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评 述 血管稳态与重构的调控机制专辑

靶向细胞衰老在血管疾病中的作用及其潜在应用

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摘要 血管衰老是指血管发生形态、结构改变及功能失常的一系列退行性变化的过程. 随着血管衰老、动脉粥样硬化等血管性疾病的发病风险显著增加, 血管衰老成为探索衰老相关性血管疾病干预策略的重要方向, 改变血管衰老的进程有望延缓心血管疾病发生. 细胞衰老是血管衰老的重要基础, 以往的研究集中于血管内皮细胞衰老在血管衰老和血管性疾病中的作用, 而对于血管平滑肌细胞衰老的认识较少. 本文重点讨论了血管细胞中平滑肌细胞的衰老及其在血管性疾病发病中的研究进展, 并阐述了血管衰老细胞的清除(senotherapy)在延缓衰老和衰老相关血管性疾病中的作用, 以期为促进血管健康和延缓衰老相关血管性疾病提供新策略.

关键词 血管衰老, 平滑肌细胞衰老, 血管性疾病, senotherapy

衰老是生命过程中机体发生的随时间推移而形成的退行性变化. 机体衰老伴随着生理过程和解剖结构复杂性的丧失[1]. 随着社会经济和医疗科技的发展以及我国人口平均寿命的延长, 我国已进入老龄化社会. 据估计, 到2030年, 65岁以上的人口将占总人口的20%. 导致该年龄组人口死亡的首要因素是心血管疾病, 占比高达40%, 针对心血管疾病治疗的费用也将增加两倍[2,3]. 在机体衰老过程中, 衰老细胞在心血管组织和器官上逐渐累积, 机体发生组织修复能力的丧失和功能障碍. 因此, 减少细胞衰老, 进而延缓衰老相关心血管疾病的发生具有重要意义.

细胞衰老是以细胞周期阻滞为特征的细胞状态[4], 主要体现为细胞大小增加、永久的生长停滞、端粒缩短、衰老相关的β-半乳糖苷酶(SA-β-gal)表达活性增加、细胞周期蛋白依赖性激酶抑制剂表达升高、出现衰老相关的分泌表型(senescence-associated secretory phenotype, SASP)以及衰老相关的异染色质聚集现象等[5,6]. 细胞衰老在多种生物学过程中发挥了重要作用, 可以分为复制性衰老(replicative senescence)和应激性衰老(stress-induced premature senescence, SISP)两种类型. 复制性衰老能够诱导端粒长度的缩短, 是指细胞在有限数量的分裂后永久丧失分裂能力, 如出现端粒融合、双中心或端粒结合因子的丢失[7,8]. 应激性衰老是许多生物学事件, 如DNA损伤、氧化应激、炎症、自噬、线粒体代谢障碍以及肾素-血管紧张素-醛固酮系统(renin angiotensin aldosterone system, RAAS)功能障碍等产生的结果[9]. 虽然SIPS具有许多复制性衰老的形态学和分子特征, 但SIPS通常不具有端粒缩短的特征.

衰老细胞是导致衰老相关心血管疾病的重要原因

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之一, 为治疗衰老相关疾病提供新靶点. 近年来, 研究表明, 靶向衰老细胞有望延缓衰老相关心血管疾病的发生[10], 为延缓衰老和防治心血管疾病提供新机遇. 以往对于血管内皮细胞的衰老在血管衰老中的作用已有较多阐述[11,12]. 本文将综述血管平滑肌细胞(vascular smooth muscle cell, VSMC)在血管衰老中的主要研究进展, 并重点讨论衰老细胞作为防治衰老相关血管性疾病的治疗靶点(senotherapy)的潜在价值及局限性, 展望通过预防细胞衰老, 延缓衰老相关血管性疾病和促进机体健康的可能性.

1　血管平滑肌细胞与血管衰老

VSMC是血管中层重要的组成部分, 能够分泌血管细胞外基质, 调控血管的收缩, 维持血管的外周阻力, 调节血压、血流的分布和再分配[13]. 血管平滑肌细胞分为两种表型, 即合成型和收缩型. 在胚胎发育过程中, VSMC伴随着血管成熟从合成型转变为收缩型, 进而实现血管张力的维持. 不同于骨骼肌和心肌细胞, 高度分化的收缩型血管平滑肌细胞仍具有可塑性, 可从收缩表型转变为合成表型. 合成型血管平滑肌细胞能够减少收缩相关蛋白的合成, 促进炎症细胞因子的分泌、上调基质金属蛋白酶(matrix metalloproteinase, MMP)的表达, 导致细胞迁移、增殖和分泌增加[14]. 合成型血管平滑肌细胞可以重新获得收缩表型的许多特征, 具有可逆性.

正常血管中平滑肌细胞的增殖率较低. 在动脉粥样硬化早期和血管损伤时, 啮齿动物衰老的平滑肌细胞与年轻动物相比, 其增殖率上升[15~17], 但在临床样本的研究中发现, 衰老兼动脉粥样硬化晚期的人类血管平滑肌增殖减少[18]. 衰老的平滑肌细胞增加了钙沉积和相关钙调因子的表达, 最终导致平滑肌细胞和血管的钙化[19,20]. 与正常的中膜平滑肌细胞相比, 动脉斑块中衰老的平滑肌细胞具有以下特征: 大而扁平, 高表达p16和p21, SA-β-gal活性增加, 端粒缩短等[21,22]. 在动脉粥样硬化的小鼠模型中, 平滑肌细胞表达SA-β-gal的活性和端粒相关的DNA损伤增加[23,24]. 在腹主动脉瘤小鼠的平滑肌细胞中也观察到SA-β-gal的活性显著增加[25]. 衰老平滑肌细胞的另一个典型表型是SASP. 衰老的血管平滑肌细胞分泌SASP相关因子, 作用于单核-巨噬细胞, 刺激血管平滑肌细胞分泌细胞因子, 表达一系列黏连分子, 如出现趋化因子(如CCL2)、黏附分子(如ICAM-1)和先天免疫受体(如TLR4)的上调[26,27], 在促炎环境下, 进一步促进炎症细胞的迁移, 引起血管性疾病. 衰老的血管平滑肌细胞上调炎症小体等相关组分的表达, 分泌基质金属蛋白酶, 加重动脉粥样硬化斑块的不稳定性[27]. 在ApoE$^{-/-}$小鼠的血管平滑肌细胞中过表达TRF2, 能降低血管的DNA损伤, 提高斑块的稳定性. 且在血管平滑肌TRF2突变的小鼠中发现动脉粥样硬化斑块的产生与DNA损伤以及衰老有较强的相关性[28]. 此外, 衰老是腹主动脉瘤最主要的危险因素, 本团队研究发现, 腹主动脉瘤病人血管病变组织的SIRT1表达显著降低, 而SIRT1的降低增加了p53的乙酰化水平, 激活p21的表达, 促进趋化分子MCP-1导致的血管炎症, 加速腹主动脉瘤的发生和破裂[25]. 能量限制能增加血管平滑肌SIRT1的表达, 有效减少腹主动脉瘤的发生和破裂[29]. 因此, 血管平滑肌细胞的衰老在血管衰老和相关的血管疾病中具有重要作用.

引起平滑肌细胞衰老的因素是复杂多样的[30], 主要包括端粒缩短、DNA损伤、氧化应激、炎症、自噬、线粒体代谢障碍以及RAAS功能障碍等, 其中, 氧化应激和炎症是主要影响因素. 已有研究证实, 氧化应激能加速血管平滑肌细胞复制性衰老的出现[21]. 在氧化应激存在下, 平滑肌细胞的表型存在很大的可塑性, 例如在动脉粥样硬化斑块中, 平滑肌细胞显示出间充质干细胞样的作用, 可以增殖分化, 实现分泌基质成分、促进炎症、钙化等作用[31]. 组蛋白修饰是氧化应激中调控ROS产生的重要因素, 组蛋白的甲基化和乙酰化与衰老密切相关. 在自然衰老或H_2O_2诱导的小鼠模型中, H3-Ac, H3K9-Ac和H3K4-tri-Me能调控p66^{shc}的表达, 促进ROS的产生, 导致细胞衰老[32]. H3Lys-4甲基化和H4的过度甲基化也被证明参与了H_2O_2诱导的细胞衰老. 线粒体氧化应激和NADPH氧化酶是产生ROS的两种途径, 在血管平滑肌细胞衰老和衰老相关血管疾病研究中受到广泛关注[33]. 衰老进程中, 线粒体生物合成相关的转录因子和细胞色素C氧化酶表达降低, 线粒体的氧化应激水平增加[30,34]. NOX-4是NADPH氧化酶中调控ROS产生的关键酶, 激活或增加NOX-4的表达能增加H_2O_2水平, 使平滑肌细胞进入前炎性状态[35]. 原癌基因*Ras*能诱导血管平滑肌细胞衰老, 血管产生炎症, 加速动脉粥样硬化斑块的形成[27].

p53在响应Ras激活而发生的衰老过程中起重要作用[36,37]. Ras通过增加ROS的产生来触发p53依赖的损伤反应. 而细胞衰老过程主要受两条效应通路控制, 即p53-p21和p16-视网膜母细胞瘤蛋白(pRB)通路[38]. 炎症因子和血管壁微环境能够对血管平滑肌细胞产生持续性的应激刺激, 促进细胞衰老和损伤血管功能, 在血管病理改变中发挥关键作用. 衰老的平滑肌细胞显著表达NF-κB, 通过分泌炎症相关因子诱导血管的慢性炎症, 致使动脉粥样硬化发生[39,40]. 局部炎症不仅可以直接诱导细胞衰老, 还参与由于年龄增长造成的动脉壁结构改变的过程. 研究表明, 白细胞介素和生长因子可以引起血管平滑肌细胞衰老, 最终导致血管衰老, 引发相关的生化炎性指标改变, 出现内皮功能障碍, 细胞外基质组成改变[41~43]. 在临床研究中发现, 衰老人群中衰老血管平滑肌数目和血浆炎性蛋白水平, 如CRP, IL-6, NF-κB都显著升高[44].

在动脉粥样硬化发展过程中, 血管平滑肌细胞和巨噬细胞的转分化作用是疾病发展的关键因素[13]. 在血管衰老过程中, AngII信号通路被激活, 出现炎症级联反应以及氧化应激, 进一步加剧机体的免疫应答, 导致高血压和动脉粥样硬化的发生[45]. 血管平滑肌细胞表达Toll样受体, 参与先天免疫反应中的炎症小体的激活. VSMCs参与AngII诱导的Janus激酶(JAK)2的磷酸化和转录因子(STAT)3的信号转导与激活, 与T细胞产生的前炎性因子和巨噬细胞共同参与血管的适应性免疫调节. 最近的研究还发现, 粥样硬化斑块形成的早期阶段, 血管平滑肌细胞能够吞噬红细胞, 加剧氧化应激的过程[46]. 在血管衰老过程中, SIRT1的降低能促进内皮细胞和血管平滑肌细胞的早衰, 导致内皮功能障碍和动脉粥样硬化的发生. SIRT1使eNOS发生去乙酰化, 并通过靶向下游分子MMPs抑制NF-κB和AT-I介导的促炎信号促进内皮依赖性的血管舒张[47~49]. SIRT1能与其他分子协同发挥作用, 如Sm22α-酪蛋白激酶II-SIRT1复合物形成正反馈环路, 限制了VSMCs中的抗炎反应[50].

脉管系统中血管平滑肌细胞、内皮细胞和巨噬细胞是相互共存的. 最近的研究发现, 股动脉结扎后, 血管平滑肌细胞来源的SIRT1能抑制内皮细胞血管生成相关基因的表达. 当外泌体cZFP609从血管平滑肌细胞输送到内皮细胞时, 细胞质中HIF-1α逐渐累积, 最终抑制血管内皮生长因子(vascular endothelial growth factor, VEGF)的表达[51]. 在血管疾病中, 巨噬细胞主要在血管外膜浸润, 而本团队[25]之前的研究发现, 在AngII诱导的衰老模型中, SA-β-gal阳性区域紧贴于细胞外膜, 并证实血管平滑肌来源的SIRT1可能通过下调MCP-1减弱外膜对巨噬细胞的招募, 从而延缓AngII诱导的血管衰老. 此外, 与内皮细胞和巨噬细胞不同, 血管平滑肌细胞并不是终末分化的细胞, 其具有较强的可塑性, 如在动脉粥样硬化斑块中的巨噬细胞源性的泡沫细胞部分来源于血管平滑肌的分化[52]. 可见, 内皮细胞和巨噬细胞的转归和表型可能由血管平滑肌细胞来源的SIRT1决定.

综上, 有多种类型的细胞, 包括平滑肌细胞、内皮细胞、成纤维细胞和巨噬细胞等共同参与构建血管微环境. 在微环境中, 随着衰老的出现, 衰老细胞的炎症和免疫反应的不平衡状态, 增加了血管病性疾病的易感性. 可见, 靶向清除衰老细胞有望对抗衰老相关疾病. 因此, 下文将重点论述关于清除血管衰老细胞的抗衰老治疗(senotherapy)策略.

2 衰老细胞的清除

生理状态下, 机体针对外界的刺激产生应激性的衰老, 当机体中的衰老细胞在各组织器官中长期驻留, 无法及时清除时, 机体的组织器官微环境受到破坏并促进疾病的发生. 靶向于衰老细胞清除的抗衰老治疗策略主要可分为两个方面: 靶向衰老细胞显著特征的清除和利用机体免疫监视功能的清除, 该抗衰老治疗策略(senotherapy)是防治衰老相关疾病的重要方式[53].

2.1 靶向衰老细胞

衰老细胞具有显著的抵抗凋亡的特征, 能够上调抗凋亡因子, 促进细胞存活[54,55]. 靶向细胞凋亡通路, 开发选择性清除衰老细胞的化合物, 称为“senolytics”. 其中, seno-是细胞衰老的词根, -lytic意为“裂解”, 解释为“衰老细胞的杀伤”[54,56,57]. 美国梅奥医学中心的研究者在对衰老和增殖细胞的转录组进行分析时发现, 衰老细胞上调促细胞存活的关键分子, 针对该类关键分子的siRNA的干扰能杀死衰老细胞. 此后研究者利用该分子为靶点筛选出senolytic相关药物“D+Q组合”, 即达沙替尼(dasatinib, D)和槲皮素(quercetin, Q), 并在衰老小鼠中得到验证[58]. 达沙替尼和槲皮素的联合使

用能够有效清除小鼠胚胎成纤维细胞[54]，但达沙替尼和槲皮素在体外小鼠胚胎成纤维细胞中均不具有显著的清除作用. 此外, 衰老细胞还出现抗凋亡蛋白BCL-2, BCL-W和BCL-XL的表达上调, 通过抑制该类蛋白, 能诱导衰老细胞特异性死亡[59]. 例如, ABT-263能溶解人脐静脉内皮细胞株，但其在衰老的人原代脂肪细胞的清除方面作用较弱[60]；针对BCL-XL的抑制剂A1331852和A1155463被证明在肺成纤维细胞和人脐静脉内皮细胞中起到清除衰老细胞的作用；非瑟酮可以选择性诱导人脐静脉内皮细胞的凋亡，苇芰酰胺可以诱导人成纤维细胞的凋亡[61,62]. 然而，senolytics对相同的衰老细胞类型作用有所差异，如ABT-263对IMR-90肺成纤维细胞样细胞株有清除作用，但对从患者中分离得到的原代人肺成纤维细胞的清除不明显[63]. 此外, 有研究发现, 抗凋亡转录因子FOXO4在衰老细胞中高表达，FOXO4和p53复合物能维持衰老细胞的存活. 靶向药物FOXO4-DRI能有效清除p53依赖的衰老细胞[64]，改善自然衰老的小鼠和经过基因改造快速衰老的小鼠寿命. 其他靶向于p53相关通路的调节，针对肽酶USP7和HSP90伴侣蛋白的抑制以及通过激活caspase蛋白增加凋亡驱动等相关靶向药物正在研究中[65~67].

SASP的产生是细胞衰老的一个关键标志, 通过自分泌和旁分泌两种方式发挥作用, 促进衰老的进程[68]. 靶向于SASP的抗衰老药物不直接杀伤衰老的细胞, 有望成为今后研究衰老细胞靶向药物开发的重要方向[69,70]. 根据细胞类型、衰老阶段和衰老诱导因素的不同, SASPs表现出较大的差异, 具有高度异质性, 能够在多个不同水平上进行调控[71~73]. 例如，TGF-β或IL-1β是SASP的重要组成成分，在血管衰老的炎症反应中形成自我放大的反馈回路[56]，靶向于该类成分的药物能够抑制SASP, 延缓衰老的进程. SASP的调控通路主要包括NF-κB，p38，GATA4，mTOR，BRD4和cGAS/STING等，靶向于这些通路的信号分子的研究正在进行[74]. 本团队[25]研究发现，在血管紧张素 II 和$CaCl_2$诱导的小鼠腹主动脉瘤中，SIRT1的表达降低, p21的表达水平上调，增加了血管衰老和炎症，证明血管平滑肌表达的SIRT1在延缓细胞衰老中有潜在价值. 此外, 研究表明, SIRT6能影响炎症信号通路NF-κB, 与其RELA亚型相互作用, 使得NF-κB启动子组蛋白的赖氨酸9(histone H3 lysine9, H3K9)位点去乙酰化，以减弱NF-κB的信号, 减少下游炎症靶基因转录, 从而减轻细胞衰老、凋亡以及相关炎症反应等生物学效应[75]. 淫羊藿苷(icariin, ICA)可以激活SIRT6酶蛋白，使年老小鼠体内SIRT6的表达程度提高, 并且抑制NF-κB蛋白表达以及机体炎症反应，在延缓衰老和延长寿命中具有一定的潜力[76]. 最新研究表明, SIRT6通过调控端粒抑制血管平滑肌细胞的衰老，减缓动脉粥样硬化的形成[77]. 因此，SIRT6也有望成为研究抗衰老的关键靶点. 另外, 一些已知的具有多功能的小分子药物, 也被用于清除衰老细胞的研究, 例如, 二甲双胍已被证明可以改善糖尿病患者的心血管功能并延长寿命，能够直接作用于血管内皮细胞改善血管舒张功能，延缓血管衰老[78].

综上，随着组学技术的发展和相关单细胞差异基因表达图谱的构建，细胞衰老领域有了突破性的进展，目前已有较多小分子药物进入临床试验阶段[79~81]. 相应的，senolytic药物暴露出来的脱靶效应和副作用值得进一步关注. 细胞衰老是一个复杂的过程，衰老细胞的形态和功能都具有一定的异质性，不同的衰老诱导方式、来源组织、细胞自身类型等都会影响衰老表型. 此外, 细胞衰老研究模型经历了体外培养、动物模型到人体内三个阶段的差异，使得抗衰老药物研发的可行性受到严峻的考验. 而目前对于各个模型研究的差异讨论较少，且在对衰老细胞的研究中，缺乏对自然衰老细胞的特征描述，无法对衰老表型进行明确的定义. 细胞衰老具有一定的时空特性[82,83]，选择在预防阶段还是疾病发生过程中给予senolytic药物以及是否有效仍值得进一步探究[84].

2.2　增强对衰老细胞的免疫监视

免疫系统具有内源性防御机制，能够识别、杀伤并及时清除体内突变细胞，防止肿瘤发生的功能，即免疫监视. 衰老细胞本身具有免疫原性. 当衰老细胞存在时，免疫监视发挥功能，机体招募免疫细胞清除衰老细胞，为组织再生提供有利的微环境[85]. 随着年龄的增长，机体免疫监视功能下降，衰老组织器官中的衰老细胞逐渐累积[86]. 因此，通过增强机体免疫系统，从内在途径消耗累积的衰老细胞是senotherapy策略中的重要内容.

衰老细胞表面通过表达不同的配体，招募相应的免疫细胞进行识别，发挥免疫监视作用[87]. 衰老细胞

上调免疫识别分子NKG2D, 招募NK细胞识别该受体消除衰老细胞[43,88]. 研究人员在衰老细胞表面发现另一种选择性的细胞表面标记物尿激酶型纤溶酶原激活剂受体(urokinase-type plasminogen activator receptoru, PAR), 通过设计相应的嵌合抗原受体T细胞(chimeric antigen receptor T-cell, CAR-T细胞)能够实现对体内靶向的衰老细胞的清除[87,89]. 其他衰老细胞表面的特异性抗原如Band 3等, 为实现CAR-T细胞清除衰老细胞提供了新靶点[87,90]. 除了衰老细胞表面的特定抗原进行免疫清除衰老细胞外, 调节机体的免疫功能是清除衰老细胞的另一重要途径. 例如, 免疫激动剂polyI:C的使用能够促进NK细胞介导的衰老细胞清除, 减缓血管老化, 防止衰老相关的血管性疾病的发生[88].

不同的组织器官在不同时间段所表达的表面特异性配体具有动态性, 如何实现对衰老细胞表面标志物的追踪, 获得特异性和普适性的靶点, 是未来通过免疫监视作用清除衰老细胞的重要目标. 针对衰老细胞显著特征的靶向清除和通过提高免疫监视功能的衰老细胞的清除有望成为防治心血管疾病的途径, 但仍需进一步深入探究其特异性和适应性.

3 总结与展望

血管平滑肌细胞具有维持血管收缩和舒张的重要功能, 血管平滑肌细胞衰老是血管衰老发生发展的主要危险因素之一. 靶向血管中的衰老细胞有望改善衰老的病理改变并延缓血管性疾病发生. 鉴于senotherapy策略存在靶向药物特异性不足并具有毒性副作用等问题, 如何针对细胞衰老诱因进行预防, 将是抗衰老策略的重要目标[90]. 研究发现, 合理的运动可以改善血管的功能, 降低血管衰老和衰老相关性血管疾病发生的风险[91~93]. 能量限制(caloric restriction)能够增加血管抗氧化应激能力和抑制炎症水平[94~96], 对预防年龄相关的内皮细胞功能下降和血管僵硬度有重要作用, 减少衰老相关性血管疾病的发生[97]. 在能量限制的预防策略中, sirtuin家族SIRTI能够对抗应激性刺激, 改善内皮功能障碍和延缓心血管疾病的发展[98,99]. 此外, 引起细胞衰老的诱因多种多样, 主要包括DNA损伤、氧化应激、炎症、自噬变化、线粒体代谢障碍以及RAAS功能障碍等, 它们之间存在紧密联系, 如果找到有效改善引起血管细胞衰老的诱导因素将有望减缓血管衰老、降低心血管疾病发生.

因此, 清除衰老细胞是目前抗衰老领域的热门话题, 但仍存在许多问题. (ⅰ) 机体内的不同细胞衰老特征有何区别和如何发展针对不同衰老细胞特异性防治心血管疾病的措施还需要进一步研究; (ⅱ) 引起细胞衰老的诱因错综复杂, 相互影响, 如何特异性针对诱因进行衰老的预防值得重点研究.

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The role of targeting cell senescence in vascular diseases and its potential application

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Vascular senescence refers to a series of degenerative changes in vascular morphologies, structures and functions. With vascular aging, the risk of vascular diseases, such as atherosclerosis, increases significantly. Therefore, vascular aging has become an important direction in exploring efficient measures targeting age-related vascular diseases, the delay of which may reduce the incidence of age-related vascular diseases. Cellular senescence is one of the fundamental reasons of vascular aging. Previous studies principally concentrated on the role of vascular endothelial cell senescence in vascular aging and vascular diseases, but the senescence of vascular smooth muscle cells was less understood. This paper reviews the research progress of vascular smooth muscle cell senescence and the roles of cellular senescence in the pathogenesis of aging-related vascular diseases, focusing on serotherapy strategies targeting cellular senescence. We hope to provide new strategies for promoting vascular health and delaying aging-related vascular diseases.

vascular aging, vascular smooth muscle cell senescence, vascular disease, senotherapy

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