[33]。最新研究发现[34], 硫化氢(H₂S)诱导的自噬通过激活 Sirt1 来保护 ECs 免受 Ox-LDL 诱导的细胞凋亡, 从而延缓动脉粥样硬化的发生发展。目前已证明, 能够通过调节自噬来改善内皮功能的药物, 有雷帕霉素, 二甲双胍, 海藻糖, miR-100, 辛伐他汀, 白藜芦醇、SRT1720 和烟酰胺单核苷酸等多酚[35]。总体而言, 自噬在调节动脉粥样硬化形成和动脉粥样硬化斑块稳定性中起着重要作用, 并可能为动脉粥样硬化的治疗提供新的机会。

4. 低剪切力与自噬

有体内研究发现[36], LSS 显著提高了 LC3II/LC3I 比值和自噬底物 p62 水平, 而 HSS 仅显著增加了 LC3II/LC3I 比值并降低了 P62 表达。也有研究表明[19] [22], 与生理剪切力相比, 暴露于 LSS 后, LC3II/LC3I 的水平显著降低, 自噬底物 p62 的水平增加。在小鼠颈动脉结扎模型中, 也发现 p62 聚集在动脉内皮层的 LSS 区域[9]。这表明 LSS 可能导致内皮细胞自噬通量受损, 从而导致动脉粥样硬化病变。HSS 下由转录因子 KLF2 和 KLF4 以及 SIRT1 激活 FoxO1 介导内皮细胞自噬通量上调, 而 LSS 抑制 AMPK 和激活 mTOR 通路, 阻断了自噬通量[19]。Ding 等人发现[37], LSS 是 LOX-1 表达、自噬和 ROS 生成的强大刺激因子。此外, 最新的研究表明[38], LSS 通过 Cav-1/miR-7-5p/SQSTM1 信号通路抑制内皮细胞线粒体自噬, 进而导致线粒体稳态受损及 EC 功能障碍。

5. 自噬介导低剪切力导致动脉粥样硬化的分子机制

5.1. 低剪切力介导的自噬减少了内皮细胞一氧化氮的生成

内皮型一氧化氮合酶(Endothelial nitric oxide synthase, eNOS)产生的一氧化氮(Nitric oxide, NO)是调节剪切力介导的内皮功能和血管张力的关键信号分子,但在动脉粥样硬化的条件下,eNOS变得不稳定和不耦合,表现为mt的产生而不是NO[9]。研究发现[19],在LSS作用下,自噬通量受损,eNOS解耦联,eNOSThr495的磷酸化,导致内皮细胞爆增,伴随着NO释放的减少。Leena P等人研究发现[39],EC自噬受损会损害EC糖酵解,ATP产生以及P2Y1-R通过PKC δ 介导的eNOS信号传导,最终减少剪切力诱导的NO产生。另外,自噬损伤会损害剪切应力诱导的eNOS Ser1177磷酸化,减少NO生物利用度[40]。此外,Zhang等人发现[22],低剪切应力通过下调TET2表达损害内皮细胞自噬,TET2下调又减弱了eNOS表达并刺激了内皮素-1(ET-1)表达。NO的释放减少和ET-1的产生增加是内皮功能障碍的标志,而内皮细胞功能障碍是动脉粥样硬化的第一步。

5.2. 低剪切力介导的自噬损伤了内皮细胞的抗氧化应激功能

有研究表明[36][41],LSS介导的氧化应激和JNK活化诱导了自噬,但自噬通量受损,促进了线粒体产生、线粒体DNA损伤和线粒体功能障碍,从而启动动脉粥样硬化。Anne等人发现[15],LSS区域有缺陷的内皮自噬诱导受损线粒体的积累,这导致线粒体活性氧(ROS)的形成增加,并最终导致细胞凋亡,衰老和炎症。Chen J等人研究发现[42],在LSS下,TET2表达下调,TET2的缺失通过减少组蛋白去乙酰化酶2的募集而导致线粒体呼吸复合物II亚基琥珀酸脱氢酶B(SDHB)的表达和活性上调。SDHB的过表达介导了线粒体损伤并增加了ROS的产生。Lu等人研究发现[43],与HSS相比,在LSS条件下,转录因子EB(TFEB)的表达下调。而TFEB是自噬和溶酶体融合的主要调节因子,TFEB在内皮细胞中的过表达可降低ROS的浓度,并增加了抗氧化基因HO-1和SOD2的表达,进而增强EC抗氧化应激功能起动脉粥样硬化保护作用。

5.3. 低剪切力介导的自噬促进内皮细胞炎症反应

动脉粥样硬化是由血管壁长期过度炎症引起的。血管内皮细胞长期暴露于炎症因子可导致内皮细胞的通透性增加,从而导致脂质进入的可能性增加并加重动脉粥样硬化的发生发展。已有研究发现PCSK9(Proprotein convertase subtilisin/kexin type 9)的过表达会增加动脉粥样硬化斑块的大小,它可能通过其促炎作用发挥促动脉粥样硬化的作用。低剪切力诱导PCSK9在血管ECs中的表达。同时,PCSK9可调控自噬的发展,在促炎环境中,低浓度的PCSK9可能通过激活ROS-ATM-LKB1-AMPK轴诱导自噬,高浓度的PCSK9激活细胞凋亡。自噬和细胞凋亡的平衡决定了细胞死亡,并在动脉粥样硬化起关键作用[11]。Santovito等人发现,在LSS自噬通量受损的条件下,miR-126-5p入核减少,从而使得其下游半胱天冬酶3表达上调加重内皮细胞凋亡和动脉粥样硬化[44]。Meng Q等人发现[2],HSS对内皮细胞炎症的抑制取决于HMGB1通过激活LOC107986345/miR-128-3p/EPHB2轴的核转移引起的自噬活化。HMGB1是一种无处不在的核蛋白,当细胞被激活或受损时,HMGB1会转移到细胞质或细胞外空间,细胞质HMGB1取代BECN1的Bcl-2,直接与BECN1结合诱导自噬体形成。Molica等人发现[45],LSS诱导的自噬通量减少可能通过Panx1通道增强ATP释放,ECs释放ATP以响应剪切力的变化,其随后调节炎症反应,从而平衡动脉粥样硬化中的白细胞募集。Yuan P等人发现[1],剪切力通过内皮细胞自噬调节YAP活性。在小鼠动脉粥样硬化低剪切力区域发现了核YAP的增加和磷酸化YAP的降低。LSS则可通过整合素 $\alpha 5\beta 1$ 及其下游激酶c-Abl的活化诱导YAP核易位,增强JNK活性,有利于炎症和动脉粥样硬化的发生。YAP

是一种自噬底物, 可以充当机械刺激的传感器和信号放大器, 是动脉粥样硬化的关键因素。SIRT1 和自噬可以限制 YAP 激活, 以防止动脉粥样硬化。此外, 剪切力可通过 Rac1-rab7-自噬依赖性机制引起内皮 KLF2 过表达, 从而对血管内皮起保护作用[46]。最新的研究发现[47], 层流剪切力激活 PIEZO1, 通过 Ca^{2+} /CaMKII/MEKK3/ERK5 轴诱导 KLF2/4 表达, 从而抑制 NF- κ B 信号通路以提供抗炎作用并维持内皮稳态。Emir 等人研究也发现[48], 在 LSS 区域周围, KLF2 表达明显降低, 使这些区域容易发生动脉粥样硬化。KLF2 和 KLF4 是机械敏感转录因子, 由层流剪切力诱导, 但在 LSS 下表达下降, 并通过调节多个抗炎基因(如 eNOS 和 THBD)的表达来维持内皮稳态, 起血管保护作用[47] [49]。

6. 问题与展望

动脉粥样硬化是一个复杂的多组分过程, 包括脂质代谢紊乱, 炎症, 氧化应激, 自噬障碍等。在动脉粥样硬化发生发展过程中自噬功能障碍不是因为自噬体形成中断, 而是溶酶体介导的货物降解中断, 也就是自噬通量的受损。在不同剪切力的介导下, 自噬通量有所改变, 从而在动脉粥样硬化的发生发展中起到促进或抑制作用。目前剪切力与内皮自噬之间的关系已被广泛研究, 但干预自噬来治疗动脉粥样硬化的研究还处在初期阶段, 仍需要继续开展分子和基因层面的系统研究。如何精确调节自噬通量治疗动脉粥样硬化, 为开发临床药物提供新的思路, 值得我们不断探索及思考。

参考文献

- [1] Yuan, P., Hu, Q., He, X., *et al.* (2020) Laminar Flow Inhibits the Hippo/Yap Pathway via Autophagy and sirt1-Mediated Deacetylation against Atherosclerosis. *Cell Death & Disease*, **11**, Article No. 141. <https://doi.org/10.1038/s41419-020-2343-1>
- [2] Meng, Q., Pu, L., Qi, M., *et al.* (2022) Laminar Shear Stress Inhibits Inflammation by Activating Autophagy in Human Aortic Endothelial Cells through hmgb1 Nuclear Translocation. *Communications Biology*, **5**, Article No. 425. <https://doi.org/10.1038/s42003-022-03392-y>
- [3] Souilhol, C., Serbanovic-Canic, J., Fragiadaki, M., *et al.* (2020) Endothelial Responses to Shear Stress in Atherosclerosis: A Novel Role for Developmental Genes. *Nature Reviews Cardiology*, **17**, 52-63. <https://doi.org/10.1038/s41569-019-0239-5>
- [4] Poznyak, A.V., Nikiforov, N.G., Wu, W.K., *et al.* (2021) Autophagy and Mitophagy as Essential Components of Atherosclerosis. *Cells*, **10**, Article No. 443. <https://doi.org/10.3390/cells10020443>
- [5] Sun, L., Zhao, M., Liu, A., *et al.* (2018) Shear Stress Induces Phenotypic Modulation of Vascular Smooth Muscle Cells via ampk/mtor/ulkl-Mediated Autophagy. *Cellular and Molecular Neurobiology*, **38**, 541-548. <https://doi.org/10.1007/s10571-017-0505-1>
- [6] Delbridge, L.M.D., Mellor, K.M., Taylor, D.J., *et al.* (2017) Myocardial Stress and Autophagy: Mechanisms and Potential Therapies. *Nature Reviews Cardiology*, **14**, 412-425. <https://doi.org/10.1038/nrcardio.2017.35>
- [7] Li, L., Dash, D., Gai, L.Y., *et al.* (2016) Intravascular Ultrasound Classification of Plaque in Angiographic True Bifurcation Lesions of the Left Main Coronary Artery. *Chinese Medical Journal (Engl)*, **129**, 1538-1543. <https://doi.org/10.4103/0366-6999.184456>
- [8] Zhu, L., Wang, F., Yang, H., *et al.* (2020) Low Shear Stress Damages Endothelial Function through stat1 in Endothelial Cells (ECS). *Journal of Physiology and Biochemistry*, **76**, 147-157. <https://doi.org/10.1007/s13105-020-00729-1>
- [9] Zhang, J.X., Qu, X.L., Chu, P., *et al.* (2018) Low Shear Stress Induces Vascular eNOS Uncoupling via Autophagy-Mediated eNOS Phosphorylation. *Biochimica et Biophysica Acta—Molecular Cell Research*, **1865**, 709-720. <https://doi.org/10.1016/j.bbamcr.2018.02.005>
- [10] Lin, K., Hsu, P.P., Chen, B.P., *et al.* (2000) Molecular Mechanism of Endothelial Growth Arrest by Laminar Shear Stress. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 9385-9389. <https://doi.org/10.1073/pnas.170282597>
- [11] Ding, Z., Pothineni, N.V.K., Goel, A., *et al.* (2020) Pcsk9 and Inflammation: Role of Shear Stress, Pro-Inflammatory Cytokines, and lox-1. *Cardiovascular Research*, **116**, 908-915. <https://doi.org/10.1093/cvr/cvz313>
- [12] Kheloufi, M., Vion, A.C., Hammoutene, A., *et al.* (2018) Endothelial Autophagic Flux Hampers Atherosclerotic Lesion Development. *Autophagy*, **14**, 173-175. <https://doi.org/10.1080/15548627.2017.1395114>

- [13] He, L., Zhang, C.L., Chen, Q., *et al.* (2022) Endothelial Shear Stress Signal Transduction and Atherogenesis: From Mechanisms to Therapeutics. *Pharmacology & Therapeutics*, **235**, Article ID: 108152. <https://doi.org/10.1016/j.pharmthera.2022.108152>
- [14] Marchio, P., Guerra-Ojeda, S., Vila, J.M., *et al.* (2019) Targeting Early Atherosclerosis: A Focus on Oxidative Stress and Inflammation. *Oxidative Medicine and Cellular Longevity*, **2019**, Article ID: 8563845. <https://doi.org/10.1155/2019/8563845>
- [15] Vion, A.C., Kheloufi, M., Hammoutene, A., *et al.* (2017) Autophagy Is Required for Endothelial Cell Alignment and Atheroprotection under Physiological Blood Flow. *Proceedings of the National Academy of Sciences of the United States of America*, **114**, E8675-E8684. <https://doi.org/10.1073/pnas.1702223114>
- [16] 李丽, 盖鲁粤, 杨庭树, 等. 血管内超声在冠状动脉分叉病变分型中应用的探讨[J]. 中国介入心脏病学杂志, 2009, 17(3): 143-146.
- [17] Stone, P.H., Maehara, A., Coskun, A.U., *et al.* (2018) Role of Low Endothelial Shear Stress and Plaque Characteristics in the Prediction of Nonculprit Major Adverse Cardiac Events: The Prospect Study. *JACC: Cardiovascular Imaging*, **11**, 462-471. <https://doi.org/10.1016/j.jcmg.2017.01.031>
- [18] Hung, O.Y., Molony, D., Corban, M.T., *et al.* (2016) Comprehensive Assessment of Coronary Plaque Progression with Advanced Intravascular Imaging, Physiological Measures, and Wall Shear Stress: A Pilot Double-Blinded Randomized Controlled Clinical Trial of Nebivolol versus Atenolol in Nonobstructive Coronary Artery Disease. *Journal of the American Heart Association*, **5**, e002764. <https://doi.org/10.1161/JAHA.115.002764>
- [19] Hua, Y., Zhang, J., Liu, Q., *et al.* (2022) The Induction of Endothelial Autophagy and Its Role in the Development of Atherosclerosis. *Frontiers in Cardiovascular Medicine*, **9**, Article ID: 831847. <https://doi.org/10.3389/fvrm.2022.831847>
- [20] De Meyer, G.R., Grootaert, M.O., Michiels, C.F., *et al.* (2015) Autophagy in Vascular Disease. *Circulation Research*, **116**, 468-479. <https://doi.org/10.1161/CIRCRESAHA.116.303804>
- [21] Klionsky, D.J., Abdel-Aziz, A.K., Abdelfatah, S., *et al.* (2021) Guidelines for the Use and Interpretation of Assays for Monitoring Autophagy (4th Edition). *Autophagy*, **17**, 1-382. <https://doi.org/10.1080/15548627.2020.1797280>
- [22] Yang, Q., Li, X., Li, R., *et al.* (2016) Low Shear Stress Inhibited Endothelial Cell Autophagy through tet2 Downregulation. *Annals of Biomedical Engineering*, **44**, 2218-2227. <https://doi.org/10.1007/s10439-015-1491-4>
- [23] Shpilka, T., Weidberg, H., Pietrokovski, S., *et al.* (2011) Atg8: An Autophagy-Related Ubiquitin-Like Protein Family. *Genome Biology*, **12**, Article No. 226. <https://doi.org/10.1186/gb-2011-12-7-226>
- [24] Nguyen, T.N., Padman, B.S., Usher, J., *et al.* (2016) Atg8 Family lc3/Gabarap Proteins Are Crucial for Autophagosome-Lysosome Fusion but Not Autophagosome Formation during pink1/parkin Mitophagy and Starvation. *Journal of Cell Biology*, **215**, 857-874. <https://doi.org/10.1083/jcb.201607039>
- [25] Jeong, S.J., Zhang, X., Rodriguez-Velez, A., *et al.* (2019) P62/sqstm1 and Selective Autophagy in Cardiometabolic Diseases. *Antioxidants & Redox Signaling*, **31**, 458-471. <https://doi.org/10.1089/ars.2018.7649>
- [26] Sergin, I., Evans, T.D., Zhang, X., *et al.* (2017) Exploiting Macrophage Autophagy-Lysosomal Biogenesis as a Therapy for Atherosclerosis. *Nature Communications*, **8**, Article No. 15750. <https://doi.org/10.1038/ncomms15750>
- [27] Gatica, D., Chiong, M., Lavandero, S., *et al.* (2015) Molecular Mechanisms of Autophagy in the Cardiovascular System. *Circulation Research*, **116**, 456-467. <https://doi.org/10.1161/CIRCRESAHA.114.303788>
- [28] Bravo-San Pedro, J.M., Kroemer, G. and Galluzzi, L. (2017) Autophagy and Mitophagy in Cardiovascular Disease. *Circulation Research*, **120**, 1812-1824. <https://doi.org/10.1161/CIRCRESAHA.117.311082>
- [29] Gkaliagkousi, E., Lazaridis, A., Dogan, S., *et al.* (2022) Theories and Molecular Basis of Vascular Aging: A Review of the Literature from Vascagenet Group on Pathophysiological Mechanisms of Vascular Aging. *International Journal of Molecular Sciences*, **23**, Article No. 8672. <https://doi.org/10.3390/ijms23158672>
- [30] Torisu, K., Singh, K.K., Torisu, T., *et al.* (2016) Intact Endothelial Autophagy Is Required to Maintain Vascular Lipid Homeostasis. *Aging Cell*, **15**, 187-191. <https://doi.org/10.1111/acer.12423>
- [31] Wang, K., Yang, C., Shi, J., *et al.* (2019) Ox-Ldl-Induced lncrna malat1 Promotes Autophagy in Human Umbilical Vein Endothelial Cells by Sponging mir-216a-5p and Regulating beclin-1 Expression. *European Journal of Pharmacology*, **858**, Article ID: 172338. <https://doi.org/10.1016/j.ejphar.2019.04.019>
- [32] Tang, F. and Yang, T.L. (2018) Microrna-126 Alleviates Endothelial Cells Injury in Atherosclerosis by Restoring Autophagic Flux via Inhibiting of pi3k/akt/mtor Pathway. *Biochemical and Biophysical Research Communications*, **495**, 1482-1489. <https://doi.org/10.1016/j.bbrc.2017.12.001>
- [33] Zhang, X., Ramirez, C.M., Aryal, B., *et al.* (2020) Cav-1 (caveolin-1) Deficiency Increases Autophagy in the Endothelium and Attenuates Vascular Inflammation and Atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **40**, 1510-1522. <https://doi.org/10.1161/ATVBAHA.120.314291>

- [34] Zhu, L., Duan, W., Wu, G., *et al.* (2020) Protective Effect of Hydrogen Sulfide on Endothelial Cells through sirt1-foxo1-Mediated Autophagy. *Annals of Translational Medicine*, **8**, Article No. 1586. <https://doi.org/10.21037/atm-20-3647>
- [35] Mameli, E., Martello, A. and Caporali, A. (2022) Autophagy at the Interface of Endothelial Cell Homeostasis and Vascular Disease. *The FEBS Journal*, **289**, 2976-2991. <https://doi.org/10.1111/febs.15873>
- [36] Li, R., Jen, N., Wu, L., *et al.* (2015) Disturbed Flow Induces Autophagy, but Impairs Autophagic Flux to Perturb Mitochondrial Homeostasis. *Antioxidants & Redox Signaling*, **23**, 1207-1219. <https://doi.org/10.1089/ars.2014.5896>
- [37] Ding, Z., Liu, S., Deng, X., *et al.* (2015) Hemodynamic Shear Stress Modulates Endothelial Cell Autophagy: Role of lox-1. *International Journal of Cardiology*, **184**, 86-95. <https://doi.org/10.1016/j.ijcard.2015.01.065>
- [38] Liu, W., Song, H., Xu, J., *et al.* (2022) Low Shear Stress Inhibits Endothelial Mitophagy via Caveolin-1/mir-7-5p/sqstm1 Signaling Pathway. *Atherosclerosis*, **356**, 9-17. <https://doi.org/10.1016/j.atherosclerosis.2022.07.014>
- [39] Bharath, L.P., Cho, J.M., Park, S.-K., *et al.* (2017) Endothelial Cell Autophagy Maintains Shear Stress-Induced Nitric Oxide Generation via Glycolysis-Dependent Purinergic Signaling to Endothelial Nitric Oxide Synthase. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **37**, 1646-1656. <https://doi.org/10.1161/ATVBAHA.117.309510>
- [40] Bharath, L.P., Mueller, R., Li, Y., *et al.* (2014) Impairment of Autophagy in Endothelial Cells Prevents Shear-Stress-Induced Increases in Nitric Oxide Bioavailability. *Canadian Journal of Physiology and Pharmacology*, **92**, 605-612. <https://doi.org/10.1139/cjpp-2014-0017>
- [41] Heo, K.S., Berk, B.C. and Abe, J. (2016) Disturbed Flow-Induced Endothelial Proatherogenic Signaling via Regulating Post-Translational Modifications and Epigenetic Events. *Antioxidants & Redox Signaling*, **25**, 435-450. <https://doi.org/10.1089/ars.2015.6556>
- [42] Chen, J., Zhang, J., Wu, J., *et al.* (2021) Low Shear Stress Induced Vascular Endothelial Cell Pyroptosis by tet2/sdhd/ros Pathway. *Free Radical Biology and Medicine*, **162**, 582-591. <https://doi.org/10.1016/j.freeradbiomed.2020.11.017>
- [43] Lu, H., Fan, Y., Qiao, C., *et al.* (2017) TFEB Inhibits Endothelial Cell Inflammation and Reduces Atherosclerosis. *Science Signaling*, **10**, eaah4214. <https://doi.org/10.1126/scisignal.aah4214>
- [44] Santovito, D., Egea, V., Bidzhekov, K., *et al.* (2020) Noncanonical Inhibition of Caspase-3 by a Nuclear MicroRNA Confers Endothelial Protection by Autophagy in Atherosclerosis. *Science Translational Medicine*, **12**, eaaz2294. <https://doi.org/10.1126/scitranslmed.aaz2294>
- [45] Molica, F., Hautefort, A., Idris, T., *et al.* (2022) Oscillatory Shear Stress Augments Endothelial Pannexin1 by Inhibiting Macro-Autophagy. *Cardiovascular Research*, **118**, cvac066.202. <https://doi.org/10.1093/cvr/cvac066.202>
- [46] Guixé-Muntet, S., De Mesquita, F.C., Vila, S., *et al.* (2017) Cross-Talk between Autophagy and klf2 Determines Endothelial Cell Phenotype and Microvascular Function in Acute Liver Injury. *Journal of Hepatology*, **66**, 86-94. <https://doi.org/10.1016/j.jhep.2016.07.051>
- [47] Zheng, Q., Zou, Y., Teng, P., *et al.* (2022) Mechanosensitive Channel piezo1 Senses Shear Force to Induce klf2/4 Expression via camkii/mekk3/erk5 Axis in Endothelial Cells. *Cells*, **11**, Article No. 2191. <https://doi.org/10.3390/cells11142191>
- [48] Akmeriç, E.B. and Gerhardt, H. (2022) Blood Flow Meets Mitophagy. *Journal of Cell Biology*, **221**, e202206033. <https://doi.org/10.1083/jcb.202206033>
- [49] Senbanerjee, S., Lin, Z., Atkins, G.B., *et al.* (2004) Klf2 Is a Novel Transcriptional Regulator of Endothelial Proinflammatory Activation. *Journal of Experimental Medicine*, **199**, 1305-1315. <https://doi.org/10.1084/jem.20031132>