



长寿富集代谢物二甲基甘氨酸抗衰老作用研究*

胡杰^{1,2)} 蒲臻钰²⁾ 李俊霖²⁾ 曹菊²⁾ 林芷欣²⁾ 安巍巍¹⁾ 李雪萌^{2,3)**} 安静^{1)**}⁽¹⁾ 哈尔滨医科大学肿瘤防治研究所, 哈尔滨 150081;⁽²⁾ 广东医科大学基础医学院, 湛江市人体微生态研究与临床转化重点实验室, 湛江 524023;⁽³⁾ 广东医科大学海洋与热带医学学院, 湛江市环北部湾海洋微生物研究开发重点实验室,
广东省红树林湿地药用资源开发与利用工程技术研究中心, 湛江 524023)

摘要 目的 基于本课题组前期血清代谢组学研究发现的二甲基甘氨酸 (dimethylglycine, DMG) 在长寿家系中显著富集的现象, 探讨DMG的体内和体外抗衰老效应并初步阐明其分子机制。**方法** 以复制性衰老的人胚肺成纤维细胞 WI-38 为体外模型, 采用CCK-8和乳酸脱氢酶法评估DMG的细胞安全性, 通过SA- β -Gal染色、EdU染色、活性氧类检测评估DMG对细胞衰老表型、增殖及氧化应激的影响。以秀丽隐杆线虫为体内模型, 通过线虫寿命、热应激、脂褐素及体内活性氧类和油红O染色实验, 评估DMG在模式生物中的抗衰老效果。并进一步通过酶联免疫吸附分析、实时荧光定量PCR、分子对接等技术探究DMG抗衰老的分子靶点与通路。**结果** 在细胞水平上, 50 μ mol/L DMG处理5 d可安全、显著地降低WI-38衰老细胞的SA- β -Gal阳性率 ($P<0.0001$)、活性氧类水平 ($P<0.0001$), 并促进其增殖 ($P=0.0035$), 同时, DMG能下调p16、p21衰老关键蛋白及IL-6、IL-8等衰老相关分泌表型因子的表达。在线虫模型上, DMG可延长线虫平均寿命 ($P<0.0001$), 增强热应激抵抗能力 ($P=0.017$), 并显著降低体内脂褐素水平 ($P<0.0001$)、降低活性氧类水平 ($P=0.008$) 及减少脂肪积累 ($P<0.0001$)。网络药理学预测表明, DMG可能与GST家族蛋白及CYP2C9等靶点稳定结合, 从而通过调节氧化应激与代谢通路实现抗衰老效果。**结论** DMG在细胞和动物水平均展现出显著的抗衰老活性, 其作用机制可能与缓解氧化应激、抑制核心衰老信号通路及改善代谢紊乱密切相关。本研究为DMG作为潜在的抗衰老制剂提供了实验依据。

关键词 二甲基甘氨酸, 细胞衰老, 秀丽隐杆线虫, 分子对接, 寿命延长**中图分类号** Q255**DOI:** 10.3724/j.pibb.2025.0574**CSTR:** 32369.14.pibb.20250574

全球范围内日益加剧的人口老龄化对社会经济和公共健康构成了严峻挑战, 深入解析衰老的生物学机制并寻求有效的干预策略已成为生命科学领域的迫切课题^[1-2]。在衰老研究中, 人胚肺成纤维细胞 WI-38 作为一种经典的复制性衰老模型, 被广泛用于在细胞水平揭示衰老的机制及评价药物的抗衰老作用^[3]。另外, 秀丽隐杆线虫因其寿命短、遗传工具完善等优势, 已成为在动物水平筛选和验证抗衰老化合物的首选模式生物, 研究者经常利用线虫模型筛选并验证小分子或中药提取物的抗衰老效果和机制^[4-5]。本课题组前期通过血清代谢组学研究发现, 长寿家族人群血清中二甲基甘氨酸 (dimethylglycine, DMG) 水平显著升高, 提示其可能是人类长寿的潜在相关代谢物^[6]。研究发现,

DMG是一种天然存在于人体的氨基酸代谢中间体, 其具有抗氧化与调节免疫功能的潜在功效^[7-8]。然而, DMG是否具备直接的抗衰老功能, 以及通过何种分子途径发挥作用尚不明确。因此, 本研究旨在利用WI-38体外细胞模型与线虫体内模型系统评估DMG的抗衰老表型, 并利用生物信息学的技术探究其可能的作用机制。

* 国家自然科学基金 (82301752), 黑龙江省自然科学基金联合引导项目 (LH2020H111), 广东省基础与应用基础研究基金联合基金-地区培育项目 (DGA0706) 和广东医科大学人体微生态创新创业项目基地项目 (JDXM2024169) 资助。

** 通讯联系人。

安静 Tel: 15004548046, E-mail: 210001@hrbmu.edu.cn

李雪萌 Tel: 0769-63552289, E-mail: lixuemeng@gdmu.edu.cn

收稿日期: 2025-12-30, 接受日期: 2026-03-16

1 材料与方法

1.1 对象、材料与仪器

1.1.1 实验对象与材料

人胚肺成纤维细胞 WI-38 购买自中国武汉亚科因生物技术有限公司, 初始代数 为 P3。利用传代的方式养至细胞达到复制性衰老 (细胞群体倍增次数 (population doubling, PD) >35 代) 开展细胞实验; 野生型 N₂ 线虫虫株和喂养线虫所需的大肠杆菌 OP50 购买自福建上源生物科技有限公司。

1.1.2 实验试剂与仪器

全波长多功能酶标仪 (Thermo, Varioskan

Flas 型), 荧光定量 PCR 仪 (ABI, 7900HT 型), 细胞培养箱 (Thermo Scientific, HERA cell 150i), 厌氧培养箱 (DWS, A20 型), 24 孔板 (Corning, 3524), 胎牛血清 (NEWZERUM, ce-500), MEM 完全培养基 (GIBCO, C12571500BT), 六孔板 (LABSELECT, 11110), 96 孔板 (LABSELECT, 11510), SA-β-gal 细胞衰老检测试剂盒 (No. C0602, Beyotime, 上海, 中国), EdU-555 细胞增殖检测试剂盒 (No.C0075S, Beyotime, 上海, 中国), 活性氧类检测试剂盒 (No. S0033S, Beyotime, 上海, 中国), GAPDH、p16 抗体购自 proteintech, p21 抗体购自赛维尔, 引物扩增序列见表 1。

Table 1 Sequences of primers used for the amplification of qPCR

Gene	Forward	Reverse
<i>β-Actin</i>	CCTTCCCTCCTCAGATCATTGC	ATACTCCTGCTTGCTGATCCAC
<i>IL-6</i>	TGAGGAGACTTGCCTGGTGAA	CAGCTCTGGCTTGTTCCTCAC
<i>IL-8</i>	TCCTGATTCTGCAGCTCTGT	AAATTTGGGGTGGAAGGTT
<i>P21</i>	CCCGCCTTTTCTTCTTAGC	TTCTCATGCCATTCTTTCC
<i>MMPI</i>	GGGGCTTTGATGTACCCTAGC	TGTCACACGCTTTTGGGGTTT

1.2 细胞实验

1.2.1 细胞计数试剂盒 -8 (cell counting kit-8, CCK-8) 检测代谢物的细胞毒性

取对数生长期细胞, 接种于 96 孔板。37℃、5% CO₂ 培养箱中预培养 24 h。实验分组设置对照组、空白组 (仅含培养基) 及不同浓度药物处理组, 每组设 6 个复孔。药物处理结束后, 加入 10 μl/孔 CCK-8 试剂。37℃ 孵育 2 h, 测定 A₄₅₀。细胞增殖率计算公式: 增殖率=[(实验组 A 值-空白组 A 值)/(对照组 A 值-空白组 A 值)]×100%。

1.2.2 乳酸脱氢酶 (lactate dehydrogenase, LDH) 检测代谢物的细胞毒性

取对数生长期细胞接种于 96 孔板, 37℃、5% CO₂ 培养箱中培养 24 h。实验分为 3 组: 实验组、阴性对照组和阳性对照组。阳性对照组于实验结束前 60 min 加入细胞裂解液, 充分裂解细胞。药物处理结束后, 将 96 孔板离心 5 min, 吸取上清 50 μl。加入 50 μl/孔 LDH 反应混合液, 室温避光孵育 30 min。反应结束后, 加入 50 μl/孔终止液, 使用酶标仪测定 490 nm 处吸光度。

1.2.3 衰老相关 β 半乳糖苷酶 (senescence-associated β-galactosidase, SA-β-gal) 染色

参考文献 [9], 按照说明书操作, 染成蓝色的

细胞为 β 半乳糖苷酶染色阳性细胞, 每组同时做 4 个复孔, 每孔计数 200 个细胞计算 β 半乳糖苷酶染色阳性百分率。

1.2.4 活性氧类 (reactive oxygen species, ROS) 检测

按照说明书操作后, 每组同时做 4 个复孔, 用荧光显微镜直接观察, 使用 Image J 软件计算各组的荧光强度。

1.2.5 5-乙炔基 -2'-脱氧尿苷 (5-ethynyl-2'-deoxyuridine, EdU) 染色

按照说明书操作, 将细胞接种于 8 孔板上, 培养 48 h 后加入 EdU, 37℃ 孵育 2 h 后, 用 4% 多聚甲醛室温固定 15 min。0.1% Triton X-100 PBS 溶液透化 10 min 后, 采用 EdU 试剂盒进行染色。荧光显微镜下观察, 使用 ImageJ 软件计数 EdU 阳性细胞数, 计算增殖指数=(EdU⁺细胞数/DAPI⁺总细胞数)×100%。

1.2.6 实时荧光定量聚合酶链反应 (real-time quantitative polymerase chain reaction, RT-qPCR)

总 RNA 采用 TRIzol 试剂提取, RNA 经 DNase I 消化后, 用 PrimeScript RT reagent Kit (Takara) 合成 cDNA。qPCR 反应采用 SYBR Green 法 (TB Green Premix Ex Taq II, Takara)。采用 2^{-ΔΔC_t} 法分析基因相对表达量。

1.2.7 酶联免疫吸附分析 (enzyme-linked immunosorbent assay, ELISA) 检测蛋白质表达

细胞通过胰酶消化后收集, 通过反复冻融破裂细胞膜, 4°C、12 000g 离心 15 min, 取上清。蛋白质浓度采用二喹啉甲酸 (bicinchoninic acid, BCA) 法测定, 随后使用泉州睿信生物科技有限公司的人 p16 蛋白 ELISA 检测试剂盒 (RX106982) 和人 p21 蛋白 ELISA 检测试剂盒 (RX106309) 检测蛋白质含量。

1.3 线虫实验

1.3.1 线虫的同步化

将富卵线虫用 M9 缓冲液收集并离心获得虫体。随后使用裂解液 (NaOH (1 mol/L) : NaClO (5%) : M9 体积比 1 : 1 : 8) 进行裂解, 裂解完成后立即加入 M9 缓冲液终止反应, 经多次离心洗涤, 最终将获得的虫卵置于无食物线虫生长培养基 (nematode growth medium, NGM) 平板中过夜孵化。收集孵出的 L1 期幼虫, 转接至含大肠杆菌 OP50 食物的 NGM 平板上, 在 20°C 下培养至所需虫期以供后续实验。

1.3.2 线虫热应激

将同步化处理后的线虫培养至年轻成虫期, 转移至含代谢物的 NGM 平板中培养 5 d, 随后移至空白 NGM 平板, 封口后于 35°C 热应激 5 h, 再转入 20°C 恢复 12 h。按寿命分析标准统计存活与死亡线虫数, 计算存活率; 每组不少于 60 条线虫, 实验重复 3 次。

1.3.3 线虫寿命实验

将线虫在 20°C 下正常培养两代, 经同步化获得 L1 期幼虫后, 接种于含活菌 OP50 的 NGM 平板, 在 20°C 培养至 L4/年轻成虫期。随后将线虫转移至含代谢物 (添加热灭活 OP50) 的 NGM 平板中, 于 20°C 培养, 并每两日更换 1 次平板以维持药效与食物供给。从第 10 天起每日统计死亡线虫的数量, 直至全部死亡。死亡判定标准为咽泵停止或对轻触无反应, 排除逃逸、钻入琼脂、内孵或受伤的个体。每组至少使用 60 条线虫。

1.3.4 线虫 β 半乳糖苷酶染色

随机取第 5 天线虫, 根据 Senescence-Tracker™ 衰老细胞绿色荧光染色试剂盒 (SA-Green) 操作指南, 收集线虫虫体后, 加入 1 ml 1×Endogenous β -gal Inhibitor 工作液, 孵育 1 h, 洗涤, 缓慢加入 500 μ l SA-Green 染色工作液, 孵育 1 h 后, 洗涤后固定, 进行荧光拍照。每组计数线虫不少于 30 条。

1.3.5 线虫脂褐素积累测试

随机取第 5 天线虫, 经 M9 缓冲液洗涤后, 用 1 mmol/L 左旋咪唑进行固定, 利用荧光显微镜蓝色激发光 (405~488 nm) 进行荧光成像。使用 Image J 软件分析线虫脂褐素积累水平。每组计数线虫不少于 30 条。

1.3.6 线虫 ROS 检测

将同步化后的线虫培养至年轻成虫期, 分别转移至含或不含代谢物的 NGM 平板中处理。处理结束后, 在平板上加入混有 H2DCF-DA 探针的 OP50 菌液, 染色 1 h。随后将线虫转移至仅含 OP50 的 NGM 平板上, 每半小时更换 1 次平板, 重复 3 次以洗净表面探针。最后使用荧光显微镜拍摄线虫, 每组不少于 30 条, 并通过 Image J 软件定量分析荧光强度。

1.3.7 油红 O 染色检测线虫脂质积累

将同步化后培养至年轻成虫期的线虫转移至含代谢物的 NGM 平板中, 培养 10 d 后收集线虫, 使用 M9 缓冲液清洗, 经 4% 多聚甲醛固定 10 min, 再经 -80°C 反复冻融 4 次以破坏几丁质结构。随后以 M9 缓冲液清洗 3 次, 加入 60% 油红 O 染液室温染色 45 min, 再用含 0.01% 吐温的 M9 缓冲液洗去多余染液, 最后将线虫移至 2% 琼脂糖凝胶垫上进行拍照。每组不少于 30 条线虫, 使用 Image J 软件对油红 O 染色强度进行定量分析。

1.4 生物信息学分析

1.4.1 DMG 作用靶点预测

使用 PharmMapper (<https://www.lilab-ecust.cn/pharmmapper/index.html>) 对 DMG 的作用靶点进行预测, 使用 STRING (<https://cn.string-db.org/>) 进行蛋白质相互作用 (protein-protein interaction, PPI) 网络分析和京都基因与基因组百科全书 (Kyoto Encyclopedia of Genes and Genomes, KEGG) 通路富集分析。

1.4.2 分子对接

从 Pub Chem (<https://pubchem.ncbi.nlm.nih.gov>) 获取小分子 DMG 的 sdf 文件, 从 RCSB PDB 获取 CYP2C9 蛋白的结构编号 (PDB 1R9O), 利用 pymol 软件对蛋白质结构进行处理后, 使用 Swissdock (<https://www.swissdock.ch/>) 进行对接, 并使用 Protein-Ligand Interaction Profile (<https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index>) 对小分子与蛋白质的相互作用进行分析。

1.5 统计方法

所有实验均进行至少3次生物学重复。所有数据采用 GraphPad Prism 10 进行统计分析, 实验数据均用 mean±SEM 表示。采用独立样本 *t* 检验 (双尾) 或单因素方差分析分别进行两组或多组间的统计学差异比较, 以 *P*<0.05 视为差异有统计学意义。

2 结 果

2.1 DMG对于复制性衰老WI-38细胞的影响

2.1.1 DMG对复制性衰老的WI-38细胞无细胞毒性作用

通过 CCK-8 细胞活性检测与乳酸脱氢酶 LDH 释放实验, 系统评估了 DMG 对复制性衰老 WI-38 细胞的毒性作用。结果显示, 经 DMG 处理后, 细

胞存活率呈时间依赖性上升趋势, 在处理中后期达到峰值 (图 1a~c)。不同浓度 DMG 处理组均表现出较对照组更高的增殖水平, 其中 50 和 70 μmol/L 处理 5 d 后的细胞增殖效果显著, 统计学差异极为显著 (图 1d)。该结果表明, DMG 在该实验条件下不仅未抑制细胞生长, 反而表现出促进细胞增殖的活性。LDH 释放实验进一步证实, 在相同处理条件下, DMG 实验组的 LDH 释放量与空白对照组相比无统计学差异 (图 1e), 说明 DMG 未引起细胞膜损伤或通透性改变, 细胞膜完整性得以维持。综合两项实验结果可得出结论: 50 和 70 μmol/L 处理 5 d 后, DMG 对复制性衰老的 WI-38 细胞无细胞毒性作用, 未表现出明显的生长抑制或膜损伤效应, 这一特性与其作为天然代谢调节剂相符, 为其后续生物学功能研究提供了安全性依据。

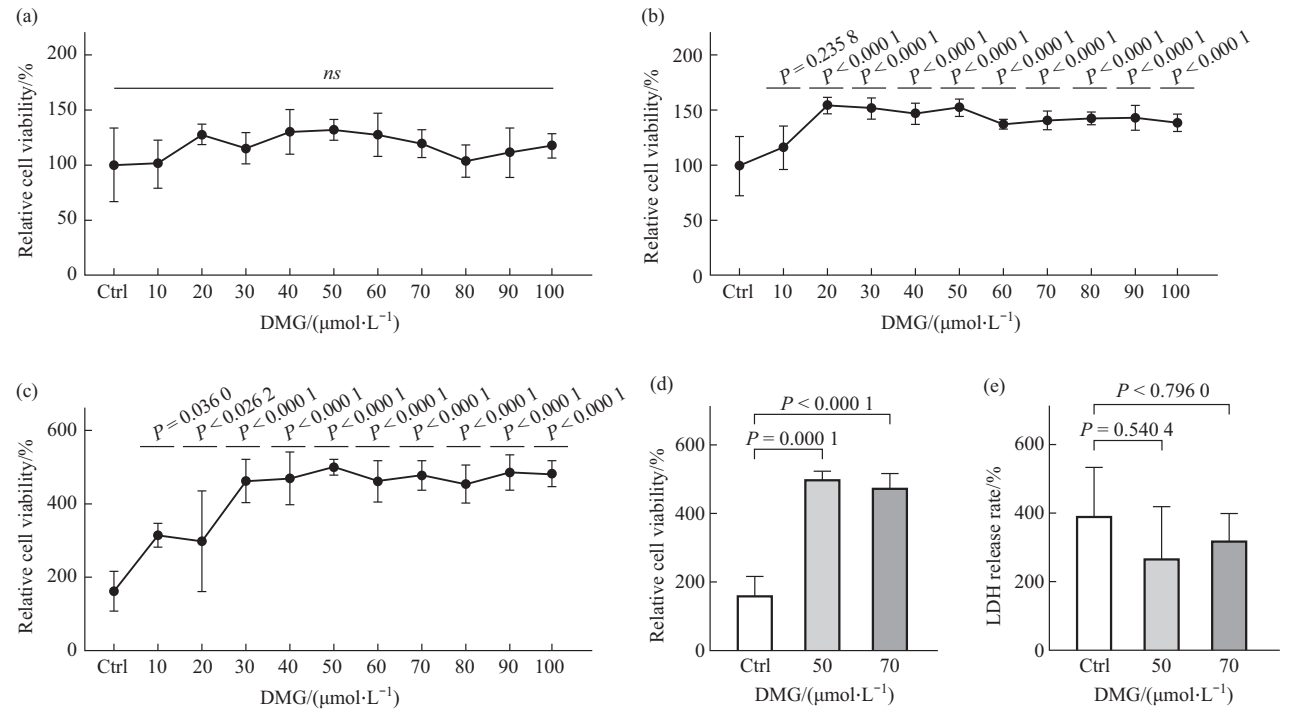


Fig. 1 DMG shows no cytotoxic effect on WI-38 cells undergoing replicative senescence

WI-38 cells were treated with DMG for 3 d (a), 4 d (b), or 5 d (c), the cell viability was measured by CCK-8 assay and expressed as percentage of control. After 50 and 70 μmol/L DMG treatment for 5 d, the cell viability (d) and LDH release (e) were measured by CCK-8 assay and LDH release assay, respectively. All experiments were performed at least 3 times independently. Comparisons were performed by two-sided *t* tests or one-way ANOVA analysis. All data are expressed as mean±SEM. ns, not significant.

2.1.2 DMG显著改善复制性衰老WI-38细胞的衰老相关功能指标

SA-β-Gal 衰老染色分析表明, DMG 处理可显著降低衰老细胞比例, 且该效应呈现浓度依赖性。其中 50 μmol/L 组的抗衰老效果最为突出, SA-β-Gal 阳性细胞率较对照组显著下降, 具有统计学差

异; 70 μmol/L 组同样观察到阳性细胞率降低, 但效果相对较弱 (图 2a~d)。基于 SA-β-Gal 染色结果, 并结合前期细胞活性与毒性实验数据, 最终选定 50 μmol/L DMG 处理细胞 5 d 作为后续实验的标准化条件。

在确定的最优条件下, DMG 展现出显著的抗

氧化功能。细胞内ROS水平检测显示,DMG处理组的荧光强度较对照组明显减弱,表明DMG能够有效清除衰老细胞内过度累积的自由基,减轻氧化应激损伤,改善细胞氧化还原状态(图2e)。

此外,EdU细胞增殖实验证实DMG具有促进衰老细胞增殖的功能。DMG处理组的EdU阳性细

胞率显著高于对照组,说明DMG可重新激活衰老WI-38细胞的DNA合成能力,提升细胞增殖活性(图2f)。上述结果综合表明,DMG通过降低衰老标志物表达、减轻氧化应激及促进细胞增殖等多重功能,显著改善复制性衰老WI-38细胞的整体生理状态。

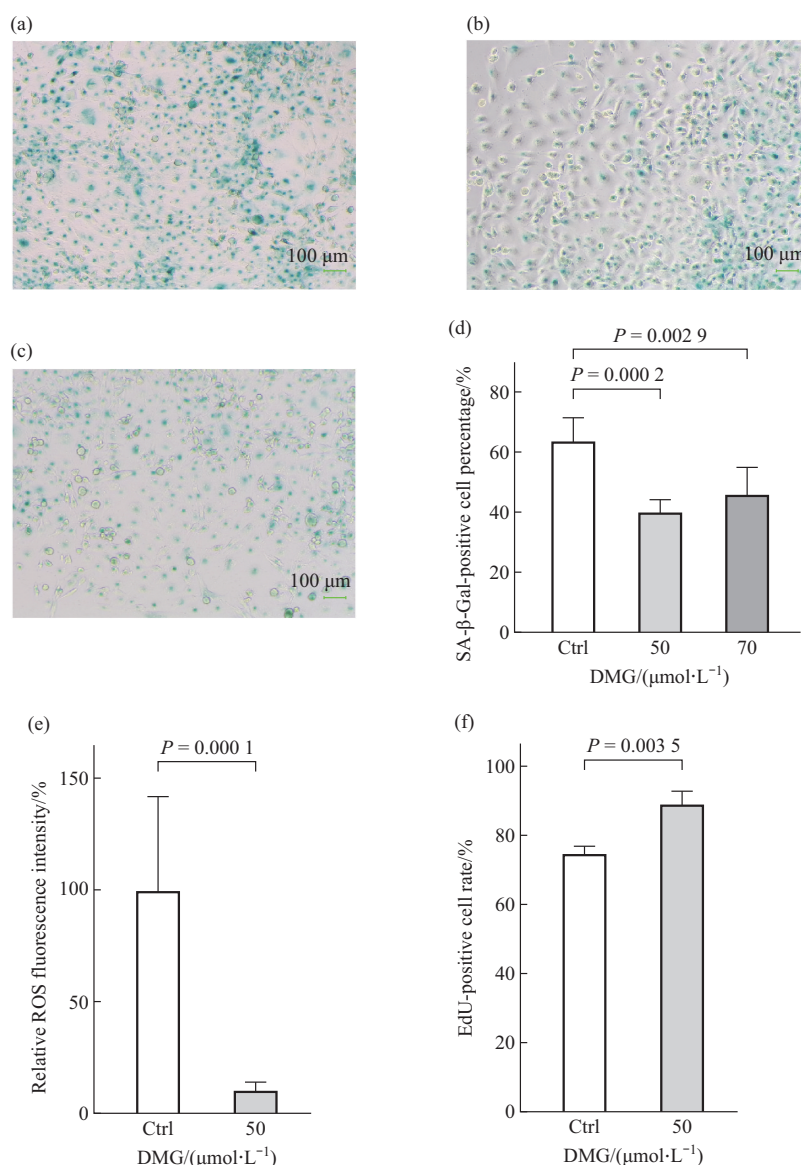


Fig. 2 DMG reduces senescence markers in replicatively senescent WI-38 cells

(a-c) Replicatively senescent WI-38 cells were treated with DMG for 5 d. (a) Control group. (b) 50 $\mu\text{mol/L}$ DMG-treated group. (c) 70 $\mu\text{mol/L}$ DMG-treated group. (d) Quantification of SA-β-Gal-positive cell percentage. (e) Effect of DMG on intracellular ROS levels in WI-38 cells. (f) Effect of DMG on the proliferation of WI-38 cells. All experiments were performed at least 3 times independently. Comparisons were performed by two-sided *t* tests or one-way ANOVA analysis. All data are expressed as mean $\pm$ SEM.

2.2 DMG对于N₂线虫的影响

2.2.1 DMG显著增强秀丽隐杆线虫模型的抗衰老功能

通过热应激存活实验与寿命分析,系统评估了

DMG对模式生物线虫抗衰老功能的影响。热应激实验结果显示,不同浓度DMG处理组均能有效提升线虫在极端温度胁迫下的存活率,且该保护效应呈浓度依赖性增强趋势,其中2 mmol/L展现出更

为显著的保护效果, 表明DMG可显著增强机体的热应激抵抗能力, 这种抗逆性提升是抗衰老功能的重要体现 (图3a)。寿命实验结果显示, DMG处理组表现出显著的生存优势。与对照组 (20.402 ± 0.256) d相比, DMG处理组的中位寿命延长至 (23.066 ± 0.356) d。此外, 最大寿命也明显延长,

且差异具有统计学意义, 这充分证明了DMG具有显著的抗衰老活性 (图3b)。综合两项经典衰老模型实验结果, DMG不仅能增强机体对环境胁迫的抵御能力, 还能从根本上延长生物体的生存周期, 从功能层面验证了DMG在整体生物学水平上的抗衰老效应。

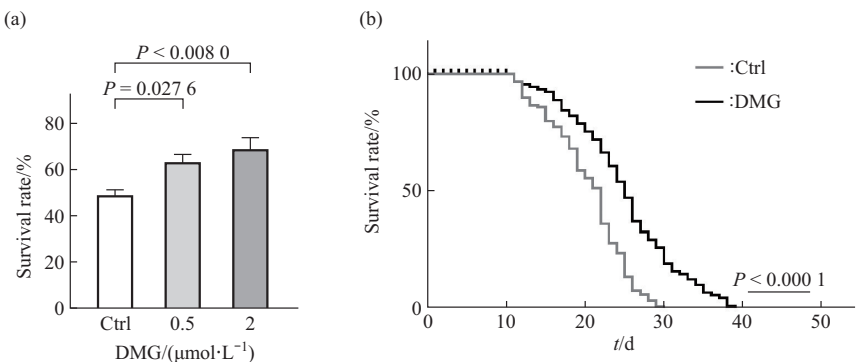


Fig. 3 DMG markedly augments the anti-aging effects in *C. elegans* models

(a) *C. elegans* were treated with DMG at 0.5 or 2 mmol/L and subjected to heat stress. Survival rates are expressed as mean±SD from 3 independent experiments. (b) Lifespan analysis of *C. elegans* treated with DMG. *C. elegans* were exposed to 2 mmol/L DMG. All experiments were performed at least 3 times independently. Comparisons were performed by two-sided *t* tests or one-way ANOVA analysis. All data are expressed as mean±SEM.

2.2.2 DMG显著改善线虫模型的衰老相关功能与代谢状态

在线虫模型中, DMG展现出多维度改善衰老表型的功能 (图4)。脂褐素含量分析显示, DMG处理组线虫体内的衰老色素积累水平较对照组显著降低, 表明DMG能有效延缓细胞衰老过程中的代谢废物堆积; 同时, SA-β-gal活性检测结果证实, DMG可显著抑制线虫体内衰老相关酶的表达水平, 进一步验证其在整体生物水平上的抗衰老功能。氧化应激状态评估显示, DMG处理组线虫体内ROS显著下降, 提示DMG具有强大的抗氧化功能, 能有效清除自由基, 维持细胞氧化还原稳态, 这与寿命延长和热应激抵抗增强的观察结果高度一致。此外, 脂质代谢分析表明, DMG处理组线虫的脂肪累积水平显著低于对照组, 说明DMG在抗衰老过程中能有效调节机体脂代谢平衡, 改善与衰老相关的代谢功能障碍。综上, DMG在线虫模型中通过降低衰老标志物表达、减轻氧化应激、改善脂质代谢等多重功能协同作用, 显著延缓机体衰老进程, 展现出全面的抗衰老效应。

2.3 延缓衰老机制的探究

2.3.1 DMG显著改善衰老细胞的分子表达特征

通过RT-qPCR技术检测发现, 与对照组相比, 经DMG处理的WI-38衰老细胞中, 多种核心衰老相关分泌表型 (senescence-associated secretory phenotype, SASP) 因子 (*IL-6*、*IL-8*、*p21* 及 *MMP-1*) 的mRNA表达水平均呈现显著下调 (图5a)。结果表明, DMG不仅能抑制细胞衰老, 还能有效缓解由衰老细胞驱动的炎性微环境, 这为其在调控机体整体衰老进程中发挥更为广泛的作用提供了分子层面的证据。细胞衰老的核心信号通路, 包括p16^{INK4A}/pRb和p53-p21通路, 其蛋白质表达水平的改变是判定衰老状态的关键分子证据。ELISA结果显示, 经DMG处理的WI-38衰老细胞中, p16蛋白水平也表现出高度显著的差异, 下降了57.3% ($P=0.0004$), 同时p21蛋白水平发生了显著变化, 相比于对照组下降了27.2% ($P=0.0007$) (图5b)。该结果从蛋白质水平证实, DMG能够同时作用于多个核心衰老信号节点, 有效缓解WI-38细胞由这些通路所介导的细胞周期阻滞状态, 为其抗衰老效应提供了坚实的分子机制依据。

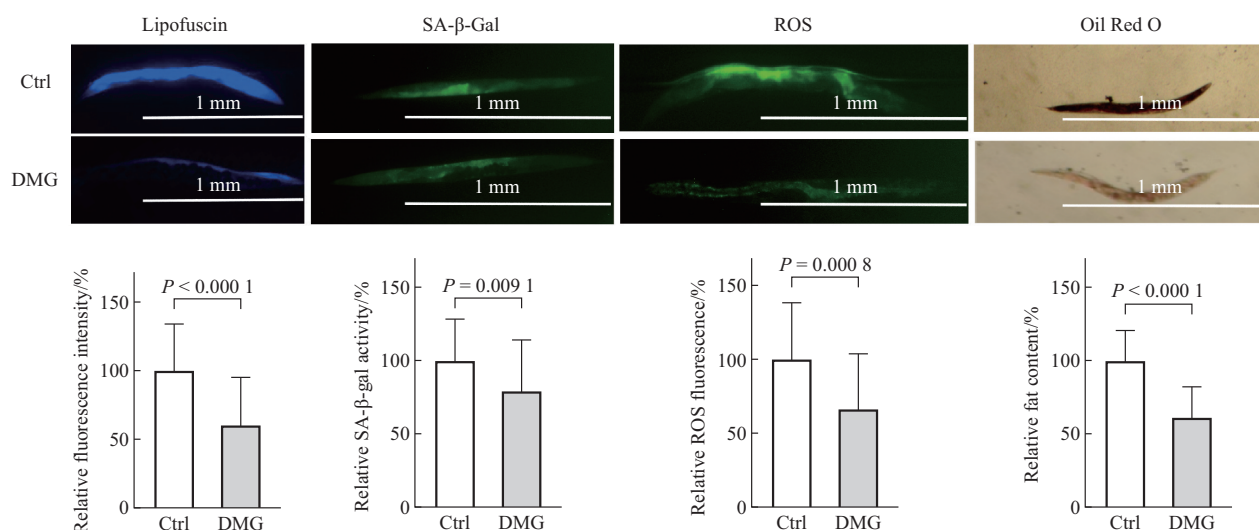


Fig. 4 Assessment of aging markers in *C. elegans*

Representative images show lipofuscin autofluorescence, SA-β-Gal staining, ROS fluorescence, and Oil Red O staining in control and DMG-treated worms (from left to right), $n > 30$. All experiments were performed at least three times independently. Comparisons were performed by two-sided t tests or one-way ANOVA analysis. All data are expressed as mean $\pm$ SEM.

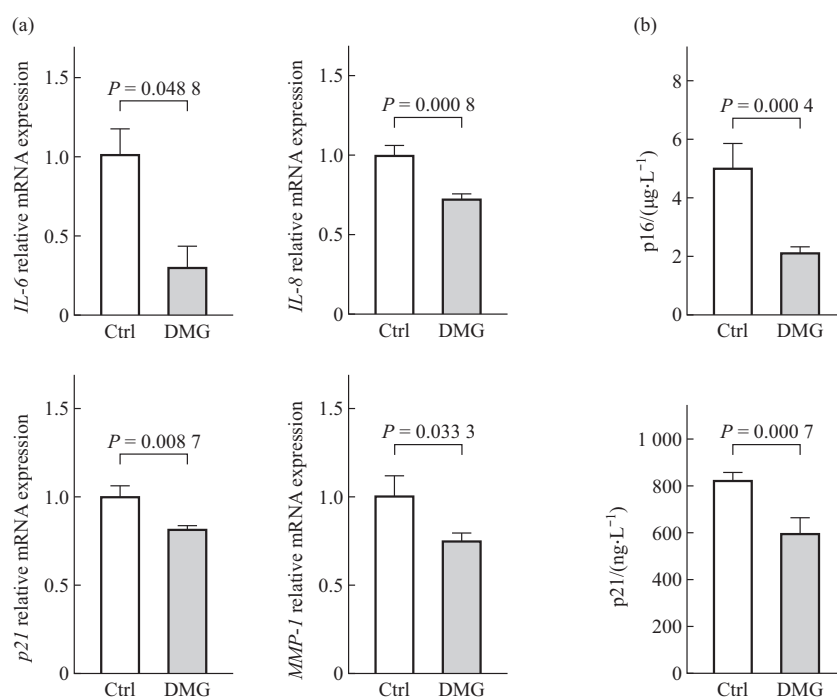


Fig. 5 DMG significantly reduces the molecular expression signature of senescence

(a) Replicative senescent WI-38 cells were treated with DMG for 5 d. Relative mRNA expression levels of *IL-6*, *IL-8*, *p21*, and *MMP-1* were measured by RT-qPCR. (b) Protein levels of p21 and p16 in cell lysates were determined using enzyme-linked immunosorbent assay (ELISA). All experiments were performed at least three times independently. Comparisons were performed by two-sided t tests or one-way ANOVA analysis. All data are expressed as mean $\pm$ SEM.

2.3.2 基于网络药理学与分子对接的DMG抗衰老靶点预测及机制验证

为探究DMG延缓衰老的潜在分子机制,通过

PharmMapper平台预测其潜在作用靶点,并借助STRING数据库构建PPI网络^[10-11]。该网络系统展示了DMG可能调控的多个靶点蛋白质及其相互作用

用关系 (图 6a)。分析发现, GST 家族蛋白 (包括 GSTA1、GSTA3、GSTP1 等) 及细胞色素 P40 家族蛋白 (如 CYP2C9) 等靶点处于网络的核心位置, 提示它们可能在 DMG 发挥生物学功能中起关键作用。

进一步通过 KEGG 通路富集分析评估 DMG 潜在靶点的生物学功能。分析结果表明, DMG 潜在靶点显著富集于药物代谢、氧化应激反应等多个与

衰老密切相关的通路, 提示 DMG 可能通过调控这些通路中的关键靶点蛋白质, 进而影响细胞代谢与氧化应激状态 (图 6b)。

综合上述结果, DMG 可能通过作用于 GST 家族蛋白、CYP2C9 等核心靶点, 调节药物代谢、氧化应激反应等关键生物学过程发挥其延缓衰老的效应。

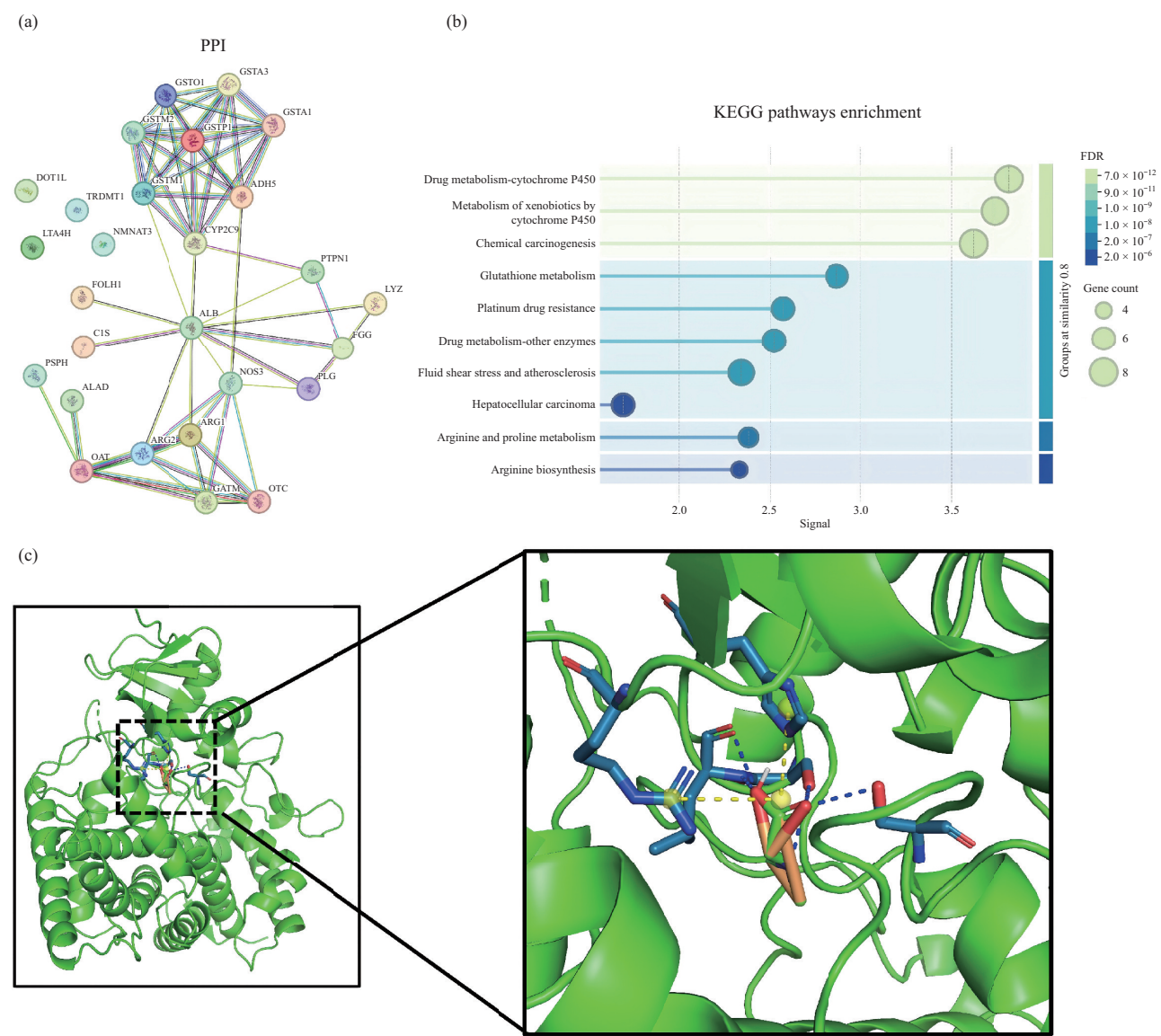


Fig. 6 Prediction and validation of anti-aging targets of DMG

(a) PPI network of DMG potential targets was constructed using PharmMapper and STRING. (b) KEGG pathway enrichment analysis. (c) Molecular docking of DMG with CYP2C9 (PDB: 1R9O). GSTA3: glutathione S-transferase alpha 3; GSTO1: glutathione S-transferase omega 1; GSTM2: glutathione S-transferase Mu 2; GSTP1: glutathione S-transferase Pi 1; GSTA1: glutathione S-transferase alpha 1; GSTM1: glutathione S-transferase Mu 1; ADH5: alcohol dehydrogenase 5; DOT1L: DOT1-like histone H3K79 methyltransferase; TRDMT1: tRNA aspartic acid methyltransferase 1; LTA4H: leukotriene A4 hydrolase; NMNAT3: nicotinamide nucleotide adenylyltransferase 3; CYP2C9: cytochrome P450 2C9; FOLH1: folate hydrolase 1; PTPN1: protein tyrosine phosphatase non-receptor type 1; C1S: complement C1S; ALB: albumin; LYZ: lysozyme; FGG: fibrinogen gamma chain; PSPH: phosphoserine phosphatase; NOS3: nitric oxide synthase 3; PLG: plasminogen; ALAD: aminolevulinatase dehydratase; OAT: ornithine aminotransferase; ARG2: arginase 2; ARG1: arginase 1; GATM: glycine amidinotransferase; OTC: ornithine transcarbamylase.

由于KEGG富集的前两位均与细胞色素P40家族有关,本研究重点关注了CYP2C9。利用Swiss Dock将DMG与CYP2C9(PDB: 1R9O)进行分子对接,得到swissparam score最优评分为-5.610 3,认为两者之间存在相互作用,通过Protein-Ligand Interaction Profiler分析相互作用^[12-13](图6c)。氢键分析结果表明,存在5个显著的氢键相互作用。其中,365位点的丝氨酸残基与配体形成了2个氢键,氢原子与受体原子的距离分别为2.85 Å和2.95 Å,供体角度分别为119.34°和112.28°。366位点的亮氨酸残基也与配体形成了2个氢键,距离分别为2.51 Å和2.74 Å,供体角度分别为141.12°和101.11°。此外,429位点的丝氨酸残基与配体形成了1个氢键,距离为2.29 Å,供体角度为149.61°。

盐桥分析结果显示,存在2个显著的盐桥相互作用。97位点的精氨酸残基与配体的羧酸基团形成了盐桥,距离为4.24 Å。368位点的组氨酸残基同样与配体的羧酸基团形成了盐桥,距离为3.95 Å。这些相互作用表明,CYP2C9蛋白与DMG小分子之间存在稳定的结合模式,其中氢键和盐桥在维持这种结合中起到了关键作用。

3 讨 论

衰老是生物体随时间推移而发生功能衰退的必然生理过程,尽管其不可避免,但通过外部干预延缓其进程并提升健康寿命已成为抗衰老研究的重要目标^[14-15]。人类长寿是一个涉及多基因、多环境因素及复杂代谢网络的系统性过程,其中代谢稳态的维持被认为是实现健康长寿的核心机制之一。正如本课题组前期基于长寿家系人群血清代谢组学的发现,DMG在长寿家族群体内呈现显著的富集态势^[6]。这一临床层面的现象强烈提示,DMG可能不仅仅是衰老过程中的一种伴随性代谢产物,更可能作为一种内源性的保护分子,主动参与了机体抵御衰老、维持长寿的生物学过程。然而,以往关于内源性代谢物与长寿的研究多停留在人群流行病学的横断面观察层面,缺乏直接的因果关系和功能学验证。本研究聚焦于这一具有抗衰潜力的小分子DMG,既往功能研究表明其在甲基转移、免疫调节及能量代谢中扮演重要角色^[16-18]。本研究尝试从体外和体内双重维度系统证实了其直接的抗衰老与延寿功能。这不仅为长寿家族内DMG的高表达提供了解释,也为开发基于DMG的抗衰老干预策略奠定了理论基础。

3.1 DMG在细胞模型中展现多重抗衰老功能

在衰老研究领域,WI-38细胞模型已被证实可用于评估白藜芦醇、雷帕霉素等小分子化合物通过调控自噬、缓解氧化应激等机制延缓复制性衰老的效果^[19-20]。尽管有观点指出连续传代可能引入细胞表观遗传的适应与改变,但大量多组学研究证实,WI-38细胞在达到复制性衰老时,仍表现出高度可重复且具有生物学意义的基因表达变化^[21-24]。此外,表观遗传改变本身即是衰老过程的固有特征,且并非决定基因表达的唯一主导因素^[25-26]。因此认为WI-38能真实模拟体内衰老细胞的核心特征与表观遗传状态。本研究是结合多维度表型指标进行验证。结果表明,DMG能显著降低WI-38细胞的SA-β-gal活性及p16/p21蛋白表达,从功能上证实了DMG具有抑制衰老标志物表达的能力。更重要的是,DMG处理不仅减少了衰老细胞的数量,还显著降低了细胞内ROS水平,展现出强大的抗氧化应激功能。同时,EdU实验证实DMG能有效提升衰老细胞的增殖活性,这种兼具“减轻氧化损伤”与“恢复增殖能力”的双重功能,为DMG促进细胞健康衰老提供了直接证据。

3.2 DMG在线虫模型中体现整体抗衰老与延寿功能

在整体生物水平上,线虫模型已被广泛用于评估小分子的抗衰老功能,亚精胺、槲皮素等小分子亦被证实可通过保守信号通路显著延长寿命^[27-28]。本研究观察到DMG可有效延长线虫寿命、提高热应激存活率,并降低线虫的ROS与脂褐素水平。特别是在热应激条件下,DMG展现出显著的保护功能,这表明DMG能够增强机体的抗逆性和应激适应能力,这是健康衰老的重要功能指标。寿命实验数据显示DMG不仅延长平均寿命,还显著增加最大寿命,说明其功能不仅限于延缓死亡,更能改善生命晚期的健康状态,体现了“健康衰老”的核心功能特征。

3.3 DMG可能靶向CYP2C9实现延缓衰老的功能

靶点预测与分子对接进一步提示,DMG可与GST家族成员(如GSTP1)及CYP2C9等蛋白质稳定结合。作为机体重要的解毒与代谢系统,谷胱甘肽S-转移酶(slutathione S-transferase, GST)家族和细胞色素P450(cytochrome P450, CYP)家族的活性与衰老进程密切相关^[29-31]。研究表明,GST功能下调会导致氧化损伤产物累积,而CYP酶系的异常则可能引发代谢稳态失衡与内源性毒性,共

同加速衰老^[32-34]。既往文献也支持, DMG作为一种重要的甲基供体, 在支持肝脏解毒、中和自由基及增强免疫等方面发挥关键的保护作用, 这些生理过程均与抵御衰老相关损伤高度契合^[7-8, 17-18]。基于此, 推测 DMG 可能通过直接作用于 GST 与 CYP2C9 等关键靶点, 增强机体的氧化应激解毒能力与代谢物清除效率, 从而从上游缓解诱发细胞衰老的核心压力。这种压力的减轻, 进而导致了下游 p21 及 p16 等核心衰老信号通路的抑制, 最终在表型上表现为细胞衰老延缓与机体寿命延长。分子对接结果显示, DMG 与 CYP2C9 之间形成多个稳定的氢键和盐桥相互作用, 这一结构基础为 DMG 的功能活性提供了理论支撑, 也提示未来可通过结构优化进一步增强其抗衰老效应。

3.4 研究局限性与未来功能验证方向

综上所述, 本研究揭示了 DMG 在延缓细胞和机体衰老方面的显著效果, 并初步探讨了其潜在的抗衰老机制。线虫与人类虽形态差异大, 但在胰岛素样生长因子 1 (insulin-like growth factor 1, IGF-1)、哺乳动物雷帕霉素靶蛋白 (mammalian target of rapamycin, mTOR) 等核心寿命相关通路上高度保守^[35-38]。凭借生命周期短、遗传工具成熟的优势, 线虫成为公认的抗衰老化合物高通量筛选与初步验证的经典模型^[39-43]。然而本研究仍存在局限性。首先, 虽然本研究利用线虫快速验证了 DMG 的抗衰老效应及机制, 但其在哺乳动物中仍需进一步验证。其次, 本研究目前主要依赖网络药理学预测 DMG 的抗衰老机制, 缺乏功能学实验验证。后续将在衰老小鼠模型中系统验证 DMG 的抗衰老机制, 以确认其靶点依赖性。

4 结 论

本研究利用 WI-38 细胞及线虫自然衰老模型, 系统证实了内源性小分子 DMG 兼具细胞与整体水平抗衰老功能。功能研究发现, DMG 通过降低 ROS、减少脂褐素积累、抑制 SASP 表达及促进细胞增殖等多重功能显著改善衰老表型。网络药理学预测与分子对接进一步揭示, DMG 可能靶向 GST 家族和 CYP2C9 等核心蛋白质, 调控药物代谢与氧化应激通路, 为其抗衰老效应提供分子基础。

参 考 文 献

- [1] Culig L, Chu X, Bohr V A. Neurogenesis in aging and age-related neurodegenerative diseases. *Ageing Res Rev*, 2022, **78**: 101636
- [2] Li J, Han X, Zhang X, *et al*. Spatiotemporal evolution of global population ageing from 1960 to 2017. *BMC Public Health*, 2019, **19**(1): 127
- [3] Wang Y, Chang J, Liu X, *et al*. Discovery of piperlongumine as a potential novel lead for the development of senolytic agents. *Aging*, 2016, **8**(11): 2915-2926
- [4] Wan Q L, Meng X, Fu X, *et al*. Intermediate metabolites of the pyrimidine metabolism pathway extend the lifespan of *C. elegans* through regulating reproductive signals. *Aging*, 2019, **11**(12): 3993-4010
- [5] Xiao Y, Zhang H, Sheng Y, *et al*. Usnic Acid extends healthspan and improves the neurodegeneration diseases via mTOR/PHA-4 signaling pathway in *Caenorhabditis elegans*. *iScience*, 2022, **25**(12): 105539
- [6] Qiu X, Mu C, Hu J, *et al*. Serum metabolic and microbial profiling yields insights into promoting effect of tryptophan-related metabolites for health longevity in centenarians. *Imeta*, 2025, **4**(3): e70025
- [7] Dhanjal D S, Bhardwaj S, Chopra C, *et al*. Millennium nutrient N, N-dimethylglycine (DMG) and its effectiveness in autism spectrum disorders. *Curr Med Chem*, 2022, **29**(15): 2632-2651
- [8] Lendvai A, Béke G, Hollósi E, *et al*. N, N-dimethylglycine sodium salt exerts marked anti-inflammatory effects in various dermatitis models and activates human epidermal keratinocytes by increasing proliferation, migration, and growth factor release. *Int J Mol Sci*, 2023, **24**(14): 11264
- [9] 彭艳华, 王跃, 刘健龙, 等. 双歧杆菌脂磷壁酸延缓细胞衰老的作用机制. *中国老年学杂志*, 2009, **29**(6): 668-670
Peng Y H, Wang Y, Liu J L, *et al*. *Chin J Gerontol*, 2009, **29**(6): 668-670
- [10] Wang X, Shen Y, Wang S, *et al*. PharmMapper 2017 update: a web server for potential drug target identification with a comprehensive target pharmacophore database. *Nucleic Acids Res*, 2017, **45**(W1): W356-W360
- [11] Szklarczyk D, Gable A L, Lyon D, *et al*. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*, 2019, **47**(D1): D607-D613
- [12] Salentin S, Schreiber S, Haupt V J, *et al*. PLIP: fully automated protein-ligand interaction profiler. *Nucleic Acids Res*, 2015, **43**(W1): W443-W447
- [13] Bugnon M, Röhrig U F, Goullieux M, *et al*. SwissDock 2024: major enhancements for small-molecule docking with Attracting Cavities and AutoDock Vina. *Nucleic Acids Res*, 2024, **52**(W1): W324-W332
- [14] Melov S. Geroscience approaches to increase healthspan and slow aging. *F1000Res*, 2016, **5**: F1000FacultyRev-F1000Faculty785
- [15] López-Otín C, Blasco M A, Partridge L, *et al*. The hallmarks of aging. *Cell*, 2013, **153**(6): 1194-1217
- [16] Lever M, McEntyre C J, George P M, *et al*. Is N, N-dimethylglycine N-oxide a choline and betaine metabolite?. *Biol Chem*, 2017, **398**(7): 775-784

- [17] Bai K, Jiang L, Wang T. Supplementation with dimethylglycine sodium salt improves lipid metabolism disorder in intrauterine growth-retarded pigs. *Anim Nutr*, 2024, **18**: 191-202
- [18] Bai K, Jiang L, Wang T, *et al.* Treatment of immune dysfunction in intrauterine growth restriction piglets *via* supplementation with dimethylglycine sodium salt during the suckling period. *Anim Nutr*, 2022, **11**: 215-227
- [19] Wang R, Yu Z, Sunchu B, *et al.* Rapamycin inhibits the secretory phenotype of senescent cells by a Nrf2-independent mechanism. *Aging Cell*, 2017, **16**(3): 564-574
- [20] Huang C Y, Chen S H, Lin T, *et al.* Resveratrol attenuates advanced glycation end product-induced senescence and inflammation in human gingival fibroblasts. *J Dent Sci*, 2024, **19**(1): 580-586
- [21] Chan M, Yuan H, Soifer I, *et al.* Revisiting the hayflick limit: insights from an integrated analysis of changing transcripts, proteins, metabolites and chromatin. *bioRxiv*, 2021. DOI: 10.1101/2021.05.03.442497
- [22] Picot C R, Perichon M, Cintrat J C, *et al.* The peptide methionine sulfoxide reductases, MsrA and MsrB (hCBS-1), are downregulated during replicative senescence of human WI-38 fibroblasts. *FEBS Lett*, 2004, **558**(1/2/3): 74-78
- [23] Sidler C, Woycicki R, Kovalchuk I, *et al.* WI-38 senescence is associated with global and site-specific hypomethylation. *Aging*, 2014, **6**(7): 564-574
- [24] Sidler C, Woycicki R, Li D, *et al.* A role for SUV39H1-mediated H3K9 trimethylation in the control of genome stability and senescence in WI38 human diploid lung fibroblasts. *Aging*, 2014, **6**(7): 545-563
- [25] Chen Y H, Zhang X, Ko K Y, *et al.* CBX4 regulates replicative senescence of WI-38 fibroblasts. *Oxid Med Cell Longev*, 2022, **2022**: 5503575
- [26] Casella G, Munk R, Kim K M, *et al.* Transcriptome signature of cellular senescence. *Nucleic Acids Res*, 2019, **47**(14): 7294-7305
- [27] Ayuda-Durán B, González-Manzano S, Miranda-Vizuete A, *et al.* Exploring target genes involved in the effect of quercetin on the response to oxidative stress in *Caenorhabditis elegans*. *Antioxidants (Basel)*, 2019, **8**(12): 585
- [28] Rhodes C H, Zhu C, Agus J, *et al.* Human fasting modulates macrophage function and upregulates multiple bioactive metabolites that extend lifespan in *Caenorhabditis elegans*: a pilot clinical study. *Am J Clin Nutr*, 2023, **117**(2): 286-297
- [29] Xu S, Hou D, Liu J, *et al.* Age-associated changes in GSH S-transferase gene/proteins in livers of rats. *Redox Rep*, 2018, **23**(1): 213-218
- [30] Miners J O, Birkett D J. Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. *Br J Clin Pharmacol*, 1998, **45**(6): 525-538
- [31] Das U N. Ageing: is there a role for arachidonic acid and other bioactive lipids? A review. *J Adv Res*, 2018, **11**: 67-79
- [32] Rousseau L, Hajdu K L, Ho P C. Meta-epigenetic shifts in T cell aging and aging-related dysfunction. *J Biomed Sci*, 2025, **32**(1): 51
- [33] Benjamin D I, Brett J O, Both P, *et al.* Multiomics reveals glutathione metabolism as a driver of bimodality during stem cell aging. *Cell Metab*, 2023, **35**(3): 472-486.e6
- [34] Huang K, Chen W, Zhu F, *et al.* RiboTag translatomic profiling of *Drosophila* oenocytes under aging and induced oxidative stress. *BMC Genomics*, 2019, **20**(1): 50
- [35] 闫灵芝. 侧流免疫层析技术在食品安全检测中的研究进展. *食品工业科技*, 2022, **43**(4): 1-11
- Yan L Z. *Sci Technol Food Ind*, 2022, **43**(4): 1-11
- [36] Russell S J, Kahn C R. Endocrine regulation of ageing. *Nat Rev Mol Cell Biol*, 2007, **8**(9): 681-691
- [37] Giacomello E, Toniolo L. Nutrition, diet and healthy aging. *Nutrients*, 2021, **14**(1): 190
- [38] Scrable H, Medrano S, Ungewitter E. Running on empty: how p53 controls INS/IGF signaling and affects life span. *Exp Gerontol*, 2009, **44**(1/2): 93-100
- [39] Silverman G A, Luke C J, Bhatia S R, *et al.* Modeling molecular and cellular aspects of human disease using the nematode *Caenorhabditis elegans*. *Pediatr Res*, 2009, **65**(1): 10-18
- [40] Martel J, Wu C Y, Peng H H, *et al.* Plant and fungal products that extend lifespan in *Caenorhabditis elegans*. *Microb Cell*, 2020, **7**(10): 255-269
- [41] Ryu D, Mouchiroud L, Andreux P A, *et al.* Urolithin A induces mitophagy and prolongs lifespan in *C. elegans* and increases muscle function in rodents. *Nat Med*, 2016, **22**(8): 879-888
- [42] Mouchiroud L, Houtkooper R H, Moullan N, *et al.* The NAD(+)/sirtuin pathway modulates longevity through activation of mitochondrial UPR and FOXO signaling. *Cell*, 2013, **154**(2): 430-441
- [43] Singh P, Gollapalli K, Mangiola S, *et al.* Taurine deficiency as a driver of aging. *Science*, 2023, **380**(6649): eabn9257

Study on The Anti-aging Effects of Longevity-enriched Metabolite Dimethylglycine*

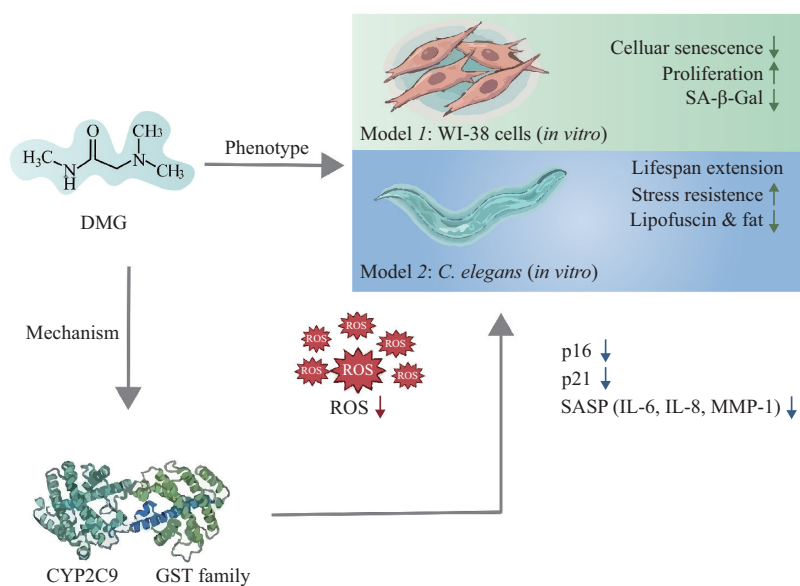
HU Jie^{1,2)}, PU Gong-Yu²⁾, LI Jun-Lin²⁾, CAO Ju²⁾, LIN Zhi-Xin²⁾, AN Wei-Wei¹⁾,
LI Xue-Meng^{2,3)**}, AN Jing^{1)**}

(¹⁾Institute of Cancer Prevention and Treatment, Harbin Medical University, Harbin 150081, China;

²⁾Zhanjiang Key Laboratory of Human Microecology Research and Clinical Translation, School of Basic Medical Sciences, Guangdong Medical University, Zhanjiang 524023, China;

³⁾Guangdong Engineering Technology Research Center for the Development and Utilization of Mangrove Wetland Medicinal Resources, The Key Lab of Zhanjiang for R&D Marine Microbial Resources in the Beibu Gulf Rim, School of Ocean and Tropical Medicine, Guangdong Medical University, Zhanjiang 524023, China)

Graphical abstract



Abstract Objective The exacerbating trend of global population aging poses profound socioeconomic and public health challenges, making the comprehensive elucidation of biological aging mechanisms and the discovery of effective anti-aging interventions an urgent priority in the life sciences. Based on our previous serum metabolomics findings that dimethylglycine, an intermediate metabolite of amino acid metabolism naturally

* This work was supported by grants from The National Natural Science Foundation of China (82301752), Heilongjiang Provincial Natural Science Foundation Joint Guidance Project (LH2020H111), Guangdong Basic and Applied Basic Research Foundation Joint Fund-Regional Cultivation Project (DGA0706), and Guangdong Medical University Human Microecology Innovation and Entrepreneurship Project Base Project (JDXM2024169).

** Corresponding author.

AN Jing. Tel: 86-15004548046, E-mail: 210001@hrbmu.edu.cn

LI Xue-Meng. Tel: 86-769-63552289, E-mail: lixuemeng@gdmu.edu.cn

Received: December 30, 2025 Accepted: March 16, 2026

present in the human body, was significantly enriched in the serum of longevity families, this study aimed to systematically investigate the anti-aging effects of dimethylglycine both in living organisms and in controlled laboratory environments, and to preliminarily elucidate its underlying molecular mechanisms. While existing literature indicates that dimethylglycine possesses antioxidant and immunomodulatory properties, its direct anti-aging efficacy and the specific molecular pathways through which it operates remain largely unexplored.

Methods To comprehensively evaluate the anti-aging properties of dimethylglycine, we utilized replicative senescent human embryonic lung fibroblasts, specifically the WI-38 cell line, as an experimental model in a controlled laboratory environment. Cell viability and safety were thoroughly assessed using Cell Counting Kit-8 and lactate dehydrogenase release assays across various concentrations of dimethylglycine. The impact of dimethylglycine on cellular senescence phenotypes, oxidative stress, and proliferative capacity was evaluated *via* senescence-associated beta-galactosidase staining, reactive oxygen species fluorescence detection, and 5-ethynyl-2'-deoxyuridine incorporation assays. Furthermore, the molecular alterations of senescence-associated secretory phenotype factors and core senescence signaling pathways were quantified using quantitative reverse transcription polymerase chain reaction for the messenger RNA levels of interleukin-6, interleukin-8, p21, and matrix metalloproteinase-1, and enzyme-linked immunosorbent assay for the measurement of p16 and p21 protein expression levels. For the living organism model, the wild-type nematode *Caenorhabditis elegans* was used to evaluate systemic physiological effects. We conducted a comprehensive lifespan analysis at 20°C, heat stress resistance survival assays at 35°C, senescence-associated beta-galactosidase staining, lipofuscin accumulation tracking, intracellular reactive oxygen species measurement, and Oil Red O staining to ascertain systemic lipid accumulation. Additionally, network pharmacology bioinformatics tools, including PharmMapper and STRING databases, and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis were utilized to predict target pathways, alongside highly detailed molecular docking simulations utilizing SwissDock and Protein-Ligand Interaction Profiler to examine interactions with the cytochrome P450 family 2 subfamily C member 9 protein.

Results The experimental outcomes robustly demonstrate the potent anti-aging capabilities of dimethylglycine. At the cellular level, toxicity analyses firmly confirmed that dimethylglycine is highly safe; continuous treatment with 50 mol/L and 70 mol/L of dimethylglycine for 5 d did not induce any cellular membrane damage or cytotoxicity, but rather actively promoted cellular proliferation. Utilizing the optimal standardized concentration of 50 mol/L, dimethylglycine treatment significantly ameliorated senescent phenotypic markers in human embryonic lung fibroblasts, which was evidenced by a drastic and highly significant reduction in the senescence-associated beta-galactosidase positive cell percentage ($P < 0.0001$) and intracellular reactive oxygen species levels ($P < 0.0001$), alongside a marked increase in the 5-ethynyl-2'-deoxyuridine-positive proliferation rate ($P = 0.0035$). On a molecular expression scale, dimethylglycine significantly downregulated the messenger RNA expression of multiple core senescence-associated secretory phenotype inflammatory factors, including interleukin-6, interleukin-8, p21, and matrix metalloproteinase-1. Concurrently, it effectively suppressed the protein expression of critical cell cycle arrest markers, diminishing p16 protein levels by 57.3% ($P = 0.0004$) and p21 protein levels by 27.2% ($P = 0.0007$). In the nematode *Caenorhabditis elegans* animal model, dimethylglycine significantly extended the mean lifespan from 20.402 d to an impressive 23.066 d ($P < 0.0001$) and notably enhanced overall survival rates under severe heat stress environmental conditions ($P = 0.017$). Furthermore, systemic dimethylglycine intervention significantly mitigated age-related physiological decline by decreasing bodily lipofuscin accumulation ($P < 0.0001$), significantly reducing senescence-associated beta-galactosidase activity, lowering systemic reactive oxygen species fluorescence ($P = 0.008$), and effectively alleviating overall fat accumulation ($P < 0.0001$). Mechanistically, extensive network pharmacology and Kyoto Encyclopedia of Genes and Genomes analyses strongly revealed that the potential targets of dimethylglycine are significantly enriched in fundamental drug metabolism and oxidative stress response pathways. Precision molecular docking simulations

conclusively demonstrated that dimethylglycine forms highly stable structural interactions with the cytochrome P450 family 2 subfamily C member 9 protein, specifically highlighting the definitive formation of 5 stable hydrogen bonds involving serine 365, leucine 366, and serine 429 residues, as well as two critical salt bridge formations with arginine 97 and histidine 368 residues. It is additionally predicted to interact favorably with glutathione S-transferase family proteins. **Conclusion** Dimethylglycine exhibits a profoundly significant and multifaceted anti-aging activity at both the cellular and entire living animal levels. By powerfully alleviating oxidative stress, heavily suppressing the core p16 and p21-dependent cellular senescence signaling pathways, and substantially mitigating the detrimental senescence-associated secretory phenotype, dimethylglycine effectively delays fundamental cellular senescence processes and drastically extends whole-organism lifespan. The biological mechanisms driving these robust protective effects are highly likely closely associated with its direct stable interactions with crucial metabolic and detoxifying enzyme systems, such as cytochrome P450 family 2 subfamily C member 9 and glutathione S-transferase family proteins, thereby systemically improving metabolic dysregulation and restoring critical redox homeostasis. This comprehensive study provides highly solid experimental evidence supporting dimethylglycine as a highly potent and safe potential anti-aging intervention agent, while simultaneously offering a clear molecular mechanistic explanation for the previously documented high abundance of dimethylglycine observed within exceptionally long-lived human populations.

Key words dimethylglycine, cellular senescence, *Caenorhabditis elegans*, molecular docking, lifespan extension

DOI: 10.3724/j.pibb.2025.0574

CSTR: 32369.14.pibb.20250574