综述

内质网应激与动脉粥样硬化的研究进展

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摘要 内质网应激是指细胞在缺血、氧化应激、钙稳态紊乱、蛋白质折叠错误或正常蛋白质表达增强等条件下发生的一系列适应性的保护反应。越来越多的研究表明, 内质网应激在动脉粥样硬化发展的所有阶段均具有重要作用。该文主要综述了动脉管壁不同类型的细胞中内质网应激和细胞凋亡在动脉粥样硬化发展中的作用, 并总结了目前调节这些途径的靶向分子以及它们在动脉粥样硬化治疗中的潜在疗效。

关键词 内质网应激; 动脉粥样硬化; 血管内皮细胞; 血管平滑肌细胞; 巨噬细胞

Progress of Endoplasmic Reticulum Stress Related to Atherosclerosis

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Abstract The endoplasmic reticulum (ER) is a complex cytoplasmic membrane structure presented in eukaryotic cells, involving in protein folding, lipid synthesis and regulation of the intracellular calcium balance. The capacity of protein folding in ER can be saturated, then induce ER stress (ERS) response. The accumulating evidence demonstrates that ERS and associated apoptosis can be induced in the pathological conditions of atherosclerotic lesions and contribute to the disease progression. Notably, they may play a role in the development of vulnerable plaques that induce thrombosis and are therefore especially dangerous. ERS response is regulated by several signaling mechanisms that involve protein kinases and transcription factors. Some of these molecules can be regarded as potential therapeutic targets to improve treatment of atherosclerosis.

Keywords endoplasmic reticulum stress; atherosclerosis; vascular endothelial cell; vascular smooth muscle cell; macrophage

动脉粥样硬化(atherosclerosis, AS)是心血管疾病发展的主要原因, 一直是西方发达国家首要的发病和死亡原因之一[1]。动脉粥样硬化性疾病发病机制复杂, 涉及多个代谢和信号转导途径。内质网(endoplasmic reticulum, ER)是真核细胞复杂的膜结构, 参与蛋白质折叠、脂质合成以及细胞内钙平衡的调

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控等。越来越多的研究表明, 内质网应激(ER stress, ERS)在AS发展的所有阶段具有重要作用[2]。高同型半胱氨酸血症、血脂异常、血糖异常、慢性炎症等心血管疾病的危险因素都可触发ERS, 促进早期AS的发展[3]。ERS过程中有多种信号转导因子参与, 这些分子有望成为AS治疗的新靶点。本文主要综述了动脉管壁不同类型细胞中ERS和细胞凋亡在AS发展中的作用, 并总结了目前调节这些途径的靶向分子以及它们在AS治疗中的潜在疗效。

1 ERS的分子机制

在缺血、氧化应激、钙稳态紊乱、蛋白质折叠错误或正常蛋白质表达增强等条件下可导致ERS, 主要特征为, 未折叠和/或错误折叠蛋白质积聚以及钙稳态失衡, 未折叠和/或错误折叠蛋白质在ER腔内大量积聚会导致一系列的适应性和保护性反应, 例如蛋白质合成抑制、内质网相关降解通路(ER-associated degradation, ERAD)激活, 这些过程称为未折叠蛋白质反应(unfolded protein response, UPR)[4]。UPR通过3种ER膜受体蛋白介导, 分别为: 双链RNA依赖的蛋白激酶样ER激酶(PER-like ER kinase, PERK)、肌醇需求酶1(inositol-requiring enzyme 1, IREl)和活化转录因子6(activating transcription factor 6, ATF6)。在生理条件下, 这3种蛋白质分别与葡萄糖调节蛋白78(glucose-regulated protein 78, GRP78)结合, 处于非活化状态。ERS时, 未折叠蛋白质在ER内堆积, 促使这3种蛋白质与GRP78解离, 诱导UPR[1]。因此, 上述3种信号转导蛋白质与GRP78的解离与结合, 是ERS的主要调控机制。

1.1 ERS早期激活UPR维持内质网稳态

未折叠蛋白质在ER累积导致PERK/GRP78复合物解离, 游离的PERK催化翻译起始因子2α(eukaryotic initiation factor 2α, eIF2α)磷酸化, 抑制蛋白质翻译, 从而减少蛋白质在ER中聚集[5]。然而, PERK激活的同时ATF4、GRP94和GRP78的表达增加, 从而上调生长停滞与DNA损伤诱导基因34(growth arrest and DNA damage-inducible gene 34, *GADD34*)的表达, 通过eIF2α的磷酸化以促进翻译恢复[6]。IRE1通过自身磷酸化激活后, 剪切X盒结合蛋白-1(X-box-binding protein-1, *XBP-1*)mRNA的中间片段, 使两端的片段形成1个拼接的RNA, 转位到细胞核, 与ERS反应元件的启动子区结合[7], 对受损细胞进行修复。ATF6包括ATF6α和ATF6β这2个亚型, ERS时, ATF6与GRP78解离, 进入高尔基体内腔, 被特异性蛋白酶裂解[8], 随后其N-端转位到细胞核, 诱导*GRP78*、CCAAT增强子结合蛋白同源蛋白(CCAAT enhancer-binding protein homologous protein, *CHOP*)和*XBP-1*等UPR相关基因的表达。

1.2 ERS晚期诱导细胞凋亡

ERS通过PERK-eIF2α和IRE-1这2条UPR通路诱导细胞发生自噬, 有效清除不能被ERAD清除的蛋白质, 发挥心血管保护作用[9-10]。在长期或严重的ERS时, UPR保护作用被抵消, 启动细胞凋亡途径。PERK长期被激活, 诱导CHOP表达增加, 促进多种细胞的凋亡[11]。CHOP可使肿瘤坏死因子相关凋亡诱导配体(tumor necrosis factor-related apoptosis-inducing ligand, TRAIL)受体表达上调, 促进外源性凋亡途径[12]。IRE1激活后, IRE1-C-jun氨基末端激酶(c-Jun N terminal kinase, JNK)-肿瘤坏死因子受体相关因子2(TNF receptor associated factors 2, TRAF2)形成复合物, 导致促凋亡JNK信号通路激活[13-14]。ERS时, 胱冬肽酶12(caspase 12)被钙蛋白酶激活, 启动一个正反馈回路, 经caspase 9-caspase 3回路增加细胞凋亡[15]。

2 动脉管壁不同类型细胞的ERS在AS中的作用

越来越多的研究表明, 在AS发生发展过程中, 动脉管壁不同类型细胞中都会发生ERS(图1)。例如, Zhou等[16]的研究证明, 高脂饮食的ApoE$^{-/-}$小鼠斑块的巨噬细胞中存在ERS, 较严重的病变部位中CHOP高表达。Myoishi等[17]在检测冠状AS患者的斑块时发现, 在薄帽样纤维斑块和破裂斑块的平滑肌细胞(smooth muscle cell, SMC)中, GRP78、GRP94和CHOP的表达比厚帽样斑块更多, 说明ERS与动脉粥样硬化严重程度有关。

2.1 内皮细胞(endothelial cell, EC)ERS

慢性长期ERS时, UPR可导致EC功能障碍, 从而导致细胞凋亡和炎症。高同型半胱氨酸血症(hyperhomocysteinemia, HHcy)作为AS的独立危险因素, 可以诱导ERS的发生。HHcy可以激活eIF2α, 从而诱导T细胞相关基因51(T-cell associated gene 51, *TDAG51*)介导的细胞黏附功能受损[18]。在AS过程中, EC的TDAG51表达升高, 而在ApoE$^{-/-}$小鼠中,

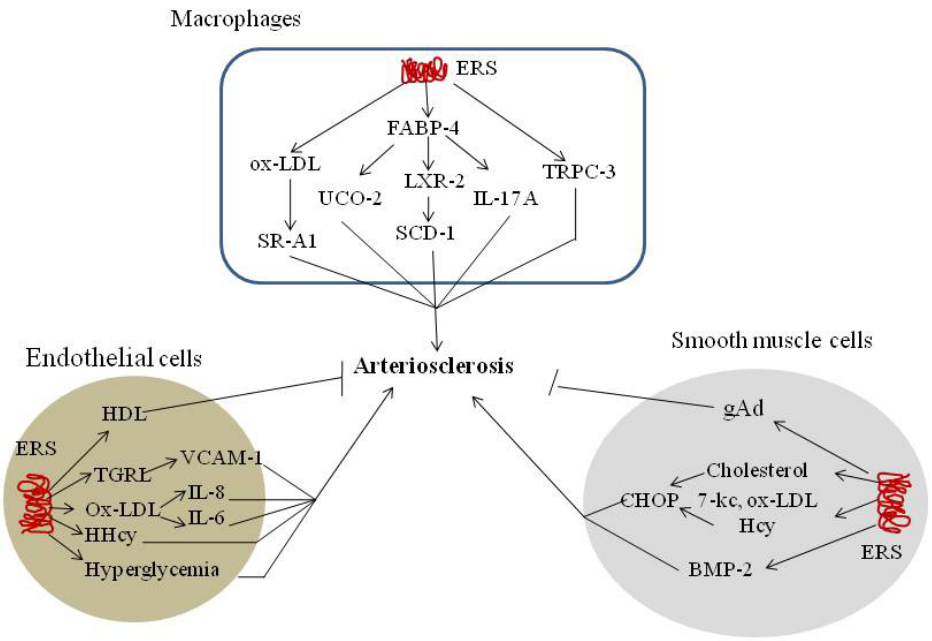

图1 动脉管壁不同类型细胞的内质网应激

Fig.1 ERS in different cell types of the artery wall

*TDAG51*缺失可减慢AS病变的发展[19]。有研究证实, 慢性局部ERS激活是血流紊乱的病理变化之一, 当血管局部血流剪切力增强时, IRE1α和ATF6途径增加, 但是PERK-ATF4途径并未激活, 表明UPR可能激活相关保护机制来减轻干扰流的影响[20]。然而, 当存在高血压、高胆固醇血症、糖尿病等基础病变时, UPR是AS的危险因素[20]。

EC中钙激活钾通道在血管张力的调节中发挥重要作用, 例如, 层流剪切力可以上调钙激活钾通道2.3(Ca^{2+}-activated K^+ channels 2.3, KCa2.3)和3.1(KCa3.1)的表达, 有助于维持EC功能稳态[21]。同型半胱氨酸(homocysteine, Hcy)可以诱导EC发生ERS, 其标志蛋白GRP78与磷酸化PERK表达均增加。进一步的膜片钳记录显示, Hcy可降低冠状动脉EC的中电导型钙激活钾通道(intermediate-conductance KCa, IKCa)和小电导型钙激活钾通道(small-conduct-ance KCa, SKCa)电流。但是在暴露于Hcy期间同时抑制ERS, 可以增强EC中IKCa与SKCa电流, 表明Hcy通过ERS介导的EC中IKCa与SKCa通道抑制, 进而导致血管舒张功能障碍[22]。

修饰的脂蛋白, 尤其是氧化型低密度脂蛋白(oxidized low density lipoprotein, ox-LDL)可通过诱导EC凋亡引发AS, 是AS的早期危险因素[23]。利用人X型磷脂酶A2水解LDL的产物(phospholipolyzed LDL, LDL-X)处理人脐静脉EC, 可以激活UPR信号通路, 诱导白介素-6(interleukin-6, IL-6)和IL-8表达。此外, 富含甘油三酯的脂蛋白(triglyceride-rich lipoproteins, TGRL)含量升高与心血管疾病风险增加相关。研究显示, 高甘油三酯血症患者产生的TGRL颗粒可以诱导主动脉EC中血管细胞黏附分子-1(vascular cell adhesion molecule-1, VCAM-1)高表达, 而正常人产生的TGRL颗粒处理细胞可以降低VCAM-1的表达[24]。同样, 肿瘤坏死因子-α(tumor necrosis factor-α, TNF-α)介导的VCAM-1表达可被促AS的TGRL颗粒增强, 而被抗AS的TGRL颗粒减弱。此外, 促AS的TGRL颗粒可激活IRE1-XBP1(X-box binding protein 1)和PERK-EIF2α信号通路, 并且活化VCAM-1转录调控因子与干扰素调节因子-1[25]。

与LDL相反, 高密度脂蛋白(high density lipoprotein, HDL)具有抗炎和抗氧化的功能, 可以抵消ox-LDL的促炎作用, 抑制ox-LDL诱导的ERS和细胞死亡。ERS通常伴随着细胞内Ca^{2+}增加和活性氧产生, 导致ER钙泵功能障碍, 而激活AMP依赖的蛋白激酶(AMP-activated protein kinase, AMPK)可以逆转这些影响[26]。

此外, 越来越多的研究证明, 1型和2型糖尿病患者AS和心血管疾病的风险增加[27-28]。有研究表明, 葡萄糖胺可以诱导人微血管EC发生UPR, 通过激活

JNK和核转录因子-κB(nuclear factor-kappa B, NF-κB)途径, 增强内皮细胞黏附分子的表达, 诱导促炎细胞因子(如C-反应蛋白、IL-6和TNF-α)的释放诱发炎症和血栓的形成[29]。

2.2 平滑肌细胞ERS

包括血小板源生长因子-BB(platelet-derived growth factor-BB, PDGF-BB)在内的多种因素刺激可使血管SMC(vascular SMC, VSMC)从静态的收缩型向动态的合成型转变。合成型VSMC中蛋白质合成增多, 蛋白质合成负荷过重时引发ERS[30]。一般来说, 轻度ERS时, UPR负责激活分子伴侣的功能以减轻细胞功能障碍或损伤。然而, 长期或严重ERS时, UPR激活可能导致VSMC凋亡, 成为AS发病机制的重要事件。SMC合成大部分的胶原, 从而稳定斑块纤维帽, 因此, SMC过度凋亡可能会破坏斑块的完整性, 导致斑块破裂[31]。在VSMC中, CHOP诱导的凋亡可被多种ERS因子触发, 包括7-酮基胆固醇(7-ketocholesterol, 7-kc)、ox-LDL、游离胆固醇、Hcy等[18]。ox-LDL引发的细胞凋亡信号是通过一个复杂序列信号调控的, 导致caspase依赖性或caspase非依赖的凋亡信号通路激活。蛋白激酶C(protein kinase C, PKC)是丝苏氨酸激酶家族的一员, 在许多类型的细胞中有促凋亡作用。将VSMC暴露于ox-LDL可以引发UPR/ERS传感器激活, 长期过度ERS可以激活促凋亡因子CHOP/生长抑制DNA损伤基因53(growth arrest and DNA-damage-inducible protein 53, *GADD53*)及其靶蛋白Bcl-2(B-cell lymphoma-2)家族中促凋亡亚家族成员(Bcl-2 interacting mediator of cell death, *BIM*)和p53上调凋亡调制物(p53 up-regulated modulator of apoptosis, *PUMA*)表达增加, 以及激活IRE1/JNK通路。此外, LDL通过生成ROS介导PKC激活, 活化的PKC通过IRE1信号通路调节ox-LDL诱导的细胞凋亡[32]。

此外, ERS还促进了VSMC氧化应激和细胞钙化[33]。研究发现, 80%的血管损伤和90%的冠状动脉疾病患者伴有血管钙化, 其主要特点是血管细胞尤其是VSMC向成骨细胞样表型转化。在血管钙化的发展过程中, 血管壁的刚度增加, 结合顺应性降低, 是AS常见病理生理学基础[34]。最近的研究表明, 在钙化血管中, VSMC的表型特征逐渐减少, 并开始具有成骨细胞的特征和功能, 合成多种骨特异性蛋白质, 具有成骨基因表达的特异性[35], 而ERS可加剧成骨细胞分化和VSMC钙化。Masuda等[36]发现, ERS的关键转录因子ATF4介导硬脂酸合成, 诱导VSMC发生钙化, 硬脂酸通过PERK-eIF2α通路和CHOP诱导ATF4表达, 加剧ERS所致的成骨细胞分化和VSMC钙化。Liberman等[37]发现, 用骨形态发生蛋白-2(bone morphogenetic protein-2, BMP-2)处理人冠状动脉SMC后, GRP78、磷酸化IRE1、XBP1和Runx2表达增加, 导致SMC向成骨细胞表型转化。Saito等[38]证实, 经PERK-eIF2α-ATF4途径介导的ERS对VSMC向成骨细胞转化和成骨细胞分化具有促进作用。ERS标志蛋白GRP78通过增加钙和磷的沉积导致矿化基质的形成, 促进VSMC钙化, 表明ERS可以通过增加Runx2的表达促进VSMC钙化。此外, 球形脂联素(globular adiponectin, gAd)可以抑制VSMC向成骨样细胞转化, 减缓AS的发展过程[39]。最近研究发现, gAd能显著抑制VSMC中ERS通路。在体外和体内给予gAd, VSMC凋亡明显减少, 骨保护素(osteoprotegerin, OPG)水平升高, Runx2水平下降, 钙化程度也显著降低[38]。

2.3 巨噬细胞ERS

巨噬细胞凋亡加重晚期AS斑块坏死, 与AS的发展和随后的斑块破裂密切相关, 这是大多数急性冠脉综合征临床表现的突出事件, 如急性心肌梗死和猝死[40]。据报道, ERS通过诱导巨噬细胞凋亡增加斑块易损性。ER是细胞蛋白质折叠和运输的主要部位, 其功能障碍激活UPR, 导致细胞核组织功能障碍[41]。长期ERS通过IRE1和CHOP引发巨噬细胞凋亡和晚期AS斑块坏死, 是促进AS进展的重要因素[42]。脂质泡沫细胞, 尤其是巨噬细胞源性泡沫细胞, 位于血管壁内皮下, 是AS的标志[43]。AS病变的发展过程中, 循环中的单核细胞黏附于动脉壁EC, 在载脂蛋白B和巨噬细胞集落刺激因子(macrophage colony stimulatory factor, M-CSF)驱动下, 迁移到内皮下间隙, 分化为M1和M2两类, 通过炎症过程在AS早期发挥作用。巨噬细胞凋亡早期, 与凋亡细胞清除程序结合, 有助于维持细胞结构和延缓病变进展, 然而, 在晚期AS病变部位, 凋亡细胞清除程序缺陷, 巨噬细胞源性的泡沫细胞凋亡导致脂质核心形成和扩展, 并产生炎症和坏死, 导致斑块的不稳定性[43]。ox-LDL可诱导巨噬细胞源性泡沫细胞形成过程中的ERS, PERK介导ox-LDL上调CHOP表达, 诱导巨噬细胞凋亡, 促进晚期AS斑块形态、纤维帽和坏死中心形成, 进一步增加炎症反应和细胞凋亡信号[44]。最

近的研究表明, 体内巨噬细胞凋亡可能不仅是ERS引发的, 也有可能是某些称为“二次打击”的刺激, 如模式识别受体(pattern recognition receptors, PRRs)激活的。PRRs包括各种Toll样受体(Toll-like receptors, TLR)和清道夫受体(scavenger receptor, SR), 可以被位于AS病变部位的氧化脂质、修饰的LDL和饱和脂肪酸激活。巨噬细胞中激活的PRRs通过CD36-TLR2和NADPH氧化酶介导的氧化应激诱导细胞凋亡。NADPH氧化酶上调激活PERK-CHOP促凋亡信号通路[45]。Yao等[46]研究表明, 巨噬细胞ERS参与调节ox-LDL诱导的SR-A1的表达。ox-LDL诱导SR-A1表达显著增加, 同时激活ERS, GRP78表达增加。

此外, Solanki等[47]发现, 钙通道瞬时受体电位3(transient receptor potential canonical 3, TRPC3)缺乏时, ERS介导的巨噬细胞凋亡增加。TRPC3可以减少巨噬细胞的清除, 从而使这些凋亡细胞发生继发性坏死, 增加斑块中血栓形成[48]。巨噬细胞ERS诱导的凋亡可能是中性胆固醇酯水解酶1(neutral cholesterol ester hydrolase 1, Nceh1)导致的。Sekiya等[49]报道, Nceh1缺失后, 腹腔巨噬细胞易被氧化型胆固醇, 尤其是25-羟基胆甾醇(25-hydroxycholesterol, 25-HC)诱导凋亡。用25-HC培养巨噬细胞可引起25-HC在ER大量聚集, 从而诱导ERS信号通路蛋白CHOP等表达增加。脂肪酸结合蛋白-4(fatty acid-binding protein-4, FABP-4)是一种调节细胞脂质代谢、接收脂质信号、促进AS发展的细胞内脂质分子伴侣。FABP-4促进AS也与ERS有关。Gao等[50]报道, FABP-4可诱导巨噬细胞发生ERS, 此外, FABP-4介导的ERS参与了IL-17A诱导的AS的进展。Erbay等[51]报道, 在AS病变部位的巨噬细胞中, 脂质诱导的ERS和细胞凋亡需要FABP-4的参与, 巨噬细胞中FABP-4缺失可通过肝X受体α(liver-x-receptor α, LXRα)介导的硬脂酰辅酶A1去饱合酶-1(stearoyl co-A1 desaturase-1, SCD-1)激活从头合成途径, 从而产生棕榈酸酯和抵抗ERS。也有报道认为, FABP-4通过调节解偶联蛋白2(uncoupling protein 2, UCP2)的表达间接调控巨噬细胞ERS[52]。

3 ERS作为AS治疗的新靶点

ERS是AS的一个关键的调节因子, 调控AS的全过程。因此, ERS是药物设计的潜在靶点, 调节ERS的药物可能是AS治疗的新选择。

3.1 分子伴侣

一些化学分子伴侣, 如苯丁酸(phenylbutyric acid, PBA)和牛磺脱氧胆酸(tauroursodeoxycholic acid, TUDCA), 可以促进蛋白质非选择性折叠和运输, 减少应力条件下ER中蛋白质负荷, 减轻ERS[53]。用PBA治疗患AS的ApoE$^{-/-}$小鼠, 在巨噬细胞丰富的病变区, PBA可以降低ERS, 抑制CD36、GRP78和IRE1磷酸化, 减少修饰后的LDL诱导的ATF6、IRE1、XBP1和GRP78信号活化[54]。此外, TUDCA可以减少ERS, 减缓AS病变进展[55]。有研究表明, TUDCA可以通过降低ERS减缓饮食诱导的小鼠脂肪肝的进展[56]。因此, PBA和TUDCA在人类心血管病理学有潜在的研究价值。

3.2 信号通路抑制剂

一种被批准用于治疗双相情感障碍和癫痫的药物——丙戊酸钠, 可诱导ER伴侣BiP(immunoglobulin heavy chain binding protein)的表达, 抑制CHOP和caspase 12的活化, 该药对患AS的ApoE$^{-/-}$小鼠疗效良好, 为AS的治疗提供了可能。目前开发的几种内质网氧化酶1(ER oxidase 1, ERO1)特异性抑制剂中, 已证明EN460和QM295可诱导UPR以减轻ERS[56]。

另一个与ERS调节密切相关的是AMPK信号通路, AMPK是细胞能量传感器之一, 使细胞在合成代谢与分解代谢之间转换。AMPK失活引起严重的ERS和AS, AMPK激活剂如5-氨基咪唑-4-甲酰胺-1-D-呋喃糖苷(5-aminoimidazole-4-carboxyamide-1-D-macribofuranoside, AICAR)、阿托伐他汀和PT1通过降低ERS对心肌细胞发挥保护作用[58]。研究表明, 在*AMPKα2*基因敲除小鼠中, ERS明显被激活[55]。AMPK的另一种激活剂——二甲双胍, 是广泛使用的胰岛素增敏剂和ERS抑制剂, 通过抑制软脂酸诱导的eIF2α磷酸化与JNK活化对大鼠胰岛细胞发挥保护作用, 表明二甲双胍的细胞保护作用可能与抑制ERS相关[59]。此外, 二甲双胍可以改善高脂饮食诱导的内皮功能障碍, Cheang等[60]证明, 二甲双胍可以诱导过氧化物酶增殖物激活受体(peroxisome proliferater-activated receptor, PPAR)活化, 抑制ERS, 在保护血管内皮功能中具有重要作用, 因此可用于治疗心血管疾病。

哺乳动物雷帕霉素靶蛋白(mammalian target of rapamycin, mTOR)抑制剂——雷帕霉素可激活多种

类型的细胞自噬, 从而抑制ERS。自噬小泡的形成可能来源于ERS[61], ERS介导的自噬可能在处理错误折叠的蛋白质中起着至关重要的作用, 错误折叠的蛋白质不能由ERAD清除, 从而有助于维持ER稳态。雷帕霉素通过抑制mTOR信号通路而激活自噬, 可能是抑制ERS的一种有效药物[62]。此外, Kato等[63]发现, 雷帕霉素通过选择性抑制IRE1-JNK信号通路抑制ERS诱导的细胞凋亡。

胰高血糖素样肽-1(glucagon-like peptide-1, GLP-1)对ERS信号通路的调节也受到关注。GLP-1激动剂艾塞那肽通过诱导ATF4的表达改善ERS, 从而恢复ERS诱导的胰岛素表达下调, 通过增强细胞防御基因(如*BiP*、抗凋亡蛋白*Bcl-2*和*JunB*)的表达, 保护细胞免受棕榈酸酯诱导的ERS和凋亡[64]。二肽基肽酶4(dipeptidyl peptidase 4, DPP4)可降解GLP-1。DPP4抑制剂维格列汀可通过降解C/EBP改善ERS[65]。另一个DPP4抑制剂西他列汀通过抑制CD36表达, 激活NF-κB, 进而抑制脂质过氧化和ERS, 改善蛋氨酸胆碱缺失饮食诱导的小鼠肝脂肪变性、炎症和纤维化[66]。此外, 一种新的DPP4抑制剂(gemigliptin)通过抑制AKT(又称protein kinase B, PKB)/PERK/CHOP和IRE1/JNK-P38信号通路保护大鼠心肌细胞免受ERS诱导的细胞凋亡和炎症[67]。这些研究结果表明, GLP-1受体激动剂或DPP4抑制剂通过激活GLP-1介导的信号通路改善ERS, 进而对心血管疾病发挥保护作用[62]。

3.3 天然产物

许多天然存在的化学复合物是已知调节ER的有效分子[68], 因此, 无论是天然化合物本身还是作为半合成的原料, 天然产物都将在ER药物治疗靶点方面发挥举足轻重的作用。已知天然产物包括: (1)蛋白酶体抑制剂, 如雷公藤素、扁蒴藤素、布雷菲德菌素A、衣霉素与乳胞素等; (2)SERCA抑制剂, 如毒胡萝卜素、basiliolide A1和agelasine B, 通过不可逆地抑制SERCA稳定细胞内钙离子浓度; (3)IRE1/PERK信号通路抑制剂, 如白藜芦醇与醉茄素A; (4)ER保护剂, 如黄连素、羟基酪醇等, 通过调节ATF6/IRE1α-XBP1、PERK-eIF2α信号通路, 抑制GRP78、CHOP的表达发挥作用; (5)其他活性分子, 如蜂胶乙醇提取物(ethanol extract of propolis, EEP)。

3.3.1 蛋白酶体抑制剂 蛋白酶体抑制剂是目前用于临床或进行临床试验最有代表性的化合物类。雷公藤素是从雷公藤分离出来的一种五环三萜类化合物, 研究表明, 此化合物有促进癌症细胞凋亡作用[69]。扁蒴藤素也是一种五环三萜类化合物, 可从多种植物中得到, 对多种癌细胞系具有细胞毒作用和促凋亡功能[70]。乳胞素是从某些链霉菌中得到的天然产物, 是一种蛋白酶抑制剂, 可抑制胰凝乳蛋白酶类、胰蛋白酶类和caspase 3种蛋白酶的活性, 乳胞素通过抑制蛋白酶体功能而激活ERS, 诱导多种细胞凋亡[71-72]。Agosterols是多羟基甾醇乙酸酯, 可从海绵体分离, 具有胰凝乳蛋白样酶体抑制作用[73], 可逆转P-糖蛋白(P-glycoprotein, P-gp)介导的肿瘤细胞的耐药性。衣霉素是从混合链霉菌属分离出来的核苷同系物混合物, 是细菌和真核生物N-乙酰葡萄糖胺转移酶抑制剂, 可防止N-乙酰氨基葡萄糖脂质中间体的形成。如今衣霉素是一种最广泛使用的诱发ERS的化合物之一, 在实验中常作为阳性对照。布雷菲德菌素A是一种16元大环内酯类化合物, 研究表明, 布雷菲德菌素A通过抑制蛋白质的组装和封闭囊泡的萌芽来抑制蛋白质的转运[74]。

3.3.2 SERCA抑制剂 毒胡萝卜素是一种不可逆的SERCA抑制剂, 是除了衣霉素和布雷菲德菌素A之外广泛被认可和应用的ERS诱导剂, 对稳定内质网钙离子稳态有重要意义。毒胡萝卜素刺激细胞后, 钙离子从ER迅速释放到细胞质, 其中部分钙离子被线粒体摄取, 导致细胞内凋亡途径激活。Basiliolide A1作为一种SERCA抑制剂, 在哺乳动物细胞中引起ER钙离子缓慢释放, 从而保证离子的再摄取, 导致细胞质和ER钙离子浓度的平衡[75]。但是, basiliolide A1不引起细胞凋亡。Agelasine B作为另外一种SRECA抑制剂, 可以导致ER钙离子显著释放, 通过降低Bcl-2的水平促进凋亡[76]。

3.3.3 IRE1/PERK信号通路抑制剂 研究显示, 白藜芦醇可以引起错误折叠蛋白在肿瘤细胞蓄积, 引发ERS, 同时伴随IRE1α、p-PERK、ATF6和CHOP表达升高[77]。透射电子显微镜和共聚焦电子显微镜观察结果显示, 白藜芦醇可以引发ER靶向的自噬和细胞凋亡, 发挥细胞保护作用。用caspase 12特异性抑制剂阻止白藜芦醇介导的caspase 3和caspase 9激活, 可以抑制凋亡[77]。此外, 黄连素、羟基酪醇等通过调节ATF6/IRE1a-XBP1与PERK-eIF2α信号通路, 抑制GRP78和CHOP的表达, 从而抑制自噬的发生。

3.3.4 ER保护剂 ER保护剂如黄连素, 羟基酪醇

等, 通过调节ATF6/IRE1a-XBP1、PERK-eIF2α信号通路, 抑制GRP78、CHOP的表达发挥作用。

3.3.5 *其他活性分子* 研究表明, EEP通过减少CD36介导的ox-LDL的摄取和抑制ERS-CHOP信号通路, 发挥细胞抗凋亡作用[78]。

4 展望

以上总结了ERS和AS的关系, 并讨论了ERS调节因子作为AS治疗靶点的分子机制, 然而以往研究有其局限性。首先, ERS对心血管的保护作用和损伤作用之间的界限并不明确, 它仍然是一个有争议的话题。其次, 大多数的研究都是在动物模型中进行的, 并不能完全代表人类患者。因此, 需要更多的研究来阐明在不同的情况下ERS发挥不同作用的分子机制, 确定ERS及其相关蛋白质在AS中的作用, 为AS提供新的治疗方法。

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