Nrf2: 非酒精性脂肪性肝病的新靶点

陈智伟, 孙 华*

(中国医学科学院、北京协和医学院药物研究所, 天然药物活性物质与功能国家重点实验室, 北京 100050)

摘要: 肝脏是机体重要器官, 具有代谢、解毒等多种功能。由于生活方式的急剧改变和公共卫生水平的提高, 非感染性疾病发病率显著上升, 从根本上改变了世界大多数地区的疾病特点。目前, 全球非酒精性脂肪性肝病 (non-alcoholic fatty liver disease, NAFLD) 的患病率大约为25%, 约59.10%的NAFLD患者在5年内进展为非酒精性脂肪性肝炎 (non-alcoholic steatohepatitis, NASH), 约41%的NASH患者进展为纤维化。NAFLD已成为全球最主要的肝脏疾病之一, 并可能在未来几十年内成为终末期肝病的主要原因。NAFLD和相关肝硬化会给患者、医疗保健系统和社会带来巨大经济负担。由于目前尚无获得美国食品和药品管理局 (Food and Drug Administration, FDA) 批准治疗的药物, NAFLD的治疗主要仍依靠运动和饮食等生活方式的改变。氧化应激和炎症是肝脏疾病发生和发展过程中最重要的病理过程。核因子E2相关因子2 (nuclear factor erythroid-2-related factor 2, Nrf2) 是机体抗氧化应激系统的关键调节因子, 具有抗炎、抗氧化等多种功能。多项研究表明Nrf2通路显著影响NAFLD的进展。本综述旨在总结Kelch样ECH-关联蛋白1-Nrf2-抗氧化反应元件 (Keap1-Nrf2-ARE) 信号通路在NAFLD发病机制中的调节作用, 并揭示Nrf2作为NAFLD治疗靶点的潜力。

关键词: 核因子E2相关因子2; 非酒精性脂肪性肝病; 胰岛素抵抗; 氧化应激; 药物开发

中图分类号: R966 **文献标识码**: A **文章编号**: 0513-4870(2022)11-3268-08

Nrf2: a new target for nonalcoholic fatty liver disease

CHEN Zhi-wei, SUN Hua*

(*State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China*)

Abstract: The liver is an important organ of the body, which has many functions, such as metabolism and detoxification. Due to the rapid change of lifestyle and the improvement of public health, the incidence rate of non-communicable diseases has increased significantly, which fundamentally changed the disease characteristics in most parts of the world. At present, the global prevalence of non-alcoholic fatty liver disease (NAFLD) is about 25%. Moreover, about 59.10% of NAFLD patients progress to non-alcoholic steatohepatitis (NASH) within 5 years, and about 41% of NASH patients progress to fibrosis. NAFLD has become one of the most important liver diseases in the world and may become the main cause of end-stage liver disease in the next few decades. In addition, NAFLD and related cirrhosis will bring huge economic burden to patients, health care system and society. Since there are currently no medications available that have been approved by Food and Drug Administration (FDA), NAFLD is still treated mainly through lifestyle changes such as exercise and diet. Oxidative stress and inflammation are the most important pathological processes in the occurrence and development of liver diseases. Nuclear factor erythroid-2-related factor 2 (Nrf2) is a key regulator of the body's antioxidant stress system, with anti-inflammatory, antioxidant and other functions. Many studies have shown that Nrf2 pathway significantly

收稿日期: 2022-05-09; 修回日期: 2022-06-07.

基金项目: 国家科技重大专项重大新药创制项目 (2019ZX09201001-002-004); 国家重点研发计划−中医药现代化项目 (2019YFC1708901); 中国医学科学院医学与健康科技创新工程项目 (2021-I2M-1-028).

*通讯作者 Tel: 86-10-50927122, E-mail: sunhua@imm.ac.cn

DOI: 10.16438/j.0513-4870.2022-0561

affects the progression of liver diseases. In this review, we aimed to summarize the regulatory role of the Kelch-like ECH-associating protein 1 (Keap1)-Nrf2-antioxidant response element (ARE) signaling pathway in the pathogenesis of NAFLD, and to reveal the potential of Nrf2 as a therapeutic target for NAFLD.

Key words: nuclear factor erythroid-2-related factor 2; non-alcoholic fatty liver disease; insulin resistance; oxidative stress; drug development

1 NAFLD的发病机制

非酒精性脂肪性肝病 (non-alcoholic fatty liver disease, NAFLD) 是全球范围内最常见的慢性肝病[1], 患病率大约为25%[2]。其特征为在没有肝病危险因素(如酗酒、致肝损伤药物和慢性肝病等其他病因) 的情况下, 超过5%肝细胞中出现脂质过度积累、炎症和纤维化[3,4]。NAFLD的发病机制尚未完全阐明, 已经为社会带来巨大经济负担[5]。Day等[6]在1998年提出的“二次打击”假说得到了众多科学家的认可, 是目前NAFLD的发病机制的经典学说。该假设将胰岛素抵抗 (insulin resistance, IR) 导致的肝脏新生脂肪合成增强和分解减少, 从而造成肝组织脂质沉积视为“第一次打击”的关键因素。脂质沉积使进入肝细胞的游离脂肪酸增加, 导致包括氧化应激、炎症反应和线粒体功能障碍等的“第二次打击”, 其中氧化应激起核心作用[7]。肝细胞中过度产生的活性氧 (reactive oxygen species, ROS) 和炎症因子进一步激活Kupffer细胞和肝星状细胞, 导致肝脏炎症和纤维化[8]。因此, 抑制肝脏氧化应激和ROS过量产生是NAFLD预防和治疗的关键方法[9]。

然而, 进一步研究发现, “二次打击”学说似乎过于简单, 很难概括人类NAFLD的复杂性。目前, 包括饮食、基因、表观遗传、糖脂代谢紊乱、炎症反应、肠道菌群紊乱等在内的“多重打击”学说正逐渐被广泛接受[10]。该假说认为, 饮食习惯、环境、遗传因素可导致肥胖、IR、肠道微生物改变[11]。脂质沉积进一步引发氧化应激、内质网应激、自噬等病理过程。肝脏内氧化应激和肠道通透性改变会进一步导致炎症和肝纤维化。

2 Keap1-Nrf2-ARE通路与肝细胞防御系统

肝脏具有相对较高的代谢活性, 是负责生物转化和解毒的主要器官, 这些特性使肝脏暴露于ROS的风险增加[1]。在肝细胞中, 不饱和游离脂肪酸主要在线粒体和细胞色素P450 (cytochrome P450, CYP450) 系统通过氧化和硝化产生大量ROS。肝细胞具有多种防御系统, 可防止内源性和外源性氧化剂和亲电试剂的毒性作用: ① 通过一相代谢酶如CYP450酶, 增加其极性和水溶性; ② 通过二相代谢酶如谷胱甘肽*S*转移酶 (glutathione *S*-transferase, GST) 和尿苷二磷酸−葡萄糖醛酸转移酶 (UDP-glucuronosyltransferases, UGTs) 将一相代谢酶的产物与亲水基团结合, 以促进其排泄; ③ 通过抗氧化酶如超氧化物歧化酶、谷胱甘肽过氧化物酶、过氧化氢酶, 使ROS失活; ④ 通过外排转运蛋白, 与二相代谢酶协同外排有毒代谢物; ⑤ 含有硫醇结构的还原性物质如谷胱甘肽 (glutathione, GSH) 和硫氧还蛋白, 其功能是维持细胞内的还原条件并使亲电化合物失活。重要的是, 这些细胞保护酶的编码基因多数在其启动子区域中含有抗氧化反应元件 (anti-oxidant responsive element, ARE)。ARE是一种顺式增强子序列, 可介导基因的转录激活, 以响应细胞氧化还原状态的变化[12]。

核因子E2相关因子2 (nuclear factor erythroid-2-related factor 2, Nrf2) 是一种碱性的亮氨酸拉链蛋白, 是激活ARE的关键转录因子, 广泛存在于包括肝脏、肺、肠、肾脏在内的大量组织中[13]。Nrf2的蛋白半衰期较短, 约为13～20 min[14]。在生理状态下, 细胞质中的细胞骨架锚定蛋白Kelch样ECH相关蛋白1 (Kelch-like ECH-associated protein 1, Keap1) 通过双甘氨酸重复区域 (DGR) 与Nrf2的N端部分的Nrf2-ECH同源2 (Neh2) 结构域结合, 将其转运到泛素蛋白酶体中以进行降解从而调节其活性[15]。此外, 当自噬途径受损且p62过量积累时, Keap1可与p62结合, 不能再结合Nrf2, 导致Nrf2信号传导增加[16]。当Keap1-Nrf2-ARE通路被ROS激活后, Keap1与Nrf2解离, 随后Nrf2易位到细胞核与ARE结合并促进许多抗氧化蛋白的转录[17]。Keap1含有25个半胱氨酸残基 (Cys), 在这些残基中, 包括Cys273和Cys288在内的4个Cys被鉴定为从Keap1-Nrf2复合物释放Nrf2的关键位点[18]。同时, Nrf2的磷酸化是Nrf2与Keap1分离的必要条件, 蛋白激酶C、磷脂酰肌醇3-激酶、p38丝裂原活化蛋白激酶、磷酸肌醇3-激酶 (PI3K)/蛋白激酶B (AKT) 在内的多种激酶可磷酸化Nrf2并改变其靶基因的转录[19]。Nrf2调控的下游蛋白参与多种细胞功能, 包括药物代谢、ROS清除、还原型GSH稳态等[11]。此外, Nrf2还可调节脂质代谢、炎症、细胞增殖和分化等多种基因表达[20]。

3 Nrf2与NAFLD“第一次打击”

IR导致的脂质沉积被视为NAFLD的“第一次打击”。肝脏在脂肪酸、胆固醇、磷脂等脂质代谢中起关

键作用。在生理状态下, 肝细胞将多余的葡萄糖转化为脂肪酸, 其可进一步转化为甘油三酯储存在脂滴中, 或合成磷脂转运到生物膜中[21]。正常情况下, 甘油三酯、胆固醇和磷脂一起组装成极低密度脂蛋白 (VLDL) 颗粒, 这些颗粒可分泌到血液中, 随后以脂滴形式储存在其他组织中, 从而防止甘油三酯在肝细胞中的过量积累。另一方面, 当葡萄糖摄入量不能满足机体能量需求时, 肝细胞通过溶酶体降解途径分解储存在脂滴中的甘油三酯和胆固醇, 提供游离脂肪酸 (FFA), 以维持线粒体 β-氧化速率和产生三磷酸腺苷 (ATP)。当脂肪酸含量超过细胞的 β-氧化能力时, 将导致肝细胞中脂肪的过量积累, 造成肝细胞脂肪变性[22]。肝脏脂肪变性增加了游离脂肪酸的氧化和三羧酸循环的速率, 导致线粒体呼吸链电子泄漏率增加, 造成更多的自由基生成, 并增加过氧化物酶体中的过氧化氢产生。微粒体氧化还通过 CYP2E1 和 CYP4A 促进氧化应激。游离饱和脂肪酸进入膜磷脂会损伤内质网, 导致 Ca^{2+} 释放, 这有可能进一步损伤相邻线粒体并促进细胞死亡[23]。

Nrf2 参与肝脏脂肪酸的代谢。Yates 等[24]的一项微阵列研究表明, Nrf2 的激活抑制了脂肪酸合酶等脂肪合成的关键酶的表达, 同时降低了肝脂质的水平。在 NAFLD 患者和饮食诱导的肥胖小鼠的 Nrf2 表达水平上调, 而 Nrf2 缺乏可通过抑制自噬途径导致肝脂在体内和体外积累[25]。Li 等[26]也同样发现 Nrf2 敲除 (knockout, KO) 小鼠在高脂饮食 (HFD) 诱导后表现出更严重的代谢综合征和肝脂肪变性。最近也有研究发现, 通过自噬和 p62 介导的 Nrf2 激活可改善小鼠肝脏免受脂毒性[27]。这些研究都是在年轻小鼠 (8～10 周龄) 上进行的。采用 Nrf2 缺失 (C57BL/6) 的老年小鼠 (25 周龄) 的研究表明, Nrf2 对肝脏脂肪酸代谢几乎没有影响[28], 使用大约 6 个月大的小鼠进行 HFD 诱导也未能检测到 Nrf2 对肝脏脂肪酸代谢的影响[29]。

然而, 最近许多研究也表明 Nrf2 缺失可改善 IR 和脂质积累[30]。Shin 等[31]研究发现, 在 HFD 诱导下, Nrf2 缺失小鼠的体重比野生型小鼠更轻。Pi 等[32]表明, 在 HFD 诱导下, 靶向敲除 Nrf2 可减少脂肪组织质量并缓解 IR。并且, Nrf2 敲除的小鼠在喂食高脂肪饮食时表现出比野生型小鼠更好的胰岛素敏感性[33]。Xu 等[34]发现, 在 Keap1 敲低小鼠中 (Nrf2 活性增强), 会增加高脂饮食诱导的肥胖和脂质积累。以上使用 Nrf2 全身性敲除而非组织特异性敲除的研究结果表明, Nrf2 在高脂肪饮食喂养下对 IR 和脂质积累起重要作用。

研究表明[35], 与对照小鼠相比, 特异性敲除肝细胞 Nrf2 的雌性小鼠可改善 HFD 诱导的 IR、炎症反应和肝脏脂质沉积。而在特异性敲除脂肪细胞 Nrf2 的肥胖自发突变的纯合子 (*ob*/*ob*) 小鼠中, IR、高血糖和高甘油三酯血症比对照小鼠更严重[36]。在一项研究中[37], 使用靶标特异性敲除 Nrf2 小鼠研究了 Nrf2 在不同组织中的特异性作用, 结果表明, 在肝细胞中 Nrf2 特异性缺失的小鼠表现出胰岛素敏感性的改善; 相反, 脂肪细胞中 Nrf2 的特异性缺失会加重高脂肪饮食喂养的小鼠 IR。因此, Nrf2 在 IR 中的确切作用机制尚未完全阐明。Nrf2 在不同周龄小鼠及不同组织细胞对脂质沉积和 IR 发挥完全不同甚至相反的作用。此外, 高脂肪饮食喂养时间长短的差异、小鼠肥胖严重程度和动物遗传背景都可能对 IR 产生影响。目前仍缺乏有力证据直接证实 Keap1-Nrf2-ARE 通路激活或抑制与改善 IR 之间的关系, Nrf2 对 IR 和脂质代谢的作用仍有待进一步研究 (图 1)。

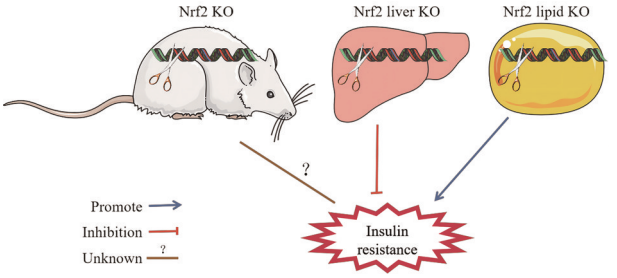

Figure 1 Nuclear factor erythroid-2-related factor 2 (Nrf2) and the non-alcoholic fatty liver disease (NAFLD) "first hit". The role of systemic knockout (KO) Nrf2 in insulin resistance is controversial and needs to be further studies; Nrf2 liver specific KO can improve insulin resistance; Nrf2 lipid specific KO further aggravates insulin resistance

4 Nrf2 激活改善 NAFLD“第二次打击”

肝脏脂质沉积引发的包括氧化应激、炎症反应、纤维化等一系列病理过程被称为 NAFLD 的“第二次打击”, 其中氧化应激发挥重要作用[38]。Keap1-Nrf2-ARE 通路在清除 ROS 中起关键作用。因此, Nrf2 的激活被认为可改善“第二次打击”来抑制 NAFLD 的进展 (图 2)。作为抗氧化反应的主要调节因子, Nrf2 增强了许多参与 ROS 清除的基因的表达[11]。与野生型小鼠相比, Keap1 敲低小鼠超氧化物歧化酶 (superoxide dismutase, SOD) 和血红素加氧 1 (heme oxygenation 1, HO-1) 等消除 ROS 的酶转录上调[39]。小鼠 Nrf2 肝脏特异性过表达可防止长期暴露于蛋氨酸胆碱缺乏饮食 (methionine/choline deficiency diet, MCD) 引起的氧化应激[40]。此外, 与对照组相比, 使用 Nrf2 激活剂氰烯酮处理后, 高脂加高果糖饮食诱导的小鼠谷胱甘肽/谷胱甘肽二硫化物 (GSH/GSSG) 的比例增加[41]。

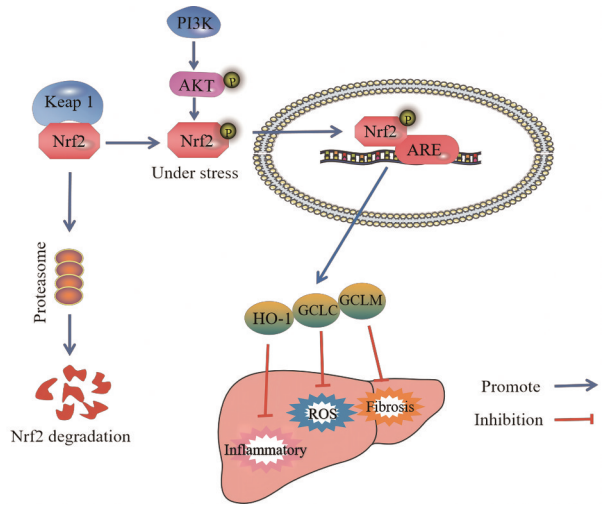

Figure 2 Nrf2 activation improves NAFLD "second hit". Under physiological conditions, Keap1 binds to Nrf2 and transports it to the ubiquitin proteasome for degradation. When the Keap1-Nrf2-ARE pathway is activated, Nrf2 dissociates from Keap1, and then translocates to the nucleus to bind to the ARE and promote the transcription of downstream proteins which can improve inflammation, oxidative stress and fibrosis in liver. Keap1: Kelch-like ECH-associated protein 1; ARE: Antioxidant responsive element; PI3K: Phosphatidylinositol-3-kinase; AKT: Protein kinase B; HO-1: Heme oxygenase 1; GCLC: Glutamate-cysteine ligase catalytic; GCLM: Glutamate-cysteine ligase modulator; ROS: Reactive oxygen species

炎症和纤维化是“第二次打击”的关键特征, 可促进 NAFLD 向非酒精性脂肪性肝炎 (non-alcoholic steatohepatitis, NASH) 的发展[42], 已发现 Nrf2 激活可防止 NASH 的发生和进展[43]。据报道, 在 Keap1 敲低的小鼠中 Nrf2 的激活可抑制脂肪性肝炎。同时, Nrf2 激活发挥了有效的抗炎作用[44]。而 Nrf2 敲除的小鼠在喂食 MCD 饮食或 HFD 时更易发生 NASH[45]。在 HFD 诱导下, Nrf2 敲除小鼠的核因子活化 B 细胞 κ 轻链增强子 (NF-κB)、白介素-6 (interleukin-6, IL-6) 和肿瘤坏死因子 α (tumor necrosis factor α, TNF-α) 的水平增加更为显著[46]。同样与野生型小鼠相比, MCD 饮食诱导的 Nrf2 敲除小鼠中 IL-1β、TNF-α、环加氧酶 2 (cyclooxygenase 2, COX2) 的转录水平显著增加[47]。

肝纤维化是慢性肝损伤和炎症所致的肝内结缔组织异常增生。当病情加重时, 肝纤维化可进展为肝硬化甚至肝癌[48]。在小鼠终末期肝硬化时, 肝 Nrf2 显著下调, 从而促进疾病进展[49]。谷氨酸-半胱氨酸连接酶催化亚基 (glutamate-cysteine ligase catalytic, GCLC) 和谷氨酸-半胱氨酸连接酶调节亚基 (glutamate-cysteine ligase modulator, GCLM) 作为 Nrf2 调控的下游蛋白可参与 GSH 合成, 具有抑制肝纤维化的作用[50]。Nrf2 在 Keap1 敲除小鼠肝细胞中的过表达可使氧化应激、炎性细胞和纤维化减少[51]。在 3,5-二乙氧羰基-1,4-二氢黏连蛋白 (DDC) 诱导的胆道纤维化模型中, Nrf2 敲除会加重肝纤维化的发生, 而在 Keap1 敲低的小鼠中肝纤维化被显著抑制[52]。Lyu 等[53]研究报道, 肝细胞特异性 Nrf2 敲除可加剧 CCL4 诱导的炎症和纤维化。川芎嗪 (ligustrazine) 是一种具有抗纤维化作用的化合物, 在 Nrf2 敲低小鼠中, 川芎嗪对肝纤维化的治疗作用降低[54]。使用 TBE-31 对 Nrf2 的药理学激活可降低高脂加高果糖饮食诱导的 NASH 小鼠肝纤维化[41]。其他 Nrf2 激活剂如 oltipraz (OPZ) 和 NK-252 都显著减弱了 NASH 大鼠肝纤维化的进展[55]。在肝脏中, Nrf2 通过促进成纤维细胞分化发挥抗纤维化作用, 且在肝星状细胞 (hepatic stellate cells, HSC) 中 Nrf2 激动显示出对 TGF-β1 的抑制作用[56]。相反, Nrf2 敲低可诱导 HSC 活化, 增加 A-平滑肌肌动蛋白表达, 并诱导 TGF-β1/Smad 通路活化[57]。总之, 已有证据表明, 激活 Keap1-Nrf2-ARE 信号通路可能是预防肝纤维化的有效策略。

此外, 有研究发现内质网应激通过自噬诱导 HSC 活化[58], 内质网应激在 NAFLD“多重打击”学说中也发挥重要作用[59]。有研究证明 Nrf2 的药理学激活可显著改善高脂肪加果糖饮食诱导的内质网应激[40]。Giudetti 等[60]同样发现, Nrf2 激活可改善 HFD 诱导的大鼠肝脏内质网应激。Morgan 等[61]报道了 Nrf2 可通过介导 CYP2A5 减少肝细胞内等内质网应激。

5 Nrf2: NAFLD 的治疗靶点

到目前为止, 生活方式的改变仍是早期 NAFLD 最有效和最可靠的干预措施, 然而接近半数的患者很难采纳和实施。尽管 NAFLD 造成的医疗和社会经济负担越来越重, 但目前仍缺乏获得 FDA 批准的药物和治疗方案。由于发病机制的复杂性, 目前针对 NAFLD 的新药研发主要从代谢、炎症、纤维化 3 个方面入手。截至 2021 年底已有超过 10 种 NASH 治疗药物进入 III 期临床试验, 其中仅 FXR 受体激动剂奥贝胆酸 (OCA) 完成 III 期临床试验, 考虑到潜在风险, FDA 尚未批准其应用于临床。Keap1-Nrf2-ARE 通路在氧化应激和炎症中的关键作用使其激动剂有望治疗 NAFLD。其中腺苷酸活化蛋白激酶 (AMPK) 和 Nrf2 激动剂 Oltipraz 已经进入 III 期临床阶段。已知的大多数 Nrf2 激活剂是 Keap1-Nrf2 蛋白-蛋白相互作用 (PPI) 的间接抑制剂, 即通过与 Keap1 Cys 的巯基共价结合, 进而抑制 Keap1 与 Nrf2 相互作用, 诱导 Nrf2 解离, 抑制其泛素化降解促进核转位。大多数 Nrf2 激活剂具有亲电性[62], 但这些亲电性激活剂可能通过与其他重要细胞蛋白的 Cys 反应而引起“脱靶”, 因此直接抑制 Keap1-Nrf2 PPI

Table 1 Keap1-Nrf2-ARE activators in the treatment of NAFLD/NASH. NASH: Non-alcoholic steatohepatitis; HFD: High-fat diet; SD: Sprague-Dawley; MCD: Methionine/choline deficiency diet; HFF: High fructose diet; CDAA: Choline deficient *L*-amino acid defined diet

Model	Compound	Treatment	Steatosis	Oxidative stress	Inflammation	Fibrosis	Clinical phase	Ref.
Male Wistar rat, HFD, 16 weeks	Hesperetin	300 mg·kg^{-1}·d^{-1}, 16 weeks	↓	↓	↓	↓	Preclinical	[65]
Male C57BL/6 mice, HFD, 16 weeks	Apigenin	30 mg·kg^{-1}·d^{-1}, 3 weeks	↓	↓	↓	−	Preclinical	[66]
Male C57BL/6 mice, CDAA diet, 12 weeks	Aloin	20 mg·kg^{-1}·d^{-1}, 12 weeks	−	↓	↓	−	Preclinical	[67]
Male SD rat, HFD, 16 weeks	Curcumin	50 mg·kg^{-1}·d^{-1}, 16 weeks	↓	↓	↓	↓	Phase III	[68]
Male Wistar rat, HFD, 10 weeks	Sulforaphane	20 mg·kg^{-1}, 3 times a week for 10 weeks	↓	↓	−	−	Phase II	[69]
Male C57BL/6 mice were injected intraperitoneally with 500 mg·kg^{-1} tyloxapol	Geniposide	100 mg·kg^{-1}·d^{-1}	↓	↓	↓	−	Preclinical	[70]
Male C57BL/6 mice, HFF diet, 24 weeks	TBE-31	1.65 mg·kg^{-1}, 3 times a week, 6 weeks	↓	↓	↓	↓	Preclinical	[41]
Male C57BL/6 mice were injected intraperitoneally with 500 mg·kg^{-1} tyloxapol	Aucubin	20 mg·kg^{-1}·d^{-1}	↓	↓	↓	−	Preclinical	[71]
Male C57BL/6 mice, MCD, 6 weeks	Silibinin	20 mg·kg^{-1}·d^{-1}, 6 weeks	↓	↓	↓	−	Phase II	[72]
Male Fischer rats, CDAA diet, 10 weeks	Oltipraz	60 mg·kg^{-1}·d^{-1}, 9 weeks	↓	↓	↓	↓	Phase III	[54]
Male C57BL/6 mice, HFD, 12 weeks	Osteocalcin	30 mg·kg^{-1}·d^{-1}, 12 weeks	↓	↓	−	−	Phase II	[73]
Male C57BL/6 mice, MCD diet, 4 weeks	Scoparone	80 mg·kg^{-1}·d^{-1}, 4 weeks	↓	↓	↓	−	Preclinical	[74]
Male C57BL/6 mice, MCD, 4 weeks	Physalin B	30 mg·kg^{-1}·d^{-1}, 4 weeks	↓	↓	↓	↓	Preclinical	[75]
Male Wistar rat, HFD, 77 days	Oleoylethanolamide	10 mg·kg^{-1}·d^{-1}, 2 weeks	↓	↓	−	−	Preclinical	[60]
Male C57BL/6 mice, HFD, 12 weeks	Erythritol	500 mg·kg^{-1}·d^{-1}, 8 weeks	↓	↓	−	−	Preclinical	[76]

成为一种更加可靠的Nrf2激活策略[63]。近些年, 已报道了几种小分子直接抑制剂通过与Keap1 Kelch结构域的非共价结合抑制Keap1-Nrf2 PPI[64]。Nrf2的激活已成为NAFLD的新型治疗策略, 更多的Nrf2激活剂有待于被发现和开发成有效的治疗或预防药物。目前已有大量研究发现了一系列对NAFLD/NASH具有改善作用的Keap1-Nrf2-ARE通路激活剂 (表1[41,54,60,65-76])。

6 总结与展望

NAFLD (包括肝脏脂质代谢紊乱、氧化应激、炎症、纤维化) 的发病率逐年增加。由于氧化应激和炎症在NAFLD中具有重要作用, Keap1-Nrf2-ARE通路被认为是有希望治疗NAFLD的药理学治疗新靶点, 目前正在全球范围内进行研究。Nrf2在NAFLD的“第一次打击”中发挥的作用目前仍存在争议, 有待于进一步探讨。但已有足够证据证明, Nrf2的激活在NAFLD的“第二次打击”中具有明确的积极作用。目前已发现多种化合物通过激活Nrf2通路, 在NAFLD中发挥抗氧化和抗炎作用, 但迄今为止尚无相关药物获得FDA批准上市。相比于亲电性的Keap1-Nrf2 PPI的间接抑制剂带来的“脱靶”反应, Keap1-Nrf2 PPI的直接抑制剂似乎更有潜力被开发成有效治疗药物。Keap1-Nrf2-ARE通路的药理学激活是治疗NAFLD有前途的新策略。

作者贡献: 陈智伟负责文献检索及综述撰写; 孙华负责综述修改。

利益冲突: 所有作者均声明不存在利益冲突。

References

[1] Palmer M, Regev A, Regev A, et al. Consensus guidelines: best practices for detection, assessment and management of suspected acute drug-induced liver injury occurring during clinical trials in adults with chronic cholestatic liver disease [J]. Aliment Pharmacol Ther, 2020, 51: 90-109.

[2] Younossi Z, Tacke F, Arrese M, et al. Global perspectives on non-

alcoholic fatty liver disease and nonalcoholic steatohepatitis [J]. Hepatology, 2019, 69: 2672-2682.

[3] Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases [J]. Hepatology, 2018, 67: 328-357.

[4] Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes [J]. Hepatology, 2016, 64: 73-84.

[5] Bellentani S. The epidemiology of non-alcoholic fatty liver disease [J]. Liver Int, 2017, 37 Suppl 1: 81-84.

[6] Day CP, James OF. Steatohepatitis: a tale of two "hits"? [J]. Gastroenterology, 1998, 114: 842-845.

[7] Sanyal AJ. Past, present and future perspectives in nonalcoholic fatty liver disease [J]. Nat Rev Gastroenterol Hepatol, 2019, 16: 377-386.

[8] Ramos-Tovar E, Muriel P. Molecular mechanisms that link oxidative stress, inflammation, and fibrosis in the liver [J]. Antioxidants (Basel), 2020, 9: 1279.

[9] Rives C, Fougerat A, Ellero-Simatos S, et al. Oxidative stress in NAFLD: role of nutrients and food contaminants [J]. Biomolecules, 2020, 10: 1702.

[10] Loomba R, Friedman SL, Shulman GI. Mechanisms and disease consequences of nonalcoholic fatty liver disease [J]. Cell, 2021, 184: 2537-2564.

[11] Li XL, Jiang W, Fan WM, et al. Role of gut microbiota in the treatment of nonalcoholic fatty liver disease with traditional Chinese medicine [J]. Acta Pharm Sin (药学学报), 2020, 55: 15-24.

[12] Ulasov AV, Rosenkranz AA, Georgiev GP, et al. Nrf2/Keap1/ARE signaling: towards specific regulation [J]. Life Sci, 2022, 291: 120111.

[13] Tu W, Wang H, Li S, et al. The anti-inflammatory and anti-oxidant mechanisms of the Keap1/Nrf2/ARE signaling pathway in chronic diseases [J]. Aging Dis, 2019, 10: 637-651.

[14] Liu S, Pi J, Zhang Q. Mathematical modeling reveals quantitative properties of KEAP1-NRF2 signaling [J]. Redox Biol, 2021, 47: 102-139.

[15] Cuadrado A, Rojo AI, Wells G, et al. Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases [J]. Nat Rev Drug Discov, 2019, 18: 295-317.

[16] Bartolini D, Dallaglio K, Torquato P, et al. Nrf2-p62 autophagy pathway and its response to oxidative stress in hepatocellular carcinoma [J]. Transl Res, 2018, 193: 54-71.

[17] Tonelli C, Chio IIC, Tuveson DA. Transcriptional regulation by Nrf2 [J]. Antioxid Redox Signal, 2018, 29: 1727-1745.

[18] Baird L, Yamamoto M. The molecular mechanisms regulating the KEAP1-NRF2 pathway [J]. Mol Cell Biol, 2020, 40: e00099-20.

[19] Liu T, Lv YF, Zhao JL, et al. Regulation of Nrf2 by phosphorylation: consequences for biological function and therapeutic implications [J]. Free Radic Biol Med, 2021, 168: 129-141.

[20] Ikonen E, Zhou X. Cholesterol transport between cellular membranes: a balancing act between interconnected lipid fluxes [J]. Dev Cell, 2021, 56: 1430-1436.

[21] Yamamoto M, Kensler TW, Motohashi H. The KEAP1-NRF2 system: a thiol-based sensor-effector apparatus for maintaining redox homeostasis [J]. Physiol Rev, 2018, 98: 1169-1203.

[22] Chen L, Chen XW, Huang X, et al. Regulation of glucose and lipid metabolism in health and disease [J]. Sci China Life Sci, 2019, 62: 1420-1458.

[23] Rezzani R, Franco C. Liver, oxidative stress and metabolic syndromes [J]. Nutrients, 2021, 13: 301.

[24] Yates MS, Tran QT, Dolan PM, et al. Genetic *versus* chemoprotective activation of Nrf2 signaling: overlapping yet distinct gene expression profiles between Keap1 knockout and triterpenoid-treated mice [J]. Carcinogenesis, 2009, 30: 1024-1031.

[25] Qiu S, Liang Z, Wu Q, et al. Hepatic lipid accumulation induced by a high-fat diet is regulated by Nrf2 through multiple pathways [J]. FASEB J, 2022, 36: e22280.

[26] Li C, Liu Q, Xie L. Suppressing NLRP2 expression accelerates hepatic steatosis: a mechanism involving inflammation and oxidative stress [J]. Biochem Biophys Res Commun, 2018, 507: 22-29.

[27] Lee DH, Park JS, Lee YS, et al. SQSTM1/p62 activates NFE2L2/NRF2 *via* ULK1-mediated autophagic KEAP1 degradation and protects mouse liver from lipotoxicity [J]. Autophagy, 2020, 16: 1949-1973.

[28] Tanaka Y, Aleksunes LM, Yeager RL, et al. NF-E2-related factor 2 inhibits lipid accumulation and oxidative stress in mice fed a high-fat diet [J]. J Pharmacol Exp Ther, 2008, 325: 655-664.

[29] Zhang YK, Wu KC, Liu J, et al. Nrf2 deficiency improves glucose tolerance in mice fed a high-fat diet [J]. Toxicol Appl Pharmacol, 2012, 264: 305-314.

[30] Li S, Eguchi N, Lau H, et al. The role of the Nrf2 signaling in obesity and insulin resistance [J]. Int J Mol Sci, 2020, 21: 6973.

[31] Shin S, Wakabayashi J, Yates MS, et al. Role of Nrf2 in prevention of high-fat diet-induced obesity by synthetic triterpenoid CDDO-imidazolide [J]. Eur J Pharmacol, 2009, 620: 138-144.

[32] Pi J, Leung L, Xue P, et al. Deficiency in the nuclear factor E2-related factor-2 transcription factor results in impaired adipogenesis and protects against diet-induced obesity [J]. J Biol Chem, 2010, 285: 9292-9300.

[33] Meakin PJ, Chowdhry S, Sharma RS, et al. Susceptibility of Nrf2-null mice to steatohepatitis and cirrhosis upon consumption of a high-fat diet is associated with oxidative stress, perturbation of the unfolded protein response, and disturbance in the expression of metabolic enzymes but not with insulin resistance [J]. Mol Cell Biol, 2014, 34: 3305-3320.

[34] Xu J, Kulkarni SR, Donepudi AC, et al. Enhanced Nrf2 activity worsens insulin resistance, impairs lipid accumulation in adipose tissue, and increases hepatic steatosis in leptin-deficient mice [J]. Diabetes, 2012, 61: 3208-3218.

[35] Li L, Fu J, Liu D, et al. Hepatocyte-specific Nrf2 deficiency mitigates high-fat diet-induced hepatic steatosis: involvement of reduced PPAR*γ* expression [J]. Redox Biol, 2020, 30: 101412.

[36] Xue P, Hou Y, Chen Y, et al. Adipose deficiency of Nrf2 in *ob/ob* mice results in severe metabolic syndrome [J]. Diabetes, 2013, 62: 845-854.

[37] Chartoumpekis DV, Palliyaguru DL, Wakabayashi N, et al. Nrf2 deletion from adipocytes, but not hepatocytes, potentiates systemic metabolic dysfunction after long-term high-fat diet-induced obesity in mice [J]. Am J Physiol Endocrinol Metab, 2018, 315: E180-E195.

[38] Arroyave-Ospina JC, Wu Z, Geng Y, et al. Role of oxidative stress in the pathogenesis of non-alcoholic fatty liver disease: implications for prevention and therapy [J]. Antioxidants (Basel), 2021, 10: 174.

[39] Mohs A, Otto T, Schneider KM, et al. Hepatocyte-specific NRF2 activation controls fibrogenesis and carcinogenesis in steatohepatitis [J]. J Hepatol, 2021, 74: 638-648.

[40] Lee LY, Köhler UA, Zhang L, et al. Activation of the Nrf2-ARE pathway in hepatocytes protects against steatosis in nutritionally induced non-alcoholic steatohepatitis in mice [J]. Toxicol Sci, 2014, 142: 361-374.

[41] Sharma RS, Harrison DJ, Kisielewski D, et al. Experimental non-alcoholic steatohepatitis and liver fibrosis are ameliorated by pharmacologic activation of Nrf2 (NF-E2 p45-related factor 2) [J]. Cell Mol Gastroenterol Hepatol, 2018, 5: 367-398.

[42] Li L, Fu J, Sun J, et al. Is Nrf2-ARE a potential target in NAFLD mitigation? [J]. Curr Opin Toxicol, 2019, 13: 35-44.

[43] Ramos-Tovar E, Muriel P. Free radicals, antioxidants, nuclear factor-E2-related factor-2 and liver damage [J]. J Appl Toxicol, 2020, 40: 151-168.

[44] Vasileva LV, Savova MS, Amirova KM, et al. Obesity and NRF2-mediated cytoprotection: where is the missing link? [J]. Pharmacol Res, 2020, 156: 104760.

[45] Tang W, Jiang YF, Ponnusamy M, et al. Role of Nrf2 in chronic liver disease [J]. World J Gastroenterol, 2014, 20: 13079-13087.

[46] Liu Z, Dou W, Ni Z, et al. Deletion of Nrf2 leads to hepatic insulin resistance *via* the activation of NF-*κ*B in mice fed a high-fat diet [J]. Mol Med Rep, 2016, 14: 1323-1331.

[47] Chowdhry S, Nazmy MH, Meakin PJ, et al. Loss of Nrf2 markedly exacerbates nonalcoholic steatohepatitis [J]. Free Radic Biol Med, 2010, 48: 357-371.

[48] Garrido A, Djouder N. Cirrhosis: a questioned risk factor for hepatocellular carcinoma [J]. Trends Cancer, 2021, 7: 29-36.

[49] He F, Ru X, Wen T. NRF2, a transcription factor for stress response and beyond [J]. Int J Mol Sci, 2020, 21: 4777.

[50] Fu N, Li D, Li W, et al. Glutamate-cysteine ligase catalytic subunit attenuated hepatitis C virus-related liver fibrosis and suppressed endoplasmic reticulum stress [J]. Front Mol Biosci, 2020, 7: 199.

[51] Ramadori P, Drescher H, Erschfeld S, et al. Genetic Nrf2 overactivation inhibits the deleterious effects induced by hepatocyte-specific c-met deletion during the progression of NASH [J]. Oxid Med Cell Longev, 2017, 2017: 3420286.

[52] Fragoulis A, Schenkel J, Herzog M, et al. Nrf2 ameliorates DDC-induced sclerosing cholangitis and biliary fibrosis and improves the regenerative capacity of the liver [J]. Toxicol Sci, 2019, 169: 485-498.

[53] Lyu H, Wang H, Li L, et al. Hepatocyte-specific deficiency of Nrf2 exacerbates carbon tetrachloride-induced liver fibrosis *via* aggravated hepatocyte injury and subsequent inflammatory and fibrogenic responses [J]. Free Radic Biol Med, 2020, 150: 136-147.

[54] Lu C, Xu W, Zhang F, et al. Nrf2 knockdown attenuates the ameliorative effects of ligustrazine on hepatic fibrosis by targeting hepatic stellate cell transdifferentiation [J]. Toxicology, 2016, 365: 35-47.

[55] Kamisako T, Tanaka Y. Oltipraz ameliorates the progression of steatohepatitis in Nrf2-null mice fed a high-fat diet [J]. J Clin Biochem Nutr, 2022, 70: 147-153.

[56] Xu D, Xu M, Jeong S, et al. The role of Nrf2 in liver disease: novel molecular mechanisms and therapeutic approaches [J]. Front Pharmacol, 2019, 9: 1428.

[57] Prestigiacomo V, Suter-Dick L. Nrf2 protects stellate cells from Smad-dependent cell activation [J]. PLoS One, 2018, 13: e0201044.

[58] Hernández-Gea V, Hilscher M, Rozenfeld R, et al. Endoplasmic reticulum stress induces fibrogenic activity in hepatic stellate cells through autophagy [J]. J Hepatol, 2013, 59: 98-104.

[59] Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD) [J]. Metabolism, 2016, 65: 1038-1048.

[60] Giudetti AM, Vergara D, Longo S, et al. Oleoylethanolamide reduces hepatic oxidative stress and endoplasmic reticulum stress in high-fat diet-fed rats [J]. Antioxidants (Basel), 2021, 10: 1289.

[61] Morgan L, Antenos M, Kirby GM. Nrf2-mediated induction of Cyp2a5 partially protects against reductive endoplasmic reticulum stress in mouse hepatocytes [J]. Toxicology, 2022, 471: 153162.

[62] Robledinos-Antón N, Fernández-Ginés R, Manda G, et al. Activators and inhibitors of NRF2: a review of their potential for clinical development [J]. Oxid Med Cell Longev, 2019, 2019: 9372182.

[63] Jiang ZY, Lu MC, Xu LL, et al. Discovery of potent Keap1-Nrf2 protein-protein interaction inhibitor based on molecular binding determinants analysis [J]. J Med Chem, 2014, 57: 2736-2745.

[64] Liu P, Tian W, Tao S, et al. Non-covalent NRF2 activation

confers greater cellular protection than covalent activation [J]. Cell Chem Biol, 2019, 26: 1427-1435.e5.

[65] Li J, Wang T, Liu P, et al. Hesperetin ameliorates hepatic oxidative stress and inflammation *via* the PI3K/AKT-Nrf2-ARE pathway in oleic acid-induced HepG2 cells and a rat model of high-fat diet-induced NAFLD [J]. Food Funct, 2021, 12: 3898-3918.

[66] Feng X, Yu W, Li X, et al. Apigenin, a modulator of PPAR*γ*, attenuates HFD-induced NAFLD by regulating hepatocyte lipid metabolism and oxidative stress *via* Nrf2 activation [J]. Biochem Pharmacol, 2017, 136: 136-149.

[67] Xu Q, Fan Y, Loor JJ, et al. Aloin protects mice from diet-induced non-alcoholic steatohepatitis *via* activation of Nrf2/HO-1 signaling [J]. Food Funct, 2021, 12: 696-705.

[68] Abd El-Hameed NM, Abd El-Aleem SA, Khattab MA, et al. Curcumin activation of nuclear factor E2-related factor 2 gene (Nrf2): prophylactic and therapeutic effect in nonalcoholic steatohepatitis (NASH) [J]. Life Sci, 2021, 285: 119983.

[69] Lei P, Tian S, Teng C, et al. Sulforaphane improves lipid metabolism by enhancing mitochondrial function and biogenesis *in vivo* and *in vitro* [J]. Mol Nutr Food Res, 2021, 65: e2170023.

[70] Shen B, Feng H, Cheng J, et al. Geniposide alleviates non-alcohol fatty liver disease *via* regulating Nrf2/AMPK/mTOR signalling pathways [J]. J Cell Mol Med, 2020, 24: 5097-5108.

[71] Shen B, Zhao C, Wang Y, et al. Aucubin inhibited lipid accumulation and oxidative stress *via* Nrf2/HO-1 and AMPK signalling pathways [J]. J Cell Mol Med, 2019, 23: 4063-4075.

[72] Liu Y, Xu W, Zhai T, et al. Silibinin ameliorates hepatic lipid accumulation and oxidative stress in mice with non-alcoholic steatohepatitis by regulating CFLAR-JNK pathway [J]. Acta Pharm Sin B, 2019, 9: 745-757.

[73] Du J, Zhang M, Lu J, et al. Osteocalcin improves nonalcoholic fatty liver disease in mice through activation of Nrf2 and inhibition of JNK [J]. Diabetes Res Clin Pract, 2016, 120: S139.

[74] Liu B, Deng X, Jiang Q, et al. Scoparone improves hepatic inflammation and autophagy in mice with nonalcoholic steatohepatitis by regulating the ROS/P38/Nrf2 axis and PI3K/AKT/mTOR pathway in macrophages [J]. Biomed Pharmacother, 2020, 125: 109895.

[75] Zhang MH, Li J, Zhu XY, et al. Physalin B ameliorates nonalcoholic steatohepatitis by stimulating autophagy and NRF2 activation mediated improvement in oxidative stress [J]. Free Radic Biol Med, 2021, 164: 11-12.

[76] Jin M, Wei Y, Yu H, et al. Erythritol improves nonalcoholic fatty liver disease by activating Nrf2 antioxidant capacity [J]. J Agric Food Chem. 2021, 69: 13080-13092.