黄芪甲苷通过抑制PINK1/Parkin通路调控细胞线粒体自噬减轻D-半乳糖诱导的内皮细胞衰老

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摘要:目的 探究黄芪甲苷IV(AS-IV)通过PINK1/Parkin信号通路,调控细胞线粒体自噬水平减轻D-半乳糖(D-GAL)内皮细胞(HUVEC)衰老的作用机制。**方法** 将体外培养的人脐静脉内皮细胞随机分为5组:空白对照组(NC组)、D-GAL(40 g/L D-GAL)组、AS-IV(200 μmol/L)组、D-GAL+AS-IV(40 g/L D-GAL+200 μmol/L)组,D-GAL+AS-IV+MTK458(40 g/L D-GAL+200 μmol/L+25 μmol/L)组,干预48 h。评估细胞增殖、迁移和血管生成能力;检测细胞凋亡、活性氧水平、线粒体膜电位,以及检测自噬相关蛋白(LC3-II/LC3-I)和PINK1/Parkin通路蛋白的表达。**结果** 经AS-IV干预后,D-GAL对HUVEC细胞活力的抑制作用显著降低($P<0.05$),AS-IV有效缓解D-GAL诱导的HUVEC管状结构形成障碍,促进血管生成($P<0.05$)、恢复细胞的迁移能力($P<0.05$),D-GAL诱导的HUVEC细胞中衰老相关β-半乳糖苷酶(SA-β-Gal)染色阳性率也显著降低($P<0.05$),并抑制衰老相关基因P21和P53的表达。AS-IV恢复D-GAL诱导的线粒体膜电位,降低细胞内活性氧水平($P<0.05$);并抑制D-GAL诱导的HUVEC细胞中自噬体与溶酶体的融合,阻止自噬流的完成。当加入线粒体自噬激动剂MTK458(25μmol/L)后,与D-GAL+AS-IV组相比,D-GAL+AS-IV+MTK458组中细胞黄色斑点明显增加($P<0.05$),P21、P53、PINK1、Parkin、LC3、Beclin等蛋白表达上升($P<0.05$)。**结论** AS-IV通过抑制PINK1/Parkin通路调控线粒体自噬,从而减轻D-GAL诱导的内皮细胞衰老。

关键词:黄芪甲苷;PINK1/Parkin信号通路;线粒体自噬;D-半乳糖;内皮细胞;细胞衰老

Astragaloside IV alleviates D-GAL-induced endothelial cell senescence by promoting mitochondrial autophagy *via* inhibiting the PINK1/Parkin pathway

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Abstract: Objective To explore the mechanism by which astragaloside IV (AS-IV) alleviates D-galactose (D-GAL)-induced senescence in human umbilical vein endothelial cells (HUVECs). **Methods** Cultured HUVECs were treated with D-GAL (40 g/L), AS-IV (200 μmol/L), D-GAL+AS-IV, or D-GAL+AS-IV+MTK458 (a mitochondrial autophagy agonist, 25 μmol/L) for 48 h, and the changes in cell proliferation, migration, and angiogenesis capacity were evaluated. Cell apoptosis, reactive oxygen species (ROS) levels, mitochondrial membrane potential, and expressions of autophagy-related proteins (LC3-II/LC3-I) and PINK1/Parkin pathway proteins in the treated cells were detected. **Results** AS-IV treatment significantly reduced the inhibitory effect of D-GAL on HUVEC viability, effectively alleviated D-GAL-induced impairment of tube-forming ability, and promoted angiogenesis and migration ability of the cells. AS-IV also significantly reduced the rate of D-GAL-induced HUVECs positive for senescence-associated β-galactosidase (SA-β-Gal) staining and inhibited the expression of senescence-related genes P21 and P53. AS-IV restored mitochondrial membrane potential and reduced intracellular ROS levels in D-GAL-induced HUVECs, and inhibited the fusion of autophagosomes and lysosomes to prevent the completion of autophagic flux. In HUVECs treated with both D-GAL and AS-IV, the application MTK458 significantly increased the number of yellow spots and enhanced the expressions of P21, P53, PINK1, Parkin, LC3, and Beclin proteins. **Conclusion** AS-IV alleviates D-GAL-induced endothelial cell senescence by inhibiting the PINK1/Parkin pathway to regulate mitochondrial autophagy.

Keywords: astragaloside; PINK1/Parkin signaling pathway; mitochondrial autophagy; D-galactose; endothelial cells; cellular senescence

细胞衰老,作为生物体老化的核心机制之一,近年来其作为慢性疾病驱动因素的病理学价值受到深度关注。内皮细胞衰老在血管老化进程中具有重要作用,其功能障碍直接参与动脉粥样硬化斑块形成、血管弹性下降及高血压病理重构等关键环节[1-3]。这些研究强调了细胞衰老不仅是生物体老化的标志,更是多种慢性疾病的驱动因素。特别是在内皮细胞中,衰老的内皮细胞呈现线粒体动力学失衡特征,表现为活性氧(ROS)过度蓄积、线粒体膜电位崩溃及生物发生障碍,直接影响血管

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的结构和功能,进而促进心血管疾病的发展[4]。因此,寻找有效干预内皮细胞衰老的策略,对于延缓血管老化和相关疾病的进程具有重要意义。

线粒体自噬是细胞清除受损线粒体的重要途径,对于维持线粒体功能和细胞稳态至关重要[5]。PINK1/Parkin通路作为核心调控轴,通过PINK1蓄积磷酸化线粒体外膜蛋白,招募Parkin泛素化标记受损线粒体,启动自噬体包裹降解。衰老微环境中,氧化应激致Parkin转位受阻、泛素连接酶活性丧失,引发线粒体功能衰竭与自噬失代偿[6]。

黄芪甲苷(AS-Ⅳ)作为传统中药黄芪的主要活性成分,近年研究发现其可通过抑制PINK1/Parkin或者AMPK/SIRT3轴改善线粒体功能,但具体作用靶点尚未明确[7, 8]。值得注意的是,D-半乳糖(D-GAL)诱导的亚急性衰老模型能精准模拟自然衰老的线粒体代谢表型,其通过半乳糖醇代谢途径产生过氧化氢等自由基,特异性攻击线粒体复合物I,导致mtDNA损伤和呼吸链解耦联,最终导致细胞衰老[9],但针对线粒体自噬修复的干预研究仍处于空白。

尽管现有研究已证实AS-Ⅳ具有调控线粒体自噬潜能,但其对D-GAL诱导HUVEC衰老的具体机制尚未明确。本研究创新性地提出:通过AS-Ⅳ干预,能够改善D-GAL诱导的HUVEC细胞衰老现象,并深入探究AS-Ⅳ通过抑制PINK1/Parkin通路从而调控线粒体自噬的分子机制,为临床延缓血管衰老及相关疾病提供了实验基础。

1 材料和方法

1.1 细胞株

人脐静脉内皮细胞(HUVEC)采购于上海中桥新舟生物有限公司。

1.2 主要试剂

AS-Ⅳ(PERFEMIKER);D-GAL(索莱宝);人脐静脉内皮细胞完全培养基(中桥新舟);细胞衰老β-半乳糖苷酶染色试剂盒(Beyotime)、CCK-8试剂盒(Abbkine);JC-1试剂盒(翌圣生物);LC3-Ⅰ/Ⅱ、P62、Beclin、PINK和Parkin(Proteintech);P21和P53(Invitrogen);β-actin和GAPDH(Abcam)。

1.3 细胞培养及分组

HUVEC培养在含有10%胎牛血清、100 U/mL青霉素和100 mg/mL链霉素的DMEM-F12培养基中,在37 ℃、5% CO_2培养箱中进行培养。将HUVEC随机分为5组:空白对照组(NC组)、加入40 g/L D-GAL(D-GAL)组、加入200 μmol/L AS-IV(AS-IV)组、40 g/LD-GAL干预24 h后加入200 μmol/L的AS-IV继续干预24 h(D-GAL+AS-IV)组,和D-GAL+AS-IV+MTK45(40 g/L D-GAL+200 μmol/L+25 μmol/L)组,干预48 h后收取细胞样本进行实验。

1.4 CCK-8检测细胞增殖

将干预48 h后的各组HUVEC收集重悬接种于96孔板中,每孔加10 μL CCK-8工作液,孵育3 h后酶标检测仪于450 nm波长处检测吸光值$A_{450\,nm}$。

1.5 细胞划痕

将HUVEC细胞($1×10^5$/mL)接种在6孔板中并培养24 h后进行划痕实验,用无菌移液枪枪头垂直于孔板制造细胞划痕,于0和24 h拍照并测算细胞划痕的愈合率(%)。

1.6 β-半乳糖苷酶染色

将HUVEC细胞($1×10^5$/mL)接种在12孔板(3个复孔/组)中培养48 h后,按照空白对照组、AS-IV组、D-GAL组和D-GAL+AS-IV组分组给药。第2天用β-半乳糖苷酶染色,用荧光显微镜拍摄记录其结果,计算并统计阳性染色率。

1.7 细胞成血管功能检测

先于96孔板中加入50 μL/孔基质胶,后将干预48 h的各组细胞收集重悬接种于胶上,继续孵育3 h后,镜下观察小管直径(mm),取其平均值,评价成管能力。

1.8 Tunel检测细胞凋亡情况

将分组干预后的各组HUVEC采用Tunel检测试剂盒(一步法)参照说明书指示,在样品上加50 μL TUNEL检测液,37 ℃避光孵育60 min,孵育完成后在倒置光学显微镜下观察拍照。

1.9 流式细胞仪检测ROS水平

干预48 h的各组细胞收集重悬后加入1∶1000无血清培养基稀释的ROS荧光探针,间隔混匀,孵育30 min后洗脱未结合的荧光探针,PBS重悬细胞上机进行检测。

1.10 线粒体膜电位检测

JC-1工作液按1∶1000用无血清培养基稀释稀释至10 μmol/L,在6孔板中培养的各组HUVEC细胞加入1 mL JC-1工作液,37 ℃孵育20 min后用JC-1缓冲液清洗,加入2 mL不含血清培养基后在荧光倒置显微镜下采集图片。

1.11 实时荧光定量PCR

使用快速提取试剂盒提取各组HUVEC细胞RNA,测定RNA的纯度与浓度。随后反转录为cDNA,配制PCR反应体系进行基因扩增及定量检测分析。用$2^{-\Delta\Delta CT}$法推算各组HUVEC细胞中目标基因的表达。详细操作步骤严格遵循试剂盒说明书,人源的基因引物序列详见表1(上海捷瑞生物工程有限公司)。

1.12 定表达自噬双标慢病毒(mRFP-eGFP-LC3)的构建

HUVEC细胞以10^5/皿接种于共聚焦小皿,给予不

表1 qRT-PCR引物序列

Tab.1 Primers sequences for qRT-PCR

Gene	Primer sequences (5'-3')
human-GAPDH	F:GGAGCGAGATCCCTCCAAAAT
	R:GGCTGTTGTCATACTTCTCATGG
human-TP53 (P53)	F:CAGCACATGACGGAGGTTGT
	R:TCATCCAAATACTCCACACGC
human-p21	F:TGTCCGTCAGAACCCATGC
	R:AAAGTCGAAGTTCCATCGCTC
Human-PINK1	F: GCCTCATCGAGGAAAAACAGG
	R: GTCTCGTGTCCAACGGGTC
Human-parkin	F:GTGTTTGTCAGGTTCAACTCCA
	R:GAAAATCACACGCAACTGGTC
Human-p62	F:GCACCCCAATGTGATCTGC
	R:CGCTACACAAGTCGTAGTCTGG
Human-Baclin1	F:CCATGCAGGTGAGCTTCGT
	R:GAATCTGCGAGAGACACCATC

同处理培养48 h后，根据制造商的说明用mRFP-EGFP-LC3慢病毒（海星生物）处理HUVEC细胞，于激光共聚焦荧光显微镜下观察红色和绿色LC3的荧光点变化，随机选取一个视野下所有细胞进行拍照，分析自噬变化。

1.13 Western blotting法检测自噬蛋白/衰老标记蛋白表达水平

干预48 h的各组细胞收集提取蛋白，BCA法测蛋白浓度，依次进行电泳、转膜、脱脂奶粉室温封闭；分别孵育一抗LC3Ⅱ/Ⅰ（1∶1000）、P62（1∶5000）、Beclin1（1∶1000）、PINK1（1∶500）、Parkin（1∶1000）及GAPDH（1∶2000）、后孵育兔二抗（1∶10 000）。通过ImageJ软件对各个条带的灰度值进行比对分析。

1.14 统计学分析

Image J软件用于分析结果图及Werstern blotting结果，GraphPadPrism9.0软件则用于数据的统计分析及绘图。采用SPSS26.0统计软件进行数据分析。计量资料以均数±标准差表示，组间比较采用one-way ANOVA分析，$P<0.05$为差异有统计学意义。

2 结果

2.1 AS-IV对D-GAL诱导HUVEC细胞活力的影响

0~20 g/L浓度的D-GAL对正常HUVEC细胞无显著影响，当浓度达到，40 g/L时，细胞存活率降低（$P<0.05$），与NC组相比，D-GAL组细胞活力降低（$P<0.05$）；经AS-IV干预后，D-GAL+AS-IV组细胞活力升高（$P<0.05$，图1）。

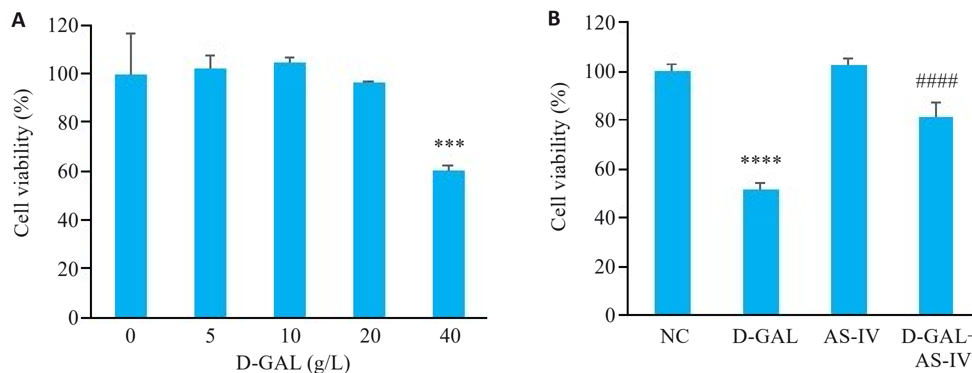

图1 AS-IV对D-GAL诱导HUVEC细胞活力的影响

Fig. 1 Effect of AS-IV on viability of D-GAL-induced human umbilical vein endothelial cells (HUVECs). **A**: Effect of different concentrations of D-GAL on HUVEC viability. **B**: Viability of HUVECs with different treatments. $^{***}P<0.001$, $^{****}P<0.0001$ *vs* NC group; $^{\#\#\#\#}P<0.0001$ *vs* D-gal group.

2.2 AS-IV对D-GAL诱导的HUVEC管状结构形成能力的影响

与NC组相比，D-GAL组管腔长度显著缩短、分支点数减少，HUVEC细胞的管状结构形成能力受到抑制（$P<0.05$），但是，经AS-IV干预后，D-GAL+AS-IV组管腔长度和分支点数显著增加，HUVEC细胞的管状结构形成能力增强（$P<0.05$，图2）。

2.3 AS-IV对D-GAL诱导的HUVEC细胞迁移能力影响

与NC组相比，D-GAL组细胞迁移能力受到显著抑制，伤口愈合率在24 h低于NC组（$P<0.05$）。经AS-IV干预后，D-GAL+AS-IV组划痕愈合率在24 h提高（$P<0.05$，图3）。

2.4 AS-IV对D-GAL诱导的HUVEC细胞半乳糖染色阳性率影响

与NC组相比，D-GAL组大量细胞呈现明显的蓝色染色，阳性细胞百分比高于NC组（$P<0.05$）。经AS-IV干预后，D-GAL+AS-IV组阳性细胞百分比降低（$P<0.05$，图4）。

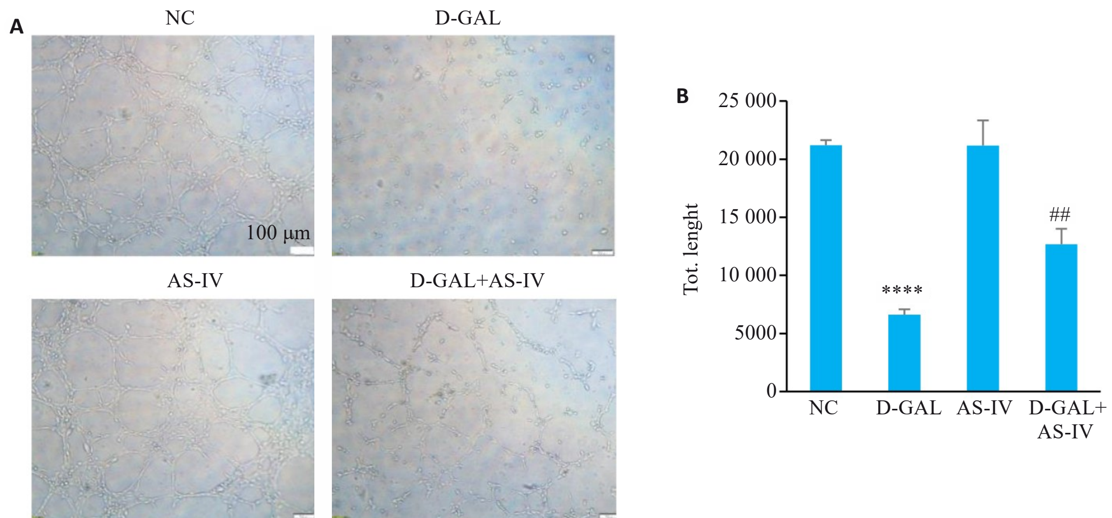

图2 AS-IV对D-GAL诱导的HUVEC管状结构形成能力的影响

Fig.2 Effect of AS-IV on tube formation ability of HUVEC induced by D-GAL. **A**: Tubule formation assay images of HUVEC cells after treatment with various groups. **B**: Statistical graph of the tubule formation assay (Scale bar=100 μm), ****P<0.0001 *vs* NC group, ##P<0.01 *vs* D-GAL group.

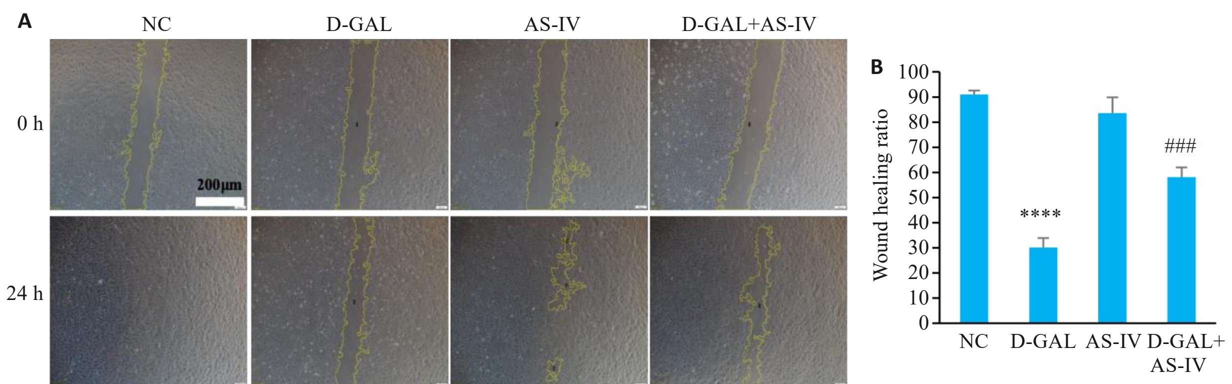

图3 AS-IV对D-GAL诱导的HUVEC细胞迁移能力影响

Fig. 3 Effect of AS-IV on migration ability of D-GAL-induced HUVECs. **A**: Observation of migration of HUVECs after different treatments (Scale bar=200 μm). **B**: Wound healing rates of HUVECs with different treatments. ****P<0.0001 *vs* NC group; ###P<0.001 *vs* D-GAL group.

2.5 AS-IV对D-GAL诱导的HUVEC细胞中P21和P53的表达影响

与NC组相比，D-GAL组P21和P53的mRNA及蛋白表达水平上调（P<0.05），经AS-IV干预后，D-GAL+AS-IV组的mRNA及蛋白表达水平下调（P<0.05，图5）。

2.6 AS-IV对D-GAL诱导的HUVEC细胞中线粒体膜电位影响

与NC组相比，D-GAL组JC-1染色显示绿光荧光强度增加（P<0.05），表明线粒体膜电位下降（P<0.05），经AS-IV干预后，D-GAL+AS-IV组的绿光荧光强度降低（P<0.05，图6）。

2.7 AS-IV对D-GAL诱导的HUVEC细胞中ROS阳性细胞百分比影响

与NC组相比，D-GAL组细胞内ROS水平显著升高，ROS阳性细胞百分比高于NC组（P<0.05），经AS-IV干预后，D-GAL+AS-IV组的ROS阳性细胞百分比降低（P<0.05，图7）。

2.8 AS-IV对D-GAL诱导的HUVEC细胞中自噬情况的影响

通过荧光显微镜观察mRFP（红色荧光）和GFP（绿色荧光）标记的LC3蛋白分布（图8）显示，与NC组相比，D-GAL组细胞中黄色斑点增加（P<0.05），表明自噬流呈现上升趋势，经AS-IV干预后，D-GAL+AS-IV组的黄色斑点减少（P<0.05）。

2.9 AS-IV对D-GAL诱导的HUVEC细胞中PINK1/Parkin通路及细胞自噬相关蛋白表达的影响

与NC组相比，D-GAL组P62 mRNA及蛋白表达水平显著下调（P<0.05），而LC3 Ⅱ/Ⅰ蛋白、Beclin、PINK和Parkin的mRNA及蛋白表达水平显著上调（P<0.05），经AS-IV干预后，D-GAL+AS-IV组的P62 mRNA及蛋白表达水平显著上调（P<0.05），而LC3 Ⅱ/Ⅰ蛋白、Beclin、PINK和Parkin的mRNA及蛋白表达水平显著下调（P<0.05，图9）。

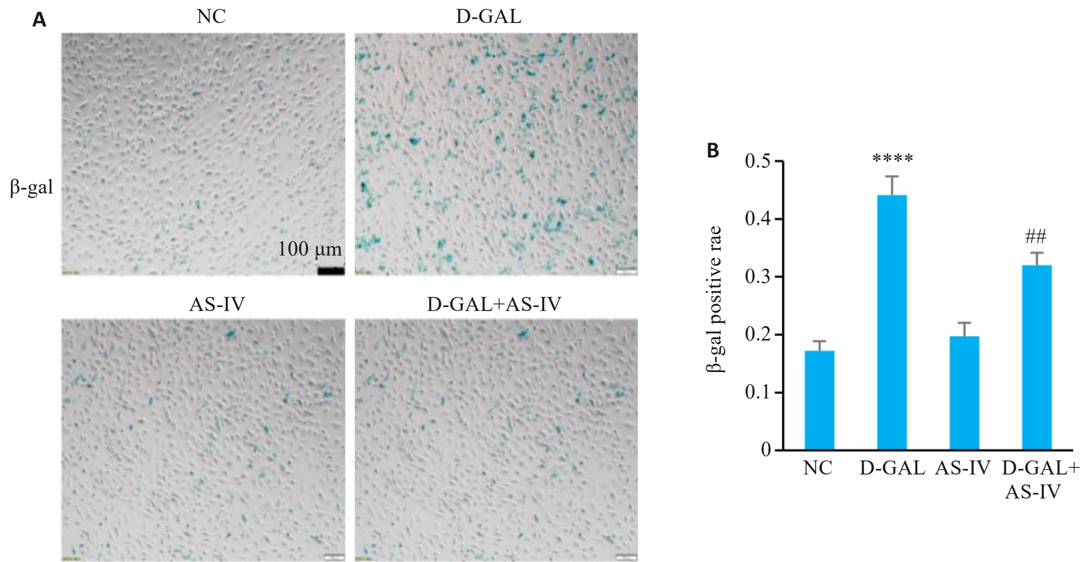

图4 AS-IV对D-GAL诱导的HUVEC细胞半乳糖染色阳性率影响

Fig. 4 Effect of AS-IV on SA-β-Gal staining positivity rate in D-GAL-induced HUVECs. **A**: SA-β-Gal staining of HUVEC cells with different treatments (Scale bar=100 μm). **B**: SA-β-Gal staining positiving rates of the cells. ****P<0.0001 *vs* NC group; ##P<0.01 *vs* D-GAL group.

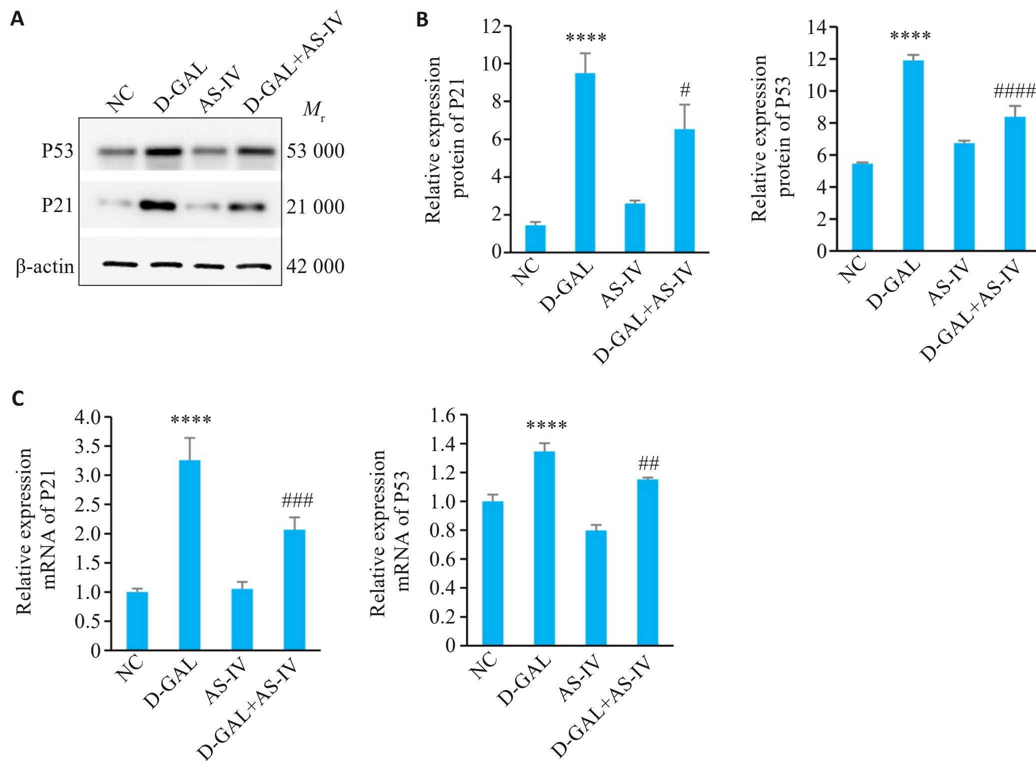

图5 AS-IV对D-GAL诱导的HUVEC细胞中P21和P53的表达影响

Fig. 5 Effect of AS-IV on the Expression of P21 and P53 in D-GAL-Induced HUVEC Cells. **A**: Protein band diagram of P53 and P21 in four groups of cells. **B**, **C**: Statistical graph of P21 and P53 gene and protein expression. ****P<0.0001 *vs* NC group; #P<0.05, ##P<0.01, ###P<0.001, ####P<0.0001 *vs* D-GAL group.

2.10 MTK458对D-GAL诱导的HUVEC细胞中自噬体与溶酶体的融合及自噬流的影响

与D-GAL+AS-IV组相比，D-GAL+AS-IV+MTK458组细胞中黄色斑点明显增多(P<0.05,图10)。

2.11 MTK458对D-GAL诱导的HUVEC细胞中PINK1/Parkin通路及细胞衰老、自噬相关蛋白表达的影响

与D-GAL+AS-IV组相比，D-GAL+AS-IV+MTK458组P62 mRNA及蛋白表达水平显著下调

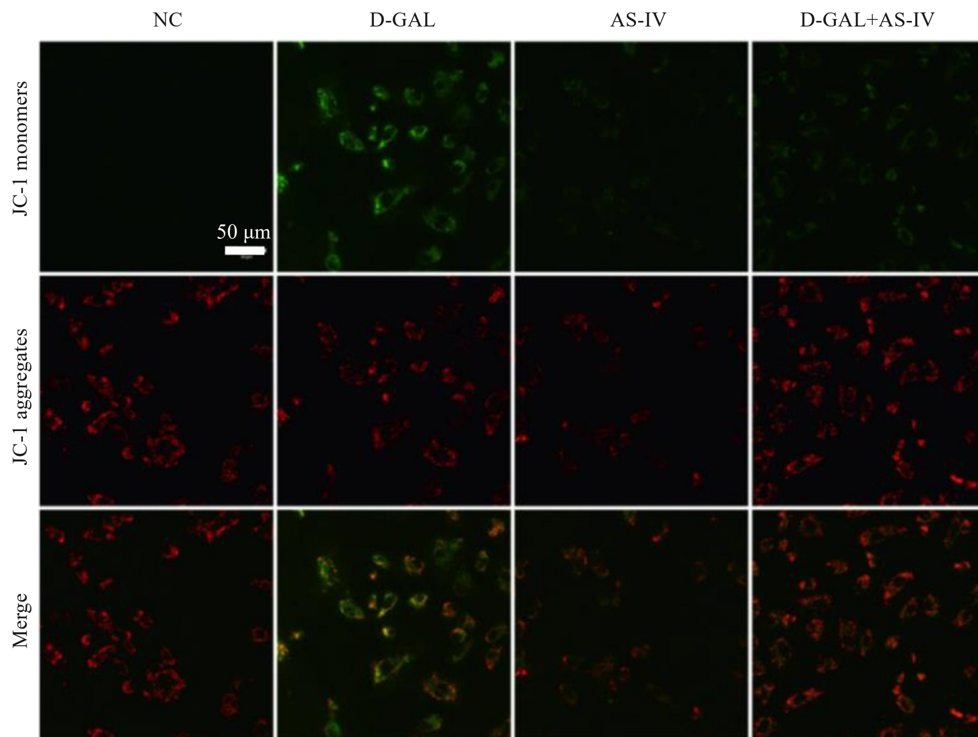

图6 AS-IV对D-GAL诱导的HUVEC细胞中线粒体膜电位影响

Fig.6 Effect of AS-IV on mitochondrial membrane potential in D-GAL-induced HUVECs (Scale bar=50 μm).

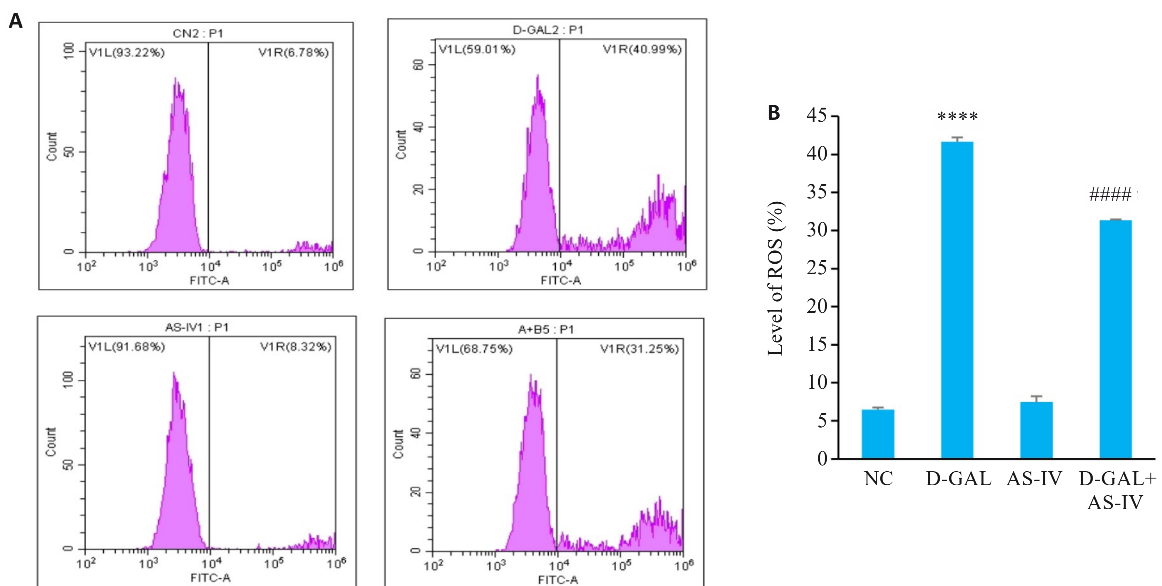

图7 AS-IV对D-GAL诱导的HUVEC细胞中ROS阳性细胞百分比影响

Fig.7 Effect of AS-IV on percentage of ROS-positive HUVECs induced by D-GAL. **A**: ROS levels in HUVEC cells with different treatments. **B**: Statistical graph of ROS levels. *****P*<0.0001 *vs* NC group; ####*P*<0.0001 *vs* D-gal group.

（*P*<0.05），而P21、P53、LC3 Ⅱ/Ⅰ蛋白、Beclin、PINK和Parkin的mRNA及蛋白表达水平显著上调（*P*<0.05，图11）。

3 讨论

内皮细胞衰老作为血管老化的核心病理环节，不仅是机体衰老进程的关键表征，更是动脉粥样硬化、高血压等重大心血管疾病发生发展的核心驱动因素[10-12]。D-GAL作为一种经典的衰老诱导剂，其通过非酶促糖基化反应生成大量ROS，ROS具有强氧化性，可攻击线粒体，导致线粒体氧化应激，使线粒体膜电位改变、呼吸链功能受损。同时，ROS还会引发内膜脂质过氧化，破坏细胞膜的完整性和流动性。这些变化进一步激活p53/p21信号通路，p53作为转录因子，可上调p21的表

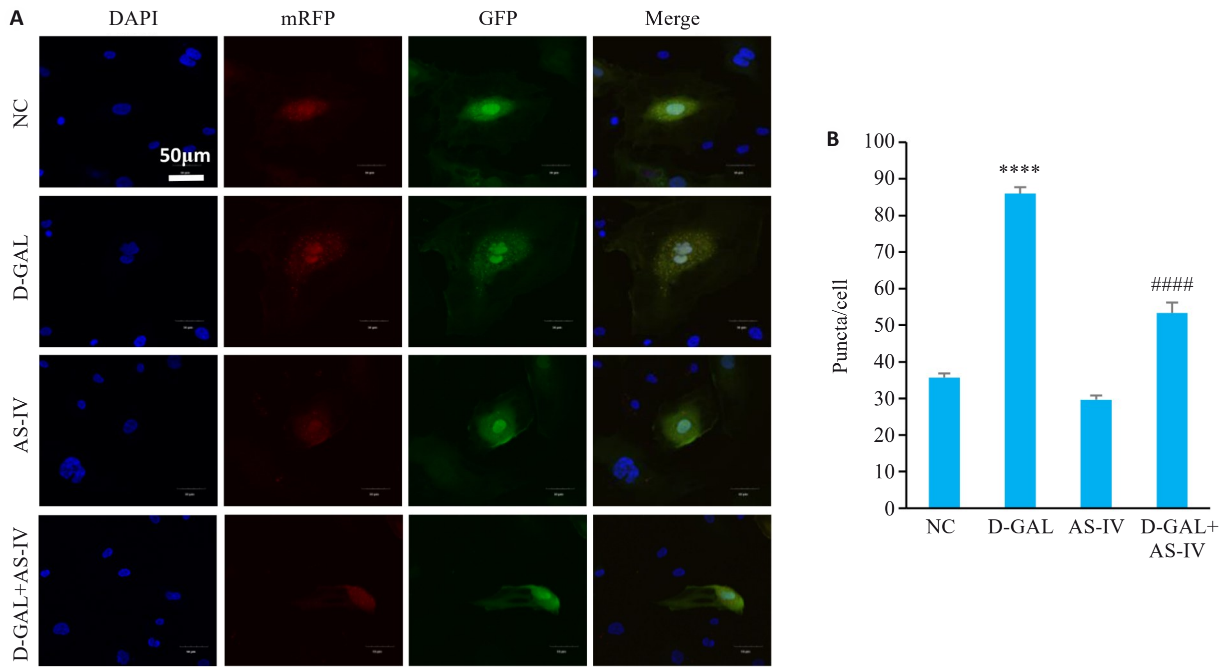

图8 AS-IV对D-GAL诱导的HUVEC细胞中自噬体与溶酶体的融合及自噬流的影响

Fig.8 Effect of AS-IV on autophagosome-lysosome fusion and autophagy flux in D-GAL-induced HUVECs. **A**: Autophagy flux assay of HUVECs with different treatments (Scale bar=50 μm). **B**: Statistical graph of puncta counts. ****P<0.0001 *vs* control group; ####P<0.0001 *vs* D-GAL group.

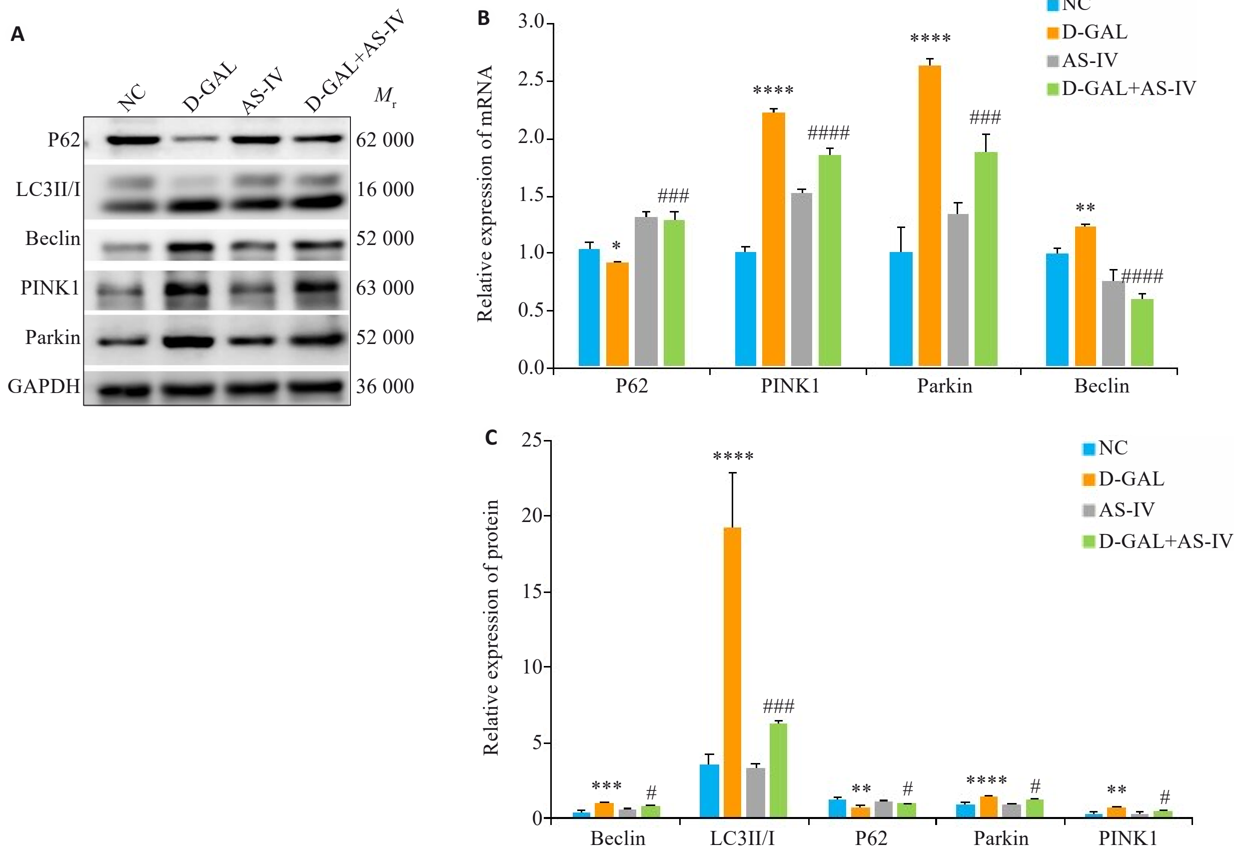

图9 AS-IV对D-GAL诱导的HUVEC细胞中PINK1/Parkin通路及细胞自噬相关蛋白表达的影响

Fig.9 Effect of AS-IV on expression of PINK1/Parkin pathway and autophagy-related proteins in D-GAL-induced HUVECs. **A**: Protein bands of P62, LC3II/I, Beclin, PINK1, and Parkin in the 4 groups. **B**: Relative mRNA expressions of P62, Beclin, PINK, and Parkin. **C**: Statistical graph of grayscale values of the protein bands of P62, LC3II/I, Beclin, PINK1, and Parkin. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 *vs* NC group; #P<0.05, ###P<0.001, ####P<0.0001 *vs* D-gal group.

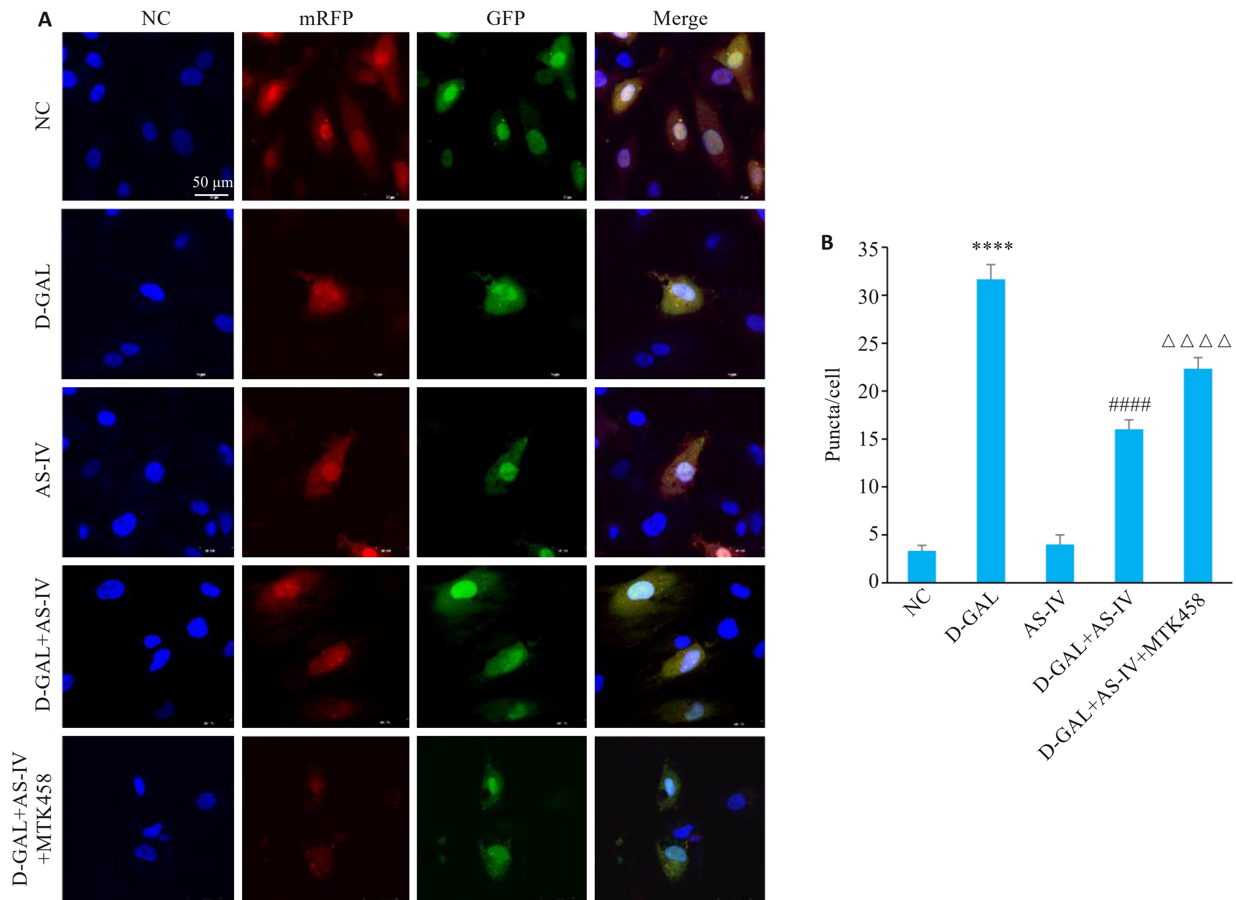

图10 MTK458对D-GAL诱导的HUVEC细胞中自噬体与溶酶体的融合及自噬流的影响

Fig.10 Effect of MTK458 on autophagosome-lysosome fusion and autophagy flux in D-GAL-induced HUVECs. **A**: Autophagy flux assay of HUVECs with different treatments (Scale bar=50 μm). **B**: Statistical graph of puncta counts. *****P*<0.0001 *vs* NC group; ####*P*<0.0001 *vs* D-gal group; △△△△*P*<0.0001 *vs* D-gal+AS-IV group.

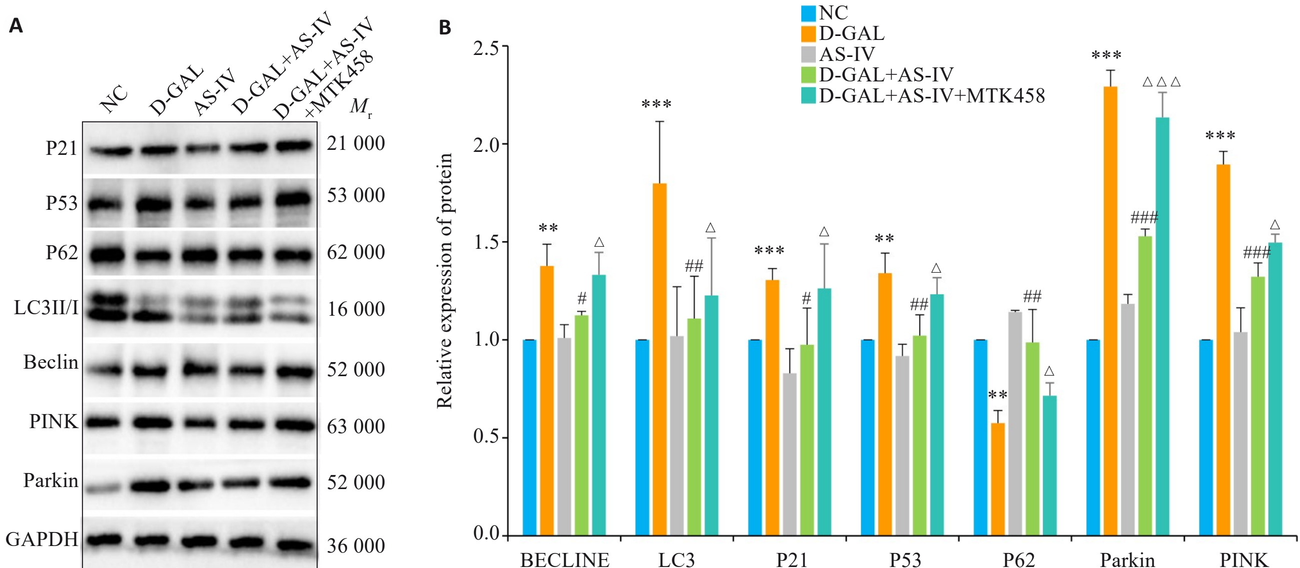

图11 MTK458对D-GAL诱导的HUVEC细胞中PINK1/Parkin通路及细胞衰老、自噬相关蛋白表达的影响

Fig. 11 Effect of MTK458 on the PINK1/Parkin pathway and expressions of proteins related to cellular senescence and autophagy in D-GAL-induced HUVECs. **A**: Protein bands in the 5 groups of cells. **B**: Statistical graph of grayscale values of the protein bands. ***P*<0.01, ****P*<0.001 *vs* NC group; #*P*<0.05, ##*P*<0.01, ###*P*<0.001 *vs* D-gal group; △*P*<0.05, △△△*P*<0.001 *vs* D-gal+AS-IV group.

达,p21通过抑制细胞周期蛋白依赖性激酶,使细胞周期停滞在G1期,最终诱导细胞呈现衰老表型[13, 14]。本研究中,D-GAL处理显著降低了HUVEC细胞活力、抑制血管生成能力、削弱迁移能力,并伴随衰老相关β-半乳糖苷酶(SA-β-Gal)阳性率升高及衰老相关基因(P21、P53)表达上调。这些结果与D-半乳糖诱导衰老的经典机制一致,说明本实验模型构建成功。

AS-IV作为传统中药黄芪的主要活性成分,其抗氧化、抗炎和抗衰老作用已被广泛研究[15, 16]。AS-IV可通过清除自由基、抑制脂质过氧化以及激活抗氧化酶来减轻氧化应激损伤,同时调节免疫反应,减轻炎症对细胞的损伤[17, 18]。近年来,AS-IV在调节细胞自噬和线粒体功能方面的作用逐渐受到关注[19-21]。本研究中,AS-IV干预后,D-GAL诱导的HUVEC细胞衰老表型得到显著逆转,包括细胞活力恢复、血管生成能力增强、迁移能力改善以及SA-β-Gal阳性率下降。这些结果与AS-IV的抗氧化和抗炎作用密切相关[22]。AS-IV可能通过减轻氧化应激和炎症反应,改善细胞内环境,从而逆转细胞衰老。

线粒体自噬是细胞维持自身稳态的重要机制,通过自噬溶酶体机制选择性清除受损线粒体,对于维持线粒体质量和细胞功能至关重要[23, 24]。本研究中,D-GAL处理导致线粒体膜电位下降、细胞内ROS水平升高,并伴随自噬体与溶酶体的融合,自噬流增加。这一现象与D-半乳糖诱导的线粒体功能障碍和自噬机制相符[25, 26]。D-半乳糖可通过降低线粒体生物发生关键蛋白(如PGC-1α)的表达,阻碍线粒体新生和稳态[27, 28]。AS-IV干预后,线粒体膜电位恢复、ROS水平降低,并伴随自噬体与溶酶体分离,表明自噬流受到抑制。这一结果与AS-IV抑制线粒体自噬的作用一致[29]。AS-IV可能通过调节相关信号通路,改善线粒体功能,抑制自噬体与溶酶体的融合,阻止自噬流。

PINK1/Parkin通路作为线粒体自噬的核心调控枢纽,其动态平衡对维持线粒体稳态至关重要[30, 31]。在正常情况下,PINK1在线粒体内膜上被切割并清除;当线粒体受损时,PINK1在线粒体外膜上积累并招募Parkin,进而激活线粒体自噬[32, 33]。本研究中,D-GAL处理后PINK1和Parkin的mRNA及蛋白表达水平显著上调,表明PINK1/Parkin通路被激活。AS-IV干预后,PINK1和Parkin的表达水平恢复正常。这一机制与AS-IV的抗氧化和抗炎作用相互协同,共同减轻D-GAL诱导的细胞衰老[34, 35]。加入线粒体自噬激动剂MTK458后,促进D-GAL诱导的HUVEC细胞自噬体与溶酶体的融合,促进自噬流的完成,PINK1/Parkin通路、细胞衰老相关蛋白、自噬相关蛋白表达均上升。证明AS-IV减轻D-GAL诱导的内皮细胞衰老,与PINK1/Parkin通路调控线粒体自噬有关。

AS-IV作为黄芪的主要活性成分,具有来源广泛、安全性高、副作用小等优点。目前,AS-IV已用于多种疾病的治疗,如心血管疾病、糖尿病及其并发症等[36, 37]。本研究结果揭示了AS-IV通过抑制PINK1/Parkin通路调控线粒体自噬的抗衰老机制,为AS-IV的临床应用提供了实验基础和理论依据。本研究表明,AS-IV还具有显著的抗衰老作用,可望用于延缓血管衰老及相关疾病的发生发展。

然而,本研究仍存在一定局限性。首先,研究仅在细胞层面进行,未涉及动物或人体实验,AS-IV在整体动物模型中的效果尚待验证。其次,PINK1/Parkin通路与其他信号通路(如mTOR、AMPK)的交互作用尚未明确,这些信号通路之间可能存在复杂的网络调控关系,共同影响细胞衰老和线粒体自噬。最后,AS-IV的剂量效应关系及长期安全性需进一步探索,不同剂量可能产生不同的效果,长期使用是否会产生不良反应也不清楚。未来研究可构建动物衰老模型,为AS-IV的广泛应用提供更坚实的理论基础和临床依据。

Declaration of interests: The authors declare no competing interests.

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