原花青素B3通过GATA3/ACSL5通路促进肝细胞脂肪酸氧化改善非酒精性脂肪肝的研究

阮诚浩[1,2], 韩　艳[1], 吴征斌[2], 田新然[2], 柳　雯[2], 徐树萍[2], 刘晴晴[3*], 谢保平[1,2*]

(1. 赣南医科大学康复学院, 江西 赣州 341000;　2. 赣南医科大学心脑血管疾病防治教育部重点实验室, 江西 赣州 341000;　3. 广州医科大学附属中医医院, 广东 广州 510000)

摘要: 本研究旨在探讨原花青素B3 (procyanidin B3, Pro B3) 对非酒精性脂肪肝 (non-alcoholic fatty liver disease, NAFLD) 的药理作用, 以及调控NAFLD中肝细胞脂肪酸氧化 (fatty acid oxidation, FAO) 的分子机制, 为Pro B3在NAFLD中的临床应用提供实验依据。采用高脂饮食 (high fat diet, HFD) 饲养12周诱导小鼠NAFLD模型, 并灌胃给药12周, 通过检测血清生化指标, H&E染色和油红O染色观察肝脏病理形态变化和脂滴形成, 全面评价Pro B3对小鼠NAFLD的药理作用。本研究方案经赣南医科大学实验动物伦理委员会批准 (批准号: 2025443)。在体外实验中, 用棕榈酸油酸混合物 (2∶1) 诱导HepG2细胞构建NAFLD的细胞模型, 并评价Pro B3对HepG2细胞毒性及细胞活力的影响, 免疫组化、免疫荧光、qRT-PCR和免疫印迹等方法检测酰基辅酶A合成酶长链家族成员5 (acyl-CoA synthetase long chain family member 5, ACSL5)、肉碱棕榈酰转移酶1 (carnitine palmitoyltransferase 1, CPT1)、乙酰辅酶A羧化酶 (acetyl-CoA carboxylase, ACC) 和GATA结合蛋白3 (GATA binding protein 3, GATA3) 等FAO相关基因的表达。机制上, 用JASPAR数据库和双荧光素酶报告基因实验探讨ACSL5的转录因子和结合位点, 用siRNA沉默GATA3, 检测Pro B3对ACSL5表达和脂质沉积的影响。结果表明, Pro B3能够显著改善小鼠NAFLD的相关指标, 降低血脂水平和HepG2细胞中脂滴沉积, 促进肝细胞FAO相关基因ACSL5、CPT1、ACC mRNA和蛋白水平的表达, 且GATA3可能是ACSL5的转录因子, 转录调节位点可能是“ACATAG”。同时, Pro B3可显著上调GATA3 mRNA和蛋白水平的表达, 沉默GATA3显著抑制ACSL5 mRNA和蛋白水平的表达, 并减弱了Pro B3对HepG2细胞脂滴沉积的抑制作用。综上所述, Pro B3可能是治疗NAFLD的有效先导化合物, 其通过调控GATA3/ACSL5通路促进肝细胞FAO, 从而改善NAFLD。

关键词: 原花青素B3; 非酒精性脂肪肝; 脂肪酸氧化; 高脂血症; 转录因子

中图分类号: R966　　**文献标识码**: A　　**文章编号**: 0513-4870(2026)08-3041-09

Procyanidin B3 promotes fatty acid oxidation and improves non-alcoholic fatty liver disease by GATA3/ACSL5 pathway

RUAN Cheng-hao[1,2], HAN Yan[1], WU Zheng-bin[2], TIAN Xin-ran[2], LIU Wen[2], XU Shu-ping[2], LIU Qing-qing[3*], XIE Bao-ping[1,2*]

(*1. Rehabilitation College of Gannan Medical University, Ganzhou 341000, China;　2. Key Laboratory for Prevention and Treatment of Cardiovascular and Cerebrovascular Diseases of the Ministry of Education, Gannan Medical University, Ganzhou 341000, China;　3. The Affiliated Traditional Chinese Medicine Hospital, Guangzhou Medical University, Guangzhou 510000, China*)

Abstract: This study aims to investigate the pharmacological effects of procyanidin B3 (Pro B3) on non-

收稿日期: 2026-02-04;　修回日期: 2026-04-23.

基金项目: 国家自然科学基金青年基金C类 (82504799); 广州市卫生健康科技项目 (20261A031011); 赣州市青年人才项目 (2025XDCE0024); 心脑血管疾病防治教育部重点实验室开放基金 (XN202505).

*通讯作者 Tel: 86-797-8169163, E-mail: xiebaoping@gmu.edu.cn; 767232603@qq.com

DOI: 10.16438/j.0513-4870.2026-0156

alcoholic fatty liver disease (NAFLD) and its molecular mechanism underlying the regulation of fatty acid oxidation (FAO) in hepatocytes during NAFLD, thereby providing experimental evidence for the clinical application of Pro B3 in NAFLD. Mice were fed a high-fat diet (HFD) for 12 weeks to induce NAFLD and received oral gavage administration for 12 weeks. The pharmacological effect of Pro B3 in improving NAFLD was assessed through serum biochemical indexes, liver pathological changes and lipid droplet formation in the liver using Hematoxylin-eosin (H&E) staining and oil red O staining. This study protocol was approved by the Experimental Animal Ethics Committee of Gannan Medical University (approval No.: 2025443). In the *in vitro* experiments, the HepG2 cells were induced with a mixture of palmitic acid and oleic acid (2∶1) to create a cellular model of NAFLD and evaluate the cytotoxicity and cell viability of ProB3 on HepG2 cells. The expressions of FAO-associated genes such as acyl-CoA synthetase long chain family member 5 (ACSL5), carnitine palmitoyltransferase 1 (CPT1), acetyl-CoA carboxylase (ACC), and GATA binding protein 3 (GATA3) were detected by immunohistochemical, immunofluorescence, qRT-PCR and Western blot. Mechanistically, the JASPAR database and dual-luciferase reporter gene assays were used to explore the transcription factors and binding sites of ACSL5. By silencing GATA3 using siRNA, the effects of Pro B3 on the expression of ACSL5 and lipid deposition were examined. The results demonstrated that Pro B3 significantly improves the NAFLD-related indicators in mice, reduces serum lipid levels and lipid droplet accumulation in HepG2 cells, and up-regulates the expression of FAO-related genes, such as ACSL5, CPT1, ACC. Furthermore, GATA3 might be a transcription factor of ACSL5, and the transcriptional regulatory site might be "ACATAG". Moreover, Pro B3 significantly upregulated the expression of GATA3 mRNA and protein levels, while silencing GATA3 significantly inhibited the expression of ACSL5 mRNA and protein levels, as well as weakened the inhibitory effect of Pro B3 on lipid accumulation in HepG2 cells. In conclusion, Pro B3 may be an effective lead compound for treating NAFLD by promoting FAO in hepatocytes through regulation of the GATA3/ACSL5 pathway, thereby ameliorating NAFLD.

Key words: procyanidin B3; non-alcoholic fatty liver disease; fatty acid oxidation; hyperlipidemia; transcription factor

非酒精性脂肪肝 (non-alcoholic fatty liver disease, NAFLD) 是除酒精和其他明确的损肝因素所致的肝细胞内脂肪过度沉积为主要特征的临床病理综合征, 是与胰岛素抵抗和遗传易感性密切相关的获得性代谢应激性肝损伤和慢性炎症反应[1,2]。目前, NAFLD 的早期干预手段主要依赖于生活方式的调整, 包括减肥、运动和饮食控制等。然而, 运动和饮食调控改善NAFLD的疗效非常有限[3,4]。因此, 研究NAFLD的发病机制, 寻找高效且安全的NAFLD治疗药物, 仍是当前医学研究的重中之重[5]。

脂质代谢异常是NAFLD的病理特征之一, 脂肪酸在肝脏中通过线粒体β氧化进行分解代谢, 由此产生的还原产物 (NADH和FADH2) 进入线粒体电子传递链 (electron transport chain, ETC), 前期研究发现, 提高肝脏线粒体呼吸活性和脂肪酸氧化 (fatty acid oxidation, FAO) 水平, 能够促进脂肪酸降解, 从而抑制肝脏脂肪堆积和NAFLD的形成[6,7]。因此, 促进FAO是NAFLD临床治疗和药物研发的新策略, 且越来越多的研究表明靶向肝细胞FAO关键激酶酰基辅酶A合成酶长链家族成员5 (acyl-CoA synthetase long chain family member 5, ACSL5) 可以显著促进脂肪酸分解代谢, 抑制脂肪酸在体内堆积[8,9], 且细胞质内Sirt6介导的ACSL5去乙酰化可以促进肝脏FAO, 从而改善NAFLD[10]。同时, ACSL5的K48去泛素化可显著提高ACSL5的表达, 从而改善NAFLD[10,11]。

原花青素B3 (procyanidin B3, Pro B3) 是从蓝莓、葡萄或金樱子等食物或药食两用中药材中提取的儿茶素类二聚体天然产物, 具有抗氧化、抗炎、抗肿瘤等药理作用[12,13]。前期研究发现, Pro B3能显著抑制β-淀粉样蛋白诱导的神经毒性, 并靶向G3BP1治疗缺血性脑卒中, 减轻神经元损伤[14,15]。然而, Pro B3在NAFLD的药理作用和作用机制尚未阐明, 特别是Pro B3对肝细胞FAO的机制尚不明确。本研究用Pro B3处理高脂饮食 (high fat diet, HFD) 诱导的NAFLD小鼠模型和棕榈酸油酸混合物诱导的HepG2细胞模型, 用Western blot、H&E染色、双荧光素酶报告基因实验、免疫组化、免疫荧光和油红O染色等方法阐明Pro B3对NAFLD和HepG2细胞FAO的影响和分子机制。

材料与方法

实验动物 本实验选用6～8周龄雄性野生型C57BL/6J小鼠35只 (23～27 g), 购自江苏华创实验动物有限责任公司 [生产许可证: SCXK (su)-20200009]。小鼠饲养在SPF屏障环境中, 自由饮食。

药品与试剂 Pro B3 (成都德思特, 纯度 ≥ 98%, Cat: DY0125-0200, Lot: DSTDY012502); 高脂饲料 (北京博泰宏达生物技术有限公司, Cat: HD012a, Lot: 2025102601); ECL发光液 (Biosharp, Cat: BL520B, Lot: 24025113AZ); 逆转录试剂盒和荧光定量试剂盒 (康为世纪, Cat: CW3371M, Lot: 02425); 低密度脂蛋白 (low density lipoprotein, LDL)、甘油三酯 (triglyceride, TG)、总胆固醇 (total cholesterol, T-CHO) 和高密度脂蛋白 (high density lipoprotein, HDL) 检测试剂盒 (南京建成, Cat: A113-1-1、A110-2-1、A111-1-1、A112-1-1, Lot: 20241217); DMEM培养基 (上海达特希尔, Cat: C3110-0550, Lot: 2515087); 肉碱棕榈酰转移酶1 (carnitine palmitoyltransferase 1, CPT1) 抗体、GATA结合蛋白3 (GATA binding protein 3, GATA3) 抗体 (爱博泰克, Cat: A27657, Lot: 6100007976、Cat: A19636, Lot: 4000000115); β-actin抗体 (贝茵莱, Cat: MAB48206, Lot: 20240910); ACSL5抗体 (华安生物, Cat: HA601166, Lot: H651360039); 高脂细胞诱导剂 (棕榈酸 + 油酸)、细胞用油红O染色试剂盒 (索莱宝, Cat: C3461, Lot: 2509180018、Cat: G1262); 细胞裂解液、胎牛血清 (赛维尔, Cat: G2002-100ML, Lot: CR2411174、Cat: G8003-100ML, Lot: SO20250425); 荧光素酶报告基因实验试剂盒 (翌圣生物, Cat: 11402ES); pGL3-vector、pGL-GATA3、pGL3-mACSL5-WT和pGL3-mACSL5-MU质粒的设计与合成由优宝生物完成。

实验仪器 多功能酶标仪 (型号: HH35000510, 美国珀金埃尔默股份有限公司); 化学发光仪 (型号: Amersham Imager 600, 美国GE医疗生命科学部); 荧光定量PCR仪 (型号: QuantStudio™ 7 Flex, 美国应用生物系统中国公司); 细胞培养箱 (型号: 371GP, 美国赛默飞科技公司); 蛋白印迹系统 (型号: BVE-4, 武汉塞维尔); 水浴锅 (型号: BLHH-4N, 上海丙林电子科技有限公司); 分析天平 (型号: ME204, 瑞士梅特勒托利多科技有限公司); 水平脱色摇床 (型号: SK-O180-S, 北京大龙兴创实验仪器股份公司)。

动物实验 动物适应性饲养1周后随机分为5组, 每组7只: 对照组、模型组 (HFD)、Pro B3低剂量 (15 mg·kg^{-1}·d^{-1}) 组、Pro B3中剂量 (30 mg·kg^{-1}·d^{-1}) 组、Pro B3高剂量 (45 mg·kg^{-1}·d^{-1}) 组。对照组饲喂正常饲料, 自由饮食, 其余各组饲喂高脂饲料, 并根据实验分组给予相应剂量的Pro B3, 对照组和模型组给予相应剂量的生理盐水。实验动物处理12周后, 小鼠经麻醉处理后处死, 取血和肝脏用于后续检测。本研究方案经赣南医科大学实验动物伦理委员会批准 (批准号: 2025443)。

血清生化检测 用生化检测试剂盒检测Pro B3对HFD诱导的小鼠血脂指标TG、T-CHO、LDL和HDL水平的影响, 按试剂盒说明书操作, 用多功能酶标仪检测吸光度, 并计算各组TG、T-CHO、LDL和HDL的浓度。

肝脏油红O染色 将肝脏组织切成厚度为100 μm大小的组织, 在4%多聚甲醛中固定24 h, 取出肝脏, 分别经过脱水、透化、浸蜡、包埋、切片与贴片、脱蜡、染色、脱水、透明等步骤获得石蜡切片, 将切片浸入60%异丙醇3 s再浸入配制好的饱和油红O工作液。在37 ℃避光条件下染色60 min后, 取出切片浸入60%异丙醇分化2 min, 用蒸馏水洗终止分化。用滤纸吸除多余的水分, 用中性树脂封片。

肝脏H&E染色 将上述获得的切片浸入Harris苏木素染液中浸染3～8 min, 自来水洗, 1%盐酸乙醇分化数秒, 自来水冲洗, 0.6%氨水返蓝, 流水冲洗。切片放入伊红染液中染色1～3 min。将切片依次放入95%乙醇I 5 min-95%酒精II 5 min-无水乙醇I 5 min-无水乙醇II 5 min-二甲苯II 5 min-二甲苯II 5 min中脱水透明, 将切片从二甲苯拿出来稍晾干, 用中性树胶封片。显微镜观察, 图像采集分析。

细胞培养和细胞活性检测 HepG2和HEK293T细胞购自美国典型培养物保藏中心, 均用含10%胎牛血清、100 u·mL^{-1}青霉素和100 μg·mL^{-1}链霉素的DMEM培养基在37 ℃、5% CO_2的湿润环境中培养。随后, 将HepG2细胞以每孔5×10^{3}个细胞的密度接种于96孔板中过夜培养, 用不同浓度的Pro B3 (0.1、1、2.5、5、10、20、40、100 μmol·L^{-1}) 处理24 h。用CCK-8试剂盒测定细胞活力, 并计算细胞增殖率。

细胞油红O染色 将HepG2细胞种于24孔板, 实验分为对照组、模型 (棕榈酸 + 油酸) 组、Pro B3 (5、10、20 μmol·L^{-1}) 组, 药物组先用不同浓度的Pro B3处理4 h后, 除对照组外均加入终浓度为200 μmol·L^{-1}棕榈酸油酸混合物处理24 h。移除细胞培养基, 用PBS洗2次, 按试剂盒说明书进行油红O染色, 加入蒸馏水覆盖细胞并在显微镜下观察脂滴的形成, 使用Image-Pro Plus 6.0分析脂滴面积。

荧光定量PCR检测 细胞分组和处理方法同"细胞油红O染色", 用Trizol试剂裂解细胞, 提取总RNA, 检测RNA浓度和纯度后, 按照试剂盒说明的步骤将其逆转录为cDNA进行实时荧光定量PCR。反应引物由生工公司合成, 引物序列见 (表1)。反应条件为: 预变性94 ℃ 30 s; 变性94 ℃ 5 s, 延伸复性60 ℃ 30 s, 40个循环。分析以β-actin表达量为内参。采用比较C_t值定量方法进行相对定量。

蛋白质印迹法 细胞分组和处理方法同"细胞油

Table 1 Primer sequences for quantitative real-time polymerase chain reaction. ACSL5: Acyl-CoA synthetase long chain family member 5; CPT1: Carnitine palmitoyltransferase 1; ACC: Acetyl-CoA carboxylase; GATA3: GATA binding protein 3

Gene name	Forward	Reverse
Acsl5	5'-CCCCCATGTCCACTTCAGTC-3'	5'-CCAACGGACCTGATGTCCA-3'
Cpt1	5'-CACGGGTGGATGTTCACTGA-3'	5'-AGGCCTCACCGACTGTAGAT-3'
Gapdh	5'-GATTTGGTCGTATTGGGCGC-3'	5'-TTCCCGTTCTCAGCCTTGAC-3'
Acc	5'-CCACCAATCTGCCTGTGCTA-3'	5'-GTGAGCACCACATAGGCTGT-3'
Gata3	5'-TCCTGTGCGAACTGTCAGAC-3'	5'-TCGGTTTCTGGTCTGGATGC-3'

红O染色”, 细胞处理24 h后, 用PBS洗2次, 用RIPA裂解液冰上裂解30 min, 用BCA试剂盒定量, 取200 μg蛋白, 加入5× loading buffer, 100 ℃变性5 min, 样品−20 ℃保存备用。蛋白质印迹方法参考之前课题组的研究[16,17], 孵育anti-GATA3、anti-CPT1、anti-β-actin、anti-ACC和anti-ACSL5抗体, 以β-actin为内参计算Pro B3对FAO相关基因蛋白水平的表达调控。

荧光素酶报告基因实验 将293T细胞种植于24孔板, 1×10^4个/孔, 按照实验分组, 将野生型和突变型质粒, 以及海肾质粒转染到293T细胞, 转染方法按照试剂盒说明书进行。转染48 h后, 收集细胞, 用荧光素酶报告基因实验试剂盒在多功能酶标仪上检测各组荧光强度。

siRNA筛选和转染 将293T细胞或HepG2细胞种植于24孔板, 1×10^4个/孔, 将实验分为control组、siRNC组、siRNA1-GATA3组、siRNA2-GATA3组和siRNA3-GATA3组, 按照实验室分组转染siRNA, 转染24 h后, 提取蛋白, 进行Western blot检测GATA3的表达。

统计学分析 用SPSS 22.0软件对数据进行统计学分析, 采用GraphPad Prism 8.0软件制图。计量资料以均值 ± 标准差 ($\bar{x} \pm s$) 表示。方差齐者多组间比较用单因素方差分析, 两两比较采用LSD-t检验法; 方差不齐者多组间比较采用Kruskal Wallis H检验。所有假设检验均采用双侧检验, $P < 0.05$为差异有统计学意义。

结果

1 Pro B3显著改善HFD诱导的小鼠血脂水平和NAFLD的形成

用HFD诱导小鼠是经典的NAFLD动物造模方法[18], 通过检测小鼠血脂水平和肝脏油红O染色等指标评价Pro B3对NAFLD的药理作用 (图1A、B)。结果表明, 饲养12周后各组小鼠体重之间无显著性差异 (图1C)。与模型组相比, Pro B3呈剂量依赖性地抑制HFD诱导的血脂水平, 降低血清中LDL、TG和T-CHO水平, 升高HDL水平 (图1D)。同时, 本研究用肝脏H&E染色和油红O染色评价了Pro B3对肝脏脂质沉积和代谢的影响。结果表明, Pro B3处理可显著抑制肝脏脂肪病变、肝细胞脂质积累和脂滴形成 (图1E、F), 提示Pro B3具有良好的治疗NAFLD的药理作用。

2 Pro B3显著抑制棕榈酸油酸混合物诱导的HepG2细胞脂滴形成

棕榈酸油酸混合物处理可以促进脂质在细胞内的沉积, 是研究NAFLD的经典细胞造模方法。因此, 本研究用棕榈酸油酸混合物诱导HepG2细胞脂质沉积, 用不同浓度的Pro B3处理HepG2细胞, 用CCK-8检测细胞活性, 油红O染色检测脂质积累。结果表明, 与对照组相比, 实验剂量下 (5、10、20 μmol·L^{-1}) 的Pro B3不会抑制HepG2细胞的增殖 (图2A)。油红O染色结果表明, Pro B3呈剂量依赖性地抑制HepG2细胞中脂质的形成 ($P < 0.05$, $P < 0.01$, $P < 0.001$, 图2B), 提示Pro B3可以显著抑制棕榈酸油酸混合物诱导的脂肪酸积累和脂滴形成。

3 Pro B3促进棕榈酸油酸混合物诱导的HepG2细胞FAO相关基因的表达

脂质代谢异常被认为是NAFLD形成的主要原因, 促进脂肪酸代谢和抑制脂肪酸合成是NAFLD治疗的有效方法, 特别是促进肝细胞FAO被认为是治疗NAFLD的新策略[19]。因此, 本研究检测了Pro B3对FAO相关基因表达的影响。结果表明, 与对照组相比, 棕榈酸油酸混合物显著抑制FAO相关基因的表达 ($P < 0.05$, $P < 0.01$, $P < 0.001$), Pro B3预处理显著促进ACSL5、ACC和CPT1的表达 ($P < 0.05$, $P < 0.01$, $P < 0.001$, 图3A)。在蛋白水平调控上, Pro B3显著促进肝细胞FAO相关基因的表达 ($P < 0.05$, $P < 0.01$, $P < 0.001$, 图3B)。提示Pro B3通过调控肝细胞FAO抑制脂质堆积, 改善NAFLD。

4 Pro B3显著促进棕榈酸油酸混合物诱导的GATA3表达

为了阐明Pro B3调控肝脏细胞FAO的分子机制, 本研究通过JASPAR数据库预测发现GATA3是ACSL5的转录因子, 双荧光素酶报告基因实验也证实GATA3是ACSL5的转录因子 (图4A), 且转录调控位点可能是“ACATAG”(图4B)。同时, 用免疫组化、免疫荧光染色、Western blot和qRT-PCR检测Pro B3对GATA3蛋白水平和mRNA水平表达的影响, 结果表明, 与模型组相比, Pro B3显著上调肝脏中GATA3的

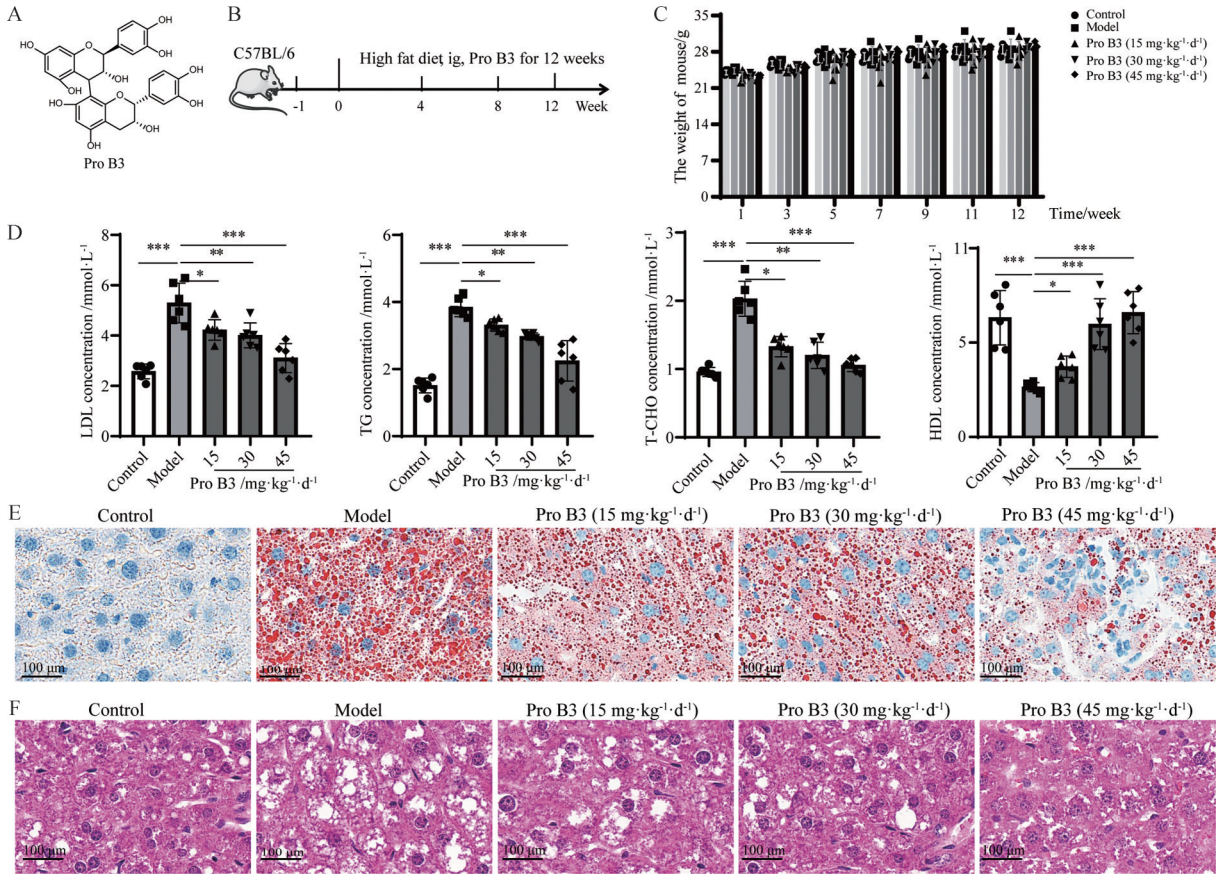


Figure 1 The effect of procyanidin B3 (Pro B3) on non-alcoholic fatty liver disease (NAFLD) induced by high fat diet (HFD). A: The chemical structure of Pro B3; B: Program of animal experiment; C: The weight of the mouse; D: The concentrations of low density lipoprotein (LDL), triglyceride (TG), total cholesterol (T-CHO), and high density lipoprotein (HDL) in serum induced by HFD; E: Oil red O staining of liver sections (scale bar: 100 μm); F: H&E staining of liver sections (scale bar: 100 μm). $n = 7, \bar{x} \pm s.$ $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

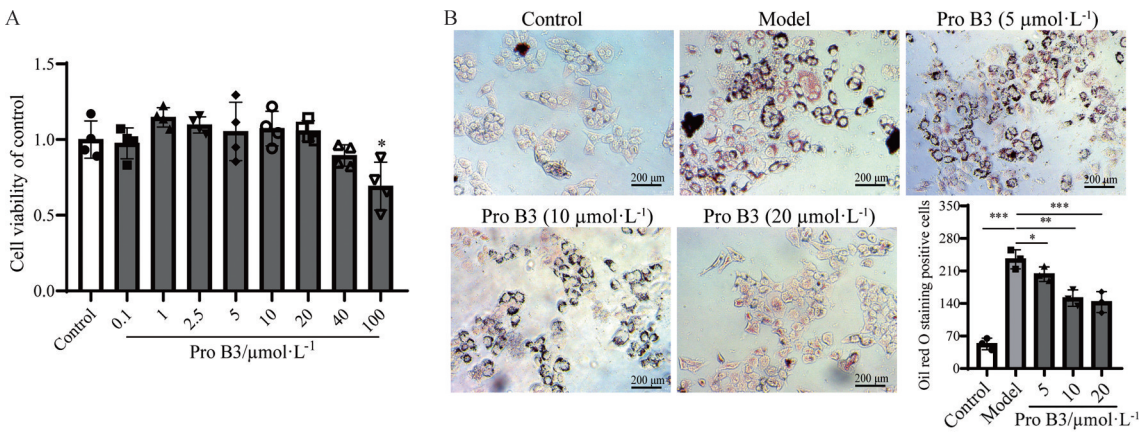


Figure 2 Effect of Pro B3 on lipid accumulation in HepG2 cells. A: Cell viability in the presence or absence of Pro B3 were detected by cell counting kit (CCK8) ($n = 5$); B: Oil red O staining of HepG2 cell induced by palm acid oleic acid mixture ($n = 3$) (scale bar: 200 μm). $\bar{x} \pm s.$ $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

表达 (图4C), 以及肝细胞中GATA3 mRNA和蛋白水平的表达 (图4D～F) ($P < 0.05$, $P < 0.01$, $P < 0.001$)。因此, GATA3是ACSL5的转录因子, 且转录结合位点可能是“ACATAG”, Pro B3可能通过GATA3/ACSL5通路促进肝细胞FAO。

5 Pro B3通过GATA3/ACSL5通路促进HepG2细胞FAO

为了进一步探讨Pro B3通过GATA3/ACSL5通路

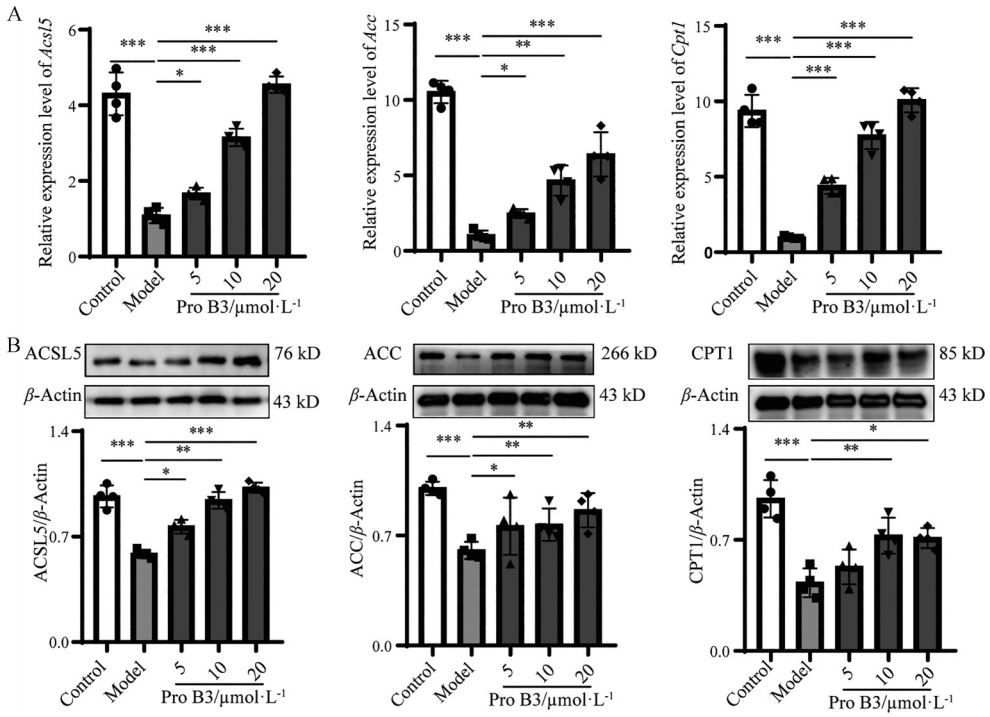


Figure 3 The effect of Pro B3 on the expression of fatty acid oxidation (FAO) related genes. A: *Acsl5*, *Acc*, and *Cpt1* mRNA expression were detected by qRT-PCR; B: Western blot was performed to measure the ACSL5, ACC, and CPT1 protein expression. $n = 4$, $\bar{x} \pm s$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

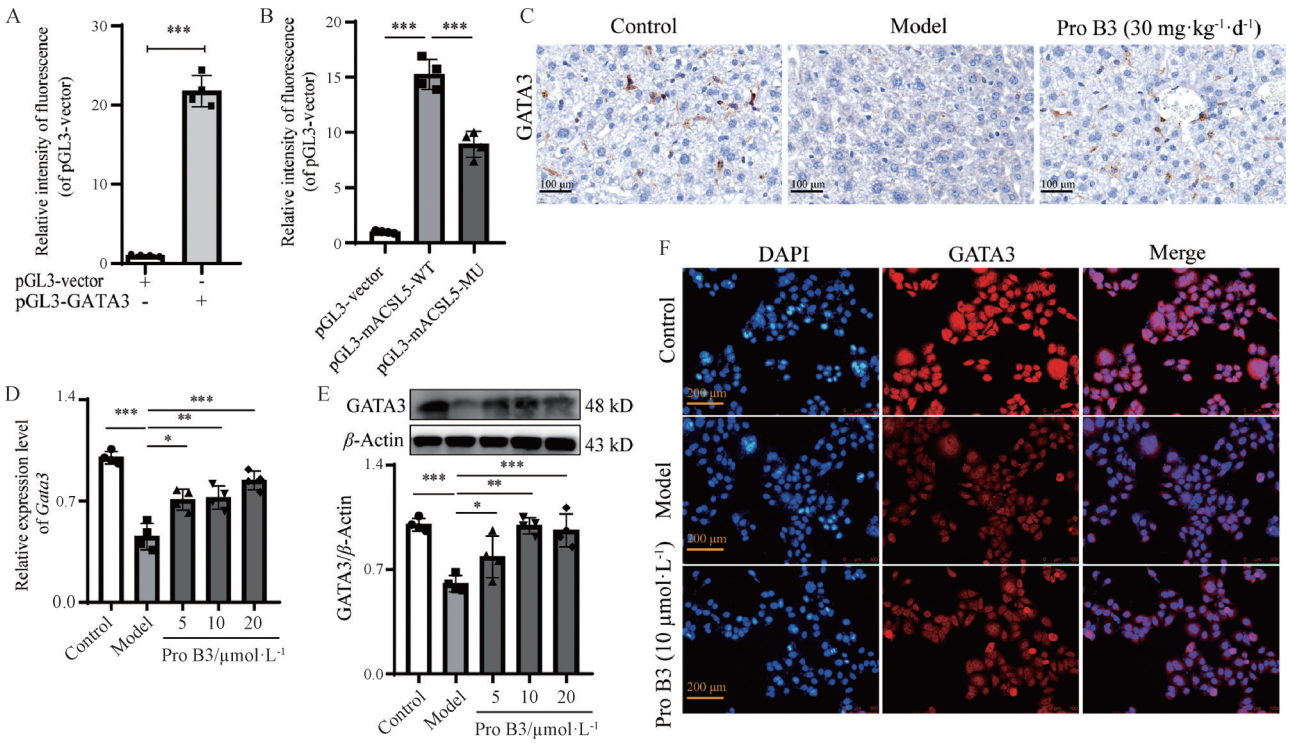


Figure 4 Pro B3 significantly promotes GATA3 expression induced by palmitate-oleic acid mixture. A, B: The dual luciferase reporter gene assay confirmed that GATA3 is a transcription factor for ACSL5 ($n = 3$); C: Immunohistochemical detection of GATA3 expression in the liver ($n = 3$) (scale bar: 100 μm); D: Detection of *Gata3* expression in liver cells by qRT-PCR ($n = 4$); E: Detection of GATA3 expression in liver cells by Western blot ($n = 3$); F: Immunofluorescence assay for the expression of GATA3 (scale bar: 200 μm)

促进肝细胞FAO的机制, 通过siRNA沉默GATA3的表达 (图5A), 检测Pro B3对棕榈酸油酸诱导的HepG2脂质沉积和FAO相关基因表达的影响。结果表明, 与模型组相比, Pro B3显著抑制HepG2脂肪堆积 ($P <$

0.01), 与 Pro B3 (10 μmol·L^{-1}) 组相比, 沉默 GATA3 显著抑制 Pro B3 对 HepG2 脂肪沉积的调控作用 (P < 0.05) (图 5B), 以及 ACSL5 mRNA 和蛋白表达的调控作用 (图 5C、D) (P < 0.05, P < 0.01)。提示 Pro B3 通过调控 GATA3/ACSL5 通路促进肝细胞 FAO, 减少脂肪酸积累。

讨论

脂肪酸在肝脏中沉积逐渐导致肝脏脂肪病变是 NAFLD 的主要病理变化[20]。肝脏对碳水化合物和脂肪酸等主要代谢能量底物的代谢能力超出其生理极限, 致使二酰甘油、神经酰胺等在肝脏内异常蓄积, 进而发展为 NAFLD 或肝硬化[21]。因此, 本研究用 HFD 诱导小鼠构建 NAFLD 的动物模型[22]。临床上常用均衡运动和降血脂药物治疗 NAFLD, 如辛伐他汀和阿托伐他汀等[23]。本研究发现从蓝莓、葡萄和金樱子中提取的 Pro B3 具有良好的抗 NAFLD 药理作用, 可以显著降低 HFD 诱导的血脂水平和高脂血症, 抑制肝脏脂肪病变和脂滴形成, 提示 Pro B3 可能是治疗 NAFLD 的先导化合物, 也为倡导居民改善饮食预防 NAFLD 提供实验依据。

肝细胞 FAO 是脂肪酸代谢的主要机制, 调节肝细胞 FAO 成为 NAFLD 基础研究和临床治疗的新热点和新策略[24]。ACSL5 是肝细胞 FAO 和临床治疗 NAFLD 的潜在靶点, 可以催化长链脂肪酸转化为脂肪酰-CoA 酯, 从而在脂质生物合成和脂肪酸降解中发挥关键作用[11,25]。前期研究发现, 上调 ACSL5 的表达显著降低 TG 的积累, 增加 FAO 水平[26]。本研究发现, Pro B3 可以显著促进 FAO 相关基因的表达, 特别是 ACSL5 的表达。在机制研究中, 我们通过生物信息学和双荧光素酶报告基因实验证实 GATA3 可能是 ACSL5 的关键转录因子, 且转录调控位点可能是“ACATAG”。前期研究发现, GATA3 可能是脂肪酸代谢调控的关键靶点[27], 抑制 AMPK-GATA3-ECHS1 通路活性可以诱导脂肪酸合成, 促进透明细胞肾细胞癌生长[28]。本研究表明, Pro B3 显著促进肝脏和肝细胞中 GATA3 mRNA 和蛋白水平的表达, 且沉默 GATA3 显著抑制 Pro B3 对脂质沉积和 ACSL5 表达的调控作用, 提示 Pro B3 通过调控 GATA3/ACSL5 通路促进肝细胞 FAO, 改善 NAFLD。

综上所述, 本研究发现 Pro B3 可能通过调控 GATA3/ACSL5 通路促进肝细胞 FAO, 从而改善 NAFLD。目前尚无 Pro B3 治疗 NAFLD 的研究报道, 本文的研究为 Pro B3 作为治疗 NAFLD 和促进肝细胞 FAO 候选药物的进一步开发奠定基础, 但是对于 Pro B3 促进肝细胞 FAO 和改善 NAFLD 的直接靶点还需

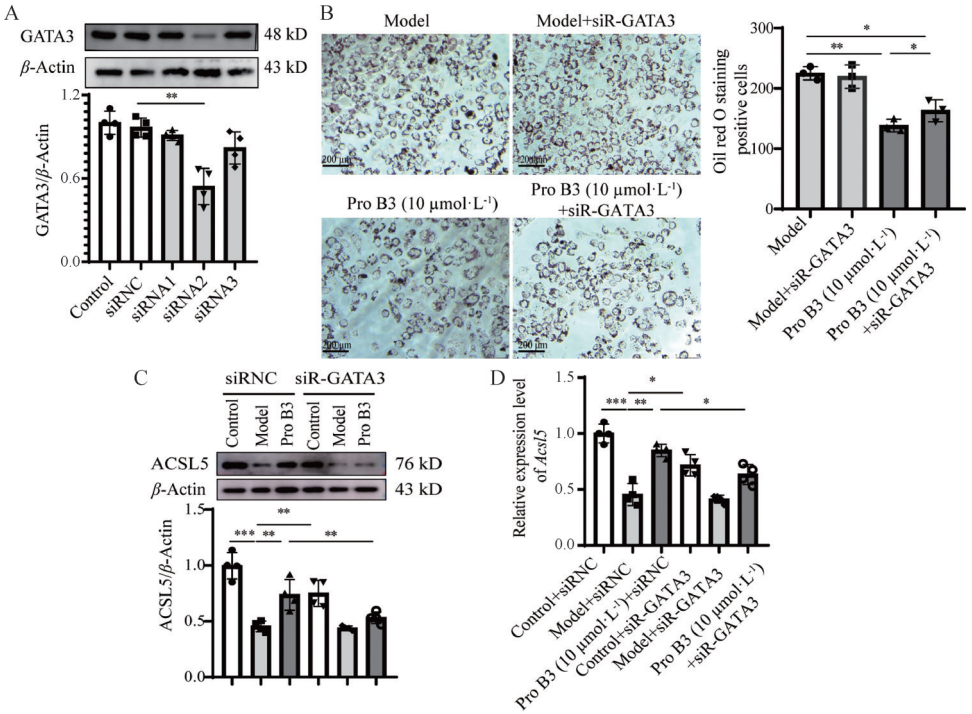


Figure 5 Pro B3 promotes FAO in HepG2 cells by GATA3/ACSL5 pathway. A: Screen for siRNAs that silence GATA3 (n = 4); B: Oil red O staining of HepG2 cells after silencing GATA3 (n = 3) (scale bar: 200 μm); C: Western blot was performed to measure the ACSL5 expression after silencing GATA3 (n = 4); D: qRT-PCR was performed to measure the *Acsl5* expression after silencing GATA3 (n = 4). $\bar{x} \pm s$. $^{*}P$ < 0.05, $^{**}P$ < 0.01, $^{***}P$ < 0.001

进一步探讨, 特别是随着修饰和非修饰小分子靶点确证方法的进展, 以及分子对接和分子动力学的发展, 为更好地阐明Pro B3促进肝细胞FAO和NAFLD的直接靶点和结合位点提供新策略[29,30]。

作者贡献: 阮诚浩、韩艳负责完成细胞实验操作、采集数据、分析数据和撰写文章; 吴征斌、田新然负责完成分子生物学实验操作; 徐树萍、柳雯负责完成动物实验操作、采集数据; 谢保平、刘晴晴负责设计、指导和完善实验方案、文章审阅和修改。

利益冲突: 本文所有作者声明不存在利益冲突关系。

References

[1] Wang X, Xie Q. Metabolic dysfunction-associated fatty liver disease (MAFLD) and viral hepatitis [J]. J Clin Transl Hepatol, 2022, 10: 128-133.

[2] Xu M, Tan J, Zhu L, et al. The deubiquitinating enzyme 13 retards non-alcoholic steatohepatitis *via* blocking inactive rhomboid protein 2-dependent pathway [J]. Acta Pharm Sin B, 2023, 13: 1071-1092.

[3] Wei S, Wang L, Evans PC, et al. NAFLD and NASH: Etiology, targets and emerging therapies [J]. Drug Discov Today, 2024, 29: 103910.

[4] Associazione Italiana per lo Studio del Fegato (AISF), Società Italiana di Diabetologia (SID), Società Italiana dell'Obesità (SIO), et al. Non-alcoholic fatty liver disease in adults 2021: A clinical practice guideline of the Italian Association for the Study of the Liver (AISF), the Italian Society of Diabetology (SID) and the Italian Society of Obesity (SIO) [J]. Nutr Metab Cardiovasc Dis, 2022, 32: 1-16.

[5] Zhou J, Zhou F, Wang W, et al. Epidemiological features of NAFLD from 1999 to 2018 in China [J]. Hepatology, 2020, 71: 1851-1864.

[6] Barbier-Torres L, Fortner KA, Iruzubieta P, et al. Silencing hepatic MCJ attenuates non-alcoholic fatty liver disease (NAFLD) by increasing mitochondrial fatty acid oxidation [J]. Nat Commun, 2020, 11: 3360.

[7] Nie K, Gao Y, Chen S, et al. Diosgenin attenuates non-alcoholic fatty liver disease in type 2 diabetes through regulating SIRT6-related fatty acid uptake [J]. Phytomedicine, 2023, 111: 154661.

[8] Al-Thihli K, Afting C, Al-Hashmi N, et al. Deficiency of acyl-CoA synthetase 5 is associated with a severe and treatable failure to thrive of neonatal onset [J]. Clin Genet, 2021, 99: 376-383.

[9] Luo Y, Zeng Y, Liu Y, et al. ACSL5 regulated acetyl-CoA to promote bladder cancer cellular senescence *via* 53BP1 acetylation [J]. Oncogene, 2025, 44: 3096-3112.

[10] Hou T, Tian Y, Cao Z, et al. Cytoplasmic SIRT6-mediated ACSL5 deacetylation impedes nonalcoholic fatty liver disease by facilitating hepatic fatty acid oxidation [J]. Mol Cell, 2022, 82: 4099-4115.

[11] Hu S, Wang Z, Zhu K, et al. USP29 alleviates the progression of MASLD by stabilizing ACSL5 through K48 deubiquitination [J]. Clin Mol Hepatol, 2025, 31: 147-165.

[12] Mizuno M, Nakanishi I, Matsubayashi S, et al. Synthesis and antioxidant activity of a procyanidin B3 analogue [J]. Bioorg Med Chem Lett, 2017, 27: 1041-1044.

[13] Aini H, Ochi H, Iwata M, et al. Procyanidin B3 prevents articular cartilage degeneration and heterotopic cartilage formation in a mouse surgical osteoarthritis model [J]. PLoS One, 2012, 7: e37728.

[14] Zhang H, Zhang Y, Chen Y, et al. Procyanidin B3 and its derivatives alleviate neuronal injury by targeting G3BP1 for ischemic stroke therapy [J]. Adv Sci (Weinh), 2025, 12: e09781.

[15] Shang P, Tang Q, Hu Z, et al. Procyanidin B3 alleviates intervertebral DISC degeneration *via* interaction with the TLR4/MD-2 complex [J]. J Cell Mol Med, 2020, 24: 3701-3711.

[16] Xie B, Tian L, Liu C, et al. Disruption of the eEF1A1/ARID3A/PKC-δ complex by neferine inhibits macrophage glycolytic reprogramming in atherosclerosis [J]. Adv Sci (Weinh), 2025, 12: e2416158.

[17] Xie B, Guo Y, Ye M, et al. Higenamine attenuates isoproterenol-induced myocardial infarction *via* regulating METTL3/TFEB pathway [J]. Acta Pharm Sin (药学学报), 2022, 57: 3106-3114.

[18] Ipsen DHJ, Lykkesfeldt J, Tveden-Nyborg P. Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease [J]. Cell Mol Life Sci, 2018, 75: 3313-3327.

[19] Wang X, Wang K, Jia W, et al. The improvement provided by harpagoside on metabolic dysfunction-associated steatohepatitis in mice [J]. Acta Pharm Sin (药学学报), 2026, 61: 163-172.

[20] Wilson RB, Chen YJ, Sutherland BG, et al. The marine compound and elongation factor 1A1 inhibitor, didemnin B, provides benefit in western diet-induced non-alcoholic fatty liver disease [J]. Pharmacol Res, 2020, 161: 105208.

[21] Chen Z, Tian R, She Z, et al. Role of oxidative stress in the pathogenesis of nonalcoholic fatty liver disease [J]. Free Radic Biol Med, 2020, 152: 116-141.

[22] Flessa C, Nasiri-Ansari N, Kyrou I, et al. Genetic and diet-induced animal models for non-alcoholic fatty liver disease (NAFLD) research [J]. Int J Mol Sci, 2022, 23: 15791.

[23] Liao J, Xie X, Wang N, et al. Formononetin promotes fatty acid β-oxidation to treat non-alcoholic steatohepatitis through SIRT1/PGC-1α/PPARα pathway [J]. Phytomedicine, 2024, 124: 155285.

[24] Zhong J, He X, Gao X, et al. Hyodeoxycholic acid ameliorates nonalcoholic fatty liver disease by inhibiting RAN-mediated PPARα nucleus-cytoplasm shuttling [J]. Nat Commun, 2023, 14: 5451.

[25] Luo Q, Das A, Oldoni F, et al. Role of ACSL5 in fatty acid metabolism [J]. Heliyon, 2023, 9: e13316.

[26] Li S, Yuan Z. More than just a histone deacetylase: Cytoplasmic

SIRT6 facilitates fatty acid oxidation through ACSL5 deacetylation [J]. Acta Biochim Biophys Sin (Shanghai), 2023, 55: 525-527.

[27] Stark JM, Tibbitt CA, Coquet JM. The metabolic requirements of Th2 cell differentiation [J]. Front Immunol, 2019, 10: 2318.

[28] Qu Y, Zhao R, Zhang H, et al. Inactivation of the AMPK-GATA3-ECHS1 pathway induces fatty acid synthesis that promotes clear cell renal cell carcinoma growth [J]. Cancer Res, 2020, 80: 319-333.

[29] Ren Y, Li H, Piao X, et al. Drug affinity responsive target stability (DARTS) accelerated small molecules target discovery: Principles and application [J]. Biochem Pharmacol, 2021, 194: 114798.

[30] Tu Y, Tan L, Tao H, et al. CETSA and thermal proteome profiling strategies for target identification and drug discovery of natural products [J]. Phytomedicine, 2023, 116: 154862.