

综述

铁死亡在非酒精性脂肪性肝病纤维化期的作用机制

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摘要: 铁死亡(ferroptosis)是一种铁依赖的程序性细胞死亡方式, 参与多种疾病的发生发展, 包括神经退行性疾病、心脑血管疾病、肿瘤和肝脏代谢性疾病等。在非酒精性脂肪性肝病(nonalcoholic fatty liver disease, NAFLD)中, 铁死亡通过芬顿反应引发细胞死亡, 从而诱导非酒精性脂肪肝(nonalcoholic fatty liver, NAFL)向非酒精性脂肪性肝炎(nonalcoholic steatohepatitis, NASH)以及肝纤维化发展。目前由NAFLD导致的肝纤维化患病人数日益增加, 引起了一系列不良后果。但研究发现, 特异性诱导肝纤维化小鼠模型中肝星状细胞(hepatic stellate cells, HSCs)铁死亡能够抑制HSCs转分化为肌成纤维细胞, 减少细胞外基质(extracellular matrix, ECM)沉积, 发挥抗纤维化作用, 提示铁死亡成为NAFLD纤维化阶段的新型治疗靶点。本文将重点探讨铁死亡在NAFLD纤维化发展中的双重作用以及肝纤维化模型中铁死亡相关分子靶点、药物缓解肝纤维化的机制, 以期为NAFLD纤维化阶段的药物研发和临床治疗提供新的思路。

关键词: 非酒精性脂肪性肝病; 铁死亡; 肝星状细胞; 纤维化; 双重作用; 药物治疗

The mechanism of ferroptosis in fibrosis stage of nonalcoholic fatty liver disease

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Abstract: Ferroptosis is an iron-dependent programmed cell death that involved in the occurrence and development of a variety of diseases, including neurodegenerative diseases, cardiovascular and cerebrovascular diseases, tumors, and liver metabolic diseases. In nonalcoholic fatty liver disease (NAFLD), ferroptosis induces cell death through the Fenton reaction, thereby causing the progression of nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH) and liver fibrosis. The number of patients with liver fibrosis caused by NAFLD is increasing, resulting in a series of adverse consequences. However, recent studies have found that specifically inducing ferroptosis in hepatic stellate cells (HSCs) in mouse models of liver fibrosis can inhibit the transdifferentiation of HSCs into myofibroblasts and reduce the deposition of extracellular matrix (ECM), exhibiting anti-fibrosis effects, suggesting ferroptosis can be a new therapeutic target for NAFLD fibrosis stage. The review will focus on exploring the dual role of ferroptosis in the development of NAFLD fibrosis, as well as the mechanisms by which ferroptosis-related targets and drugs alleviate liver

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fibrosis in liver fibrosis models, providing new insights into drug development and clinical treatment in fibrosis stage of NAFLD.

Key Words: nonalcoholic fatty liver disease; ferroptosis; hepatic stellate cells; fibrosis; dual function; drug treatment

肝硬化是世界上日益严重的主要健康问题, 2019年全球死亡率统计中因肝硬化而死亡的人数占比为2.4%, 约50%的肝硬化病例源于非酒精性脂肪性肝病(nonalcoholic fatty liver disease, NAFLD)和过度饮酒^[1,2]。NAFLD是多种慢性肝病的总称, 包括肝脂质累积、非酒精性脂肪肝(nonalcoholic fatty liver, NAFL)、非酒精性脂肪性肝炎(nonalcoholic steatohepatitis, NASH)、肝纤维化以及终末期肝硬化和肝细胞癌(hepatocellular carcinoma, HCC), 其特征是病理性脂肪异位堆积伴随慢性炎症状态^[3]。据报道, 全球NAFLD的患病率约为32.4%, 且患病率呈上升趋势, 与NAFLD相关的肝衰竭和HCC的发病率也在急剧增加, 导致NAFLD成为肝脏相关死亡率和发病率增长最快的因素以及肝移植的主要因素^[4,5]。研究表明, NAFLD不良预后最主要的判断因素是纤维化的分级, 而不是NASH的组织学特征, 即使在非常早期的纤维化阶段, 其死亡率也会小幅增加, 并且随着纤维化严重程度增加而线性上升^[6-8]。

驱动NAFL向纤维化期进展的机制包括脂滴在肝细胞中的异常积累、胰岛素抵抗、过度氧化应激、炎症细胞浸润和肝细胞死亡, 凋亡、坏死、铁死亡、焦亡等不同类型细胞死亡方式在代谢性肝病的进展中共存^[9,10]。铁死亡于2012年首次被提出, 是一种以铁元素为中心的调节性细胞死亡方式, 铁驱动多不饱和脂肪酸过氧化的积累, 引起活性氧(reactive oxygen species, ROS)的大量产生, 导致无法控制的氧化还原反应, 最终引起细胞死亡^[11,12]。铁死亡在肝纤维化中具有双重作用: 一方面, 铁死亡会导致NAFLD等慢性肝病的病变加重, 加速纤维化产生, 减少铁的积累、抑制铁相关的氧化应激和炎症反应, 从而有效减轻肝纤维化的病理进程, 因此抑制铁死亡成为治疗NAFLD纤维化的策略之一; 另一方面, 促进铁死亡也可能成为治疗NAFLD纤维化期的靶点。研究

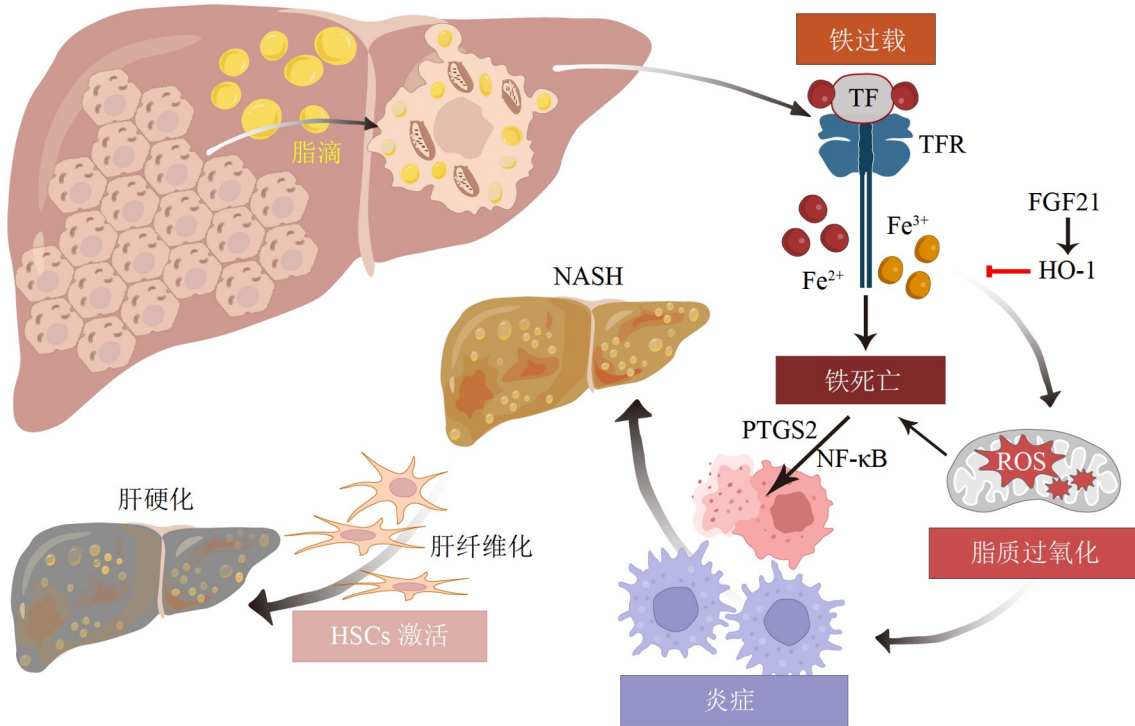
表明, 青蒿琥酯、异甘草酸镁等能特异性诱导肝纤维化模型小鼠肝内的肝星状细胞(hepatic stellate cells, HSCs)铁死亡, 逆转肝纤维化的发生, 从而改善NAFLD纤维化期的病理特征^[13-15]。阐述铁死亡的双重作用有助于揭示其在NAFLD纤维化期发生和发展中的具体机制, 为开发新的治疗策略提供理论依据。

1 铁死亡促进NAFL向肝纤维化的进展

程序性死亡方式如坏死、凋亡、铁死亡等, 能够引起细胞膜通透性的改变从而导致细胞内成分的释放, 诱导炎症反应的发生, 这将有助于免疫细胞在损伤部位聚集, 避免非正常细胞对周围组织产生影响, 同时引起HSCs的转分化和胶原蛋白的大量释放, 促进纤维化的修复, 在NAFLD的预后中起关键作用^[9,16]。但当炎症反应持续时, 炎症介质的释放和HSCs的激活导致细胞外基质(extracellular matrix, ECM)的聚集则引起纤维化的增加, 最终会导致NAFL转变为肝纤维化和肝硬化^[16]。已经在多种模式动物的NAFLD模型中发现了铁死亡的存在, 并且研究发现铁死亡是引发脂肪性肝炎的第一个因素, 早于其他细胞死亡方式^[17]。铁死亡导致NAFL到纤维化的进展主要由三个因素引起, 即游离铁积累引起的肝脏铁过载、氧化应激和诱导细胞膜变性的脂质过氧化损伤、免疫细胞浸润引起的炎症反应发生^[18](图1)。

1.1 铁过载

肝脏是体内铁的主要储存部位, 其中25%~30%的总铁以铁蛋白的形式储存在肝脏中, 铁在肝内进行吸收、利用、储存和分泌, 导致肝脏成为铁超负荷和器官毒性的主要靶器官^[19]。多种疾病可导致铁代谢异常, 如原发性血色病、2型糖尿病、肥胖和NAFLD, 而铁代谢异常导致的铁过载也会对疾病的发展产生影响^[20]。研究证明, 遗传性血色病与NAFLD之间存在很强的相关性, 并且



TF: 转铁蛋白(transferrin); TFR: 转铁蛋白受体(transferrin receptor); Fe^{2+} : 亚铁离子; Fe^{3+} : 铁离子; FGF21: 成纤维细胞生长因子21(fibroblast growth factor 21); HO-1: 血红素加氧酶1(heme oxygenase-1); PTGS2: 环加氧酶2(prostaglandin-endoperoxide synthase 2); NF- κ B: 细胞核因子- κ B(nuclear factor-kappa B)

图1 铁死亡促进NAFL到肝纤维化的进展模式图

遗传性血色病最常见的形式是由稳态铁调节因子(human homeostatic iron regulator, *HFE*)突变导致的铁调素分泌降低以及铁超载形成, 在高脂喂养(high-fat diet, HFD)条件下, 敲除小鼠*Hfe*基因会表现出更严重的肝脂肪变性和早期肝纤维化的发生^[21,22]。同时研究发现, 膳食铁的摄入量与NAFLD的患病率呈正相关, 并且晚期NAFLD患者可通过血清铁蛋白水平的升高来进行鉴别, 表明铁超负荷诱导的铁死亡可能与NAFLD的发生发展有关^[23,24]。Yu等^[25]研究发现, 敲除小鼠转铁蛋白可引起血清游离铁增加和肝内铁过载, 导致肝纤维化的形成; 进一步敲除溶质载体家族39成员14(solute carrier family 39 member 14, *SLC39A14*), 使用铁死亡抑制剂-1(ferrostatin-1, Fer-1)可逆转小鼠肝纤维化的发生。机体铁含量的增加会影响脂肪酸的摄入和合成, 铁螯合剂去铁胺(deferoxamine, DFO)能够缓解HFD诱导的肝脂质代谢异常和肝损伤的发生^[26]。肝铁过载也会加重NASH的进展, 在胆碱缺乏、补充乙硫氨酸(deficient in choline and

supplemented with ethionine, CDE)饮食诱导的小鼠NASH模型中, DFO可以缓解NASH中的细胞死亡和炎症的加重^[17]。FGF21是FGF家族中的内分泌成员, 在脂质代谢中发挥重要作用, 其能够通过促进HO-1泛素化, 加速HO-1的降解, 从而降低血红素向 Fe^{2+} 的转化, 有效降低细胞内的游离铁含量, 并最终抑制铁死亡, 缓解由右旋糖酐铁注射引起的小鼠肝纤维化^[27]。此外, 研究显示, 小鼠肝细胞特异性聚rC结合蛋白1[poly(rC)binding protein 1, PCBP1]缺失可增加肝细胞内的游离铁积累, 诱发肝脂肪变性, 加重NAFLD病理情况, 增加肝纤维化形成几率^[28]。

1.2 氧化应激和脂质过氧化

铁死亡诱导肝细胞内ROS过量生成和氧化应激的产生, 会导致线粒体膜和细胞膜等脂质过氧化的产生, 引起胞内大量线粒体功能障碍甚至肝细胞死亡, 诱发NAFL的炎症状态, 使其向肝炎和纤维化发展^[18]。研究发现, NASH患者的肝内磷脂酰胆碱/磷脂酰乙醇胺比值偏低, 磷脂酰胆碱/磷脂酰

乙醇胺比值降低将导致肝细胞损害和炎症的产生,加重NAFLD的病理进程,可能与铁死亡诱导的脂质过氧化相关^[29]。脂质过氧化产物丙二醛(malondialdehyde, MDA)和4-羟基壬烯醛(4-hydroxynonenal, 4-HNE)也被用作检测NASH患者的铁死亡标志物^[17]。此外,细胞内脂质的沉积会导致诱导性谷胱甘肽过氧化物酶4(inducible glutathione peroxidase 4, iGPX4)的产生, iGPX4与GPX4的经典转录产物(canonical gpx4, cGPX4)相互作用并促进cGPX4从单体(活性)转变为低聚物(非活性),从而造成抗氧化系统的失调, iGPX4亚型可促进铁死亡并加重NAFLD到纤维化阶段的发生发展^[30]。临床采用抗氧化剂维生素E对NASH患者进行治疗,可有效抑制脂质过氧化发生,降低肝损伤标记物血清转氨酶含量^[31]。

1.3 炎症

研究发现,铁死亡具有促炎作用,炎症的加重导致炎症因子的大量释放,引起HSCs的激活,促进肝脏中ECM过度沉积,并且铁死亡在NASH初期即可引发细胞死亡并诱发炎症的产生^[17]。作为一种炎症细胞死亡形式,铁死亡参与组织损伤过程中损伤相关模式分子(damage-associated molecular pattern, DAMP)或脂质氧化产物如4-HNE、氧化磷脂、前列腺素E₂、白三烯B₄的释放,引起HSCs的激活,诱发NAFLD纤维化的产生^[32]。嗜铁细胞通过释放高迁移率族蛋白B1(high mobility group box 1, HMGB1),激活晚期糖基化终产物特异性受体(advanced glycosylation end product-specific receptor, AGER),参与NF- κ B通路的激活, NF- κ B已被证明在NAFLD促炎和促肝纤维化中发挥重要作用^[33,34]。此外,铁死亡产生的脂质过氧化产物4-HNE也是一种促炎介质,参与NF- κ B途径来诱导肝脏细胞凋亡,活化HSCs参与肝纤维化的进展^[35,36]。同时,脂质过氧化产物引起的免疫炎症反应也被用于预测NAFLD进展为晚期纤维化的风险^[37]。除了通过释放脂质过氧化产物和参与炎症信号转导外,细胞铁死亡后还能作为类花生酸的生物合成供体,产生前列腺素、凝血恶烷、白三烯等,直接参与炎症反应,加速肝纤维化的发生发展^[38,39]。

2 铁死亡延缓肝纤维化进程

铁死亡通过铁过载引起的芬顿反应加重氧化应激的发生,导致细胞器膜和细胞膜的受损,进一步加重NAFLD炎症进展,炎症因子的释放会导致HSCs的激活和ECM的堆积,加速肝纤维化的发生。肝纤维化患者往往有严重的病理性铁过载症状,提示铁死亡在肝纤维化的进展中发挥重要作用^[40]。肝纤维化的核心事件是HSCs转分化为产生基质的肌成纤维细胞,靶向清除HSCs被认为是逆转肝纤维化的治疗方法^[41]。最近的研究表明,三结构域蛋白26(tripartite motif containing 26, TRIM26)、溴结构域蛋白(bromodomain-containing proteins, BRDs)、青蒿琥酯等能够在多种模式动物体内通过靶向HSCs铁死亡,抑制HSCs转分化为肌成纤维细胞,缓解肝纤维化,甚至用来治疗肝癌^[13,42-45](表1)。

2.1 相关分子

TRIM26是三结构域蛋白(tripartite motif-containing proteins, TRIMs)家族成员之一,该家族的许多成员作为E3泛素连接酶起作用, TRIM26通过调节特定基因的泛素化修饰参与了先天免疫应答和慢性代谢疾病等多种生物过程^[65]。研究表明, TRIM26通过泛素化下调CCAAT增强子结合蛋白 Δ (CCAAT enhancer binding protein delta, CEBPD)/缺氧诱导因子-1 α (hypoxia-inducible factor-1 α , HIF-1 α)相关炎症信号通路和一氧化氮合酶2(nitric oxide synthase 2, NOS2)信号传导,从而对NAFLD产生抑制作用,进而缓解肝脂质变性、NASH以及肝纤维化^[46,47]。在进一步探索TRIM26作用于肝纤维化的机制时, Zhu等^[44]发现,在小鼠四氯化碳(carbon tetrachloride, CCl₄)肝纤维化模型中, TRIM26在纤维化肝组织中下调,通过腺病毒过表达TRIM26会诱导脂质ROS积累,导致活化的HSCs发生铁死亡,小鼠血清谷丙转氨酶(alanine aminotransferase, ALT)、谷草转氨酶(aspartate aminotransferase, AST)和羟脯氨酸的水平显著降低, SLC7A11、 α 平滑肌肌动蛋白(α -smooth muscle actin antibody, α -SMA)和胶原蛋白I的表达下调^[44]。TRIM26可与溶质载体家族7成员11(solute carrier family 7 member 11, SLC7A11/xCT)相互作

表 1 铁死亡缓解肝纤维化的相关靶点及药物

分类	名称	参与机制	参考文献
相关分子	TRIM26	下调CEBPD/HIF-1 α 相关炎症信号通路和NOS2信号传导; 与SLC7A11相互作用, 介导其泛素化降低SLC7A11表达	[44,46,47]
	BRD7	通过与p53的N端转录激活结构域直接结合, 促进p53与SLC25A28相互作用, 增强SLC25A28的活性	[45]
	ZNF36	导致ATG16L1的mRNA衰变, 触发自噬失活和阻断自噬铁蛋白降解	[48]
	MSC-ex	介导BECN1抑制SLC7A11/xCT驱动的GPX4表达	[49]
临床药物及生物制剂	索拉非尼	抑制HIF-1 α /SLC7A11轴; 下调ZFP36的转录; 调控ELAVL1/HuR通路; 上调HSCs中BRD7水平; 抑制TGF- β 1	[45,48,50-52]
	仑伐替尼	通过FGFR4进而影响xCT转运系统	[53,54]
	异甘草酸镁	上调HO-1的表达, 抑制GPX4和DMT1的表达	[14]
	爱拉斯汀	下调ZFP36的转录	[48]
中药及其活性成分	青蒿琥酯	引起HSCs中ATG3、ATG5、ATG6/beclin1、ATG的上调和p62、FTH1、NCOA4的下调, 从而触发铁蛋白自噬	[13]
	双氢青蒿素	抑制PDGF-BB诱导的体外HSCs活化, 上调NCOA4的表达	[55]
	蒿甲醚	增加IRP2与STUB1的结合来降低IRP2的泛素化, 促进细胞内IRP2蛋白的积累; 促进p53依赖性诱导的铁死亡	[56,57]
	南蛇藤醇	抑制PRDXs的抗氧化活性并特异性上调活化HSCs中HO-1的表达	[58]
	鞣花酸	促进VAMP2在蛋白酶体依赖性途径中的降解, 抑制FPN依赖性囊泡运输到质膜, 阻断SNARE复合物的形成, 导致FPN易位和细胞内铁过载	[59]
	小檗碱	抑制HSCs中的自噬-溶酶体途径, 增加细胞内ROS的产生, 并通过泛素-蛋白酶体途径增加HSCs中铁蛋白的水解, 从而诱导肝细胞内亚铁离子过载	[60]
	姜黄素	促进HSCs的自噬, 介导NCOA4和FTH1复合物的降解; 抑制肝纤维化中的CXCL12/CXCR4生物轴, 防止HSCs活化	[61,62]
	多索茶碱	抑制TGF- β /Smad信号通路	[63]
	茺莢素	降低GPX4水平	[64]

用, 介导其泛素化, 降低SLC7A11表达, 从而促进脂质过氧化与铁死亡的发生, 抑制HSCs增殖。此外, 铁死亡抑制剂DFO处理可抑制TRIM26过表达, 引起纤维化减轻, 表明TRIM26靶向SLC7A11表达可作为肝纤维化治疗的靶点^[44]。

BRDs是识别组蛋白结合位点的乙酰赖氨酸识别器, 在基因表达调控中发挥重要作用^[66]。前期已报道, 溴结构域蛋白7(bromodomain-containing proteins 7, BRD7)敲除会增加肝内脂质累积。研究发现, BRD7通过与肿瘤抑制蛋白53(tumor protein p53, p53)的N端转录激活结构域直接结合, 促进p53线粒体易位, 使其与溶质载体家族25成员28(solute carrier family 25 member 28, SLC25A28)相互作用并形成复合物, 增强SLC25A28的活性, 引起线粒体铁的积累和电子转移链的功能亢进, 诱发HSCs铁死亡; 特异性阻断HSC的BRD7-P53-SLC25A28轴可以消除爱拉斯汀(erastin)诱导的

HSC铁死亡, 抑制erastin引起的小鼠肝纤维化改善作用^[45]。

锌指蛋白36(zinc finger protein 36, ZNF36)编码RNA结合蛋白, 促进富含腺嘌呤和尿嘧啶(adenine and uracil, AU)元素的转录本降解^[67]。ZFP36可通过与3'非翻译区中的富含AU的元件结合导致自噬相关基因16L1(autophagy related 16 like 1, ATG16L1) mRNA衰变, 从而触发自噬失活和阻断自噬铁蛋白降解, 并最终赋予对HSCs铁死亡的抵抗力^[48]。ZFP36引起的自噬依赖性铁死亡可作为治疗肝纤维化的潜在靶点, 通过下调ZFP36的表达可诱导HSCs的铁死亡, 最终抑制肝纤维化的产生^[48]。

人脐带间充质干细胞(human umbilical cord mesenchymal stem cells, hucMSCs)分泌的外泌体(MSC-ex)可以介导茺莢素1(beclin 1, BECN1), 抑制SLC7A11/xCT驱动的GPX4表达, 促进人肝星状

细胞系ROS形成、线粒体功能障碍、 Fe^{2+} 释放和脂质过氧化,从而引发HSCs铁死亡,静脉注射MSC-ex显著降低了CCL4实验小鼠纤维化肝脏中的胶原蛋白沉积^[49]。

2.2 临床药物及生物制剂

索拉非尼(sorafenib)是一种多激酶抑制剂,是针对晚期肝细胞癌的分子靶向药物中唯一获得美国食品和药物管理局批准的药物,能够作用于HSCs发挥抗肝纤维化作用^[68]。对sorafenib发挥抗纤维化作用的机制进行研究,发现在sorafenib处理的大鼠肝星状细胞(HSC-T6)中,存在铁死亡的发生,如SLC7A11、GPX4和还原型谷胱甘肽(reduced glutathione, GSH)的降低,铁、ROS和MDA的积累,同时sorafenib诱导的铁死亡并未在肝细胞或巨噬细胞中检测到^[50]。通过铁死亡抑制剂Fer-1和DFO可以消除sorafenib引发的HSCs铁死亡和ECM减少,表明sorafenib通过特异性诱导HSCs的铁死亡来发挥抗纤维化作用^[50]。此外,sorafenib还可抑制HIF-1 α /SLC7A11轴,导致HSCs中GPX4、GSH消耗和ROS生成过量^[50];调控RNA结合蛋白ELAV类蛋白1(embryonic lethal abnormal vision like protein 1, ELAVL1)/人类抗原R(human antigen R, HuR)通路,激活铁蛋白自噬从而促进HSCs的铁死亡^[51]。另外,被美国药物管理局批准用于HCC患者的一线治疗药物仑伐替尼也被报道通过成纤维细胞生长因子受体4(fibroblast growth factor receptor 4, FGFR4)影响SLC7A11/xCT转运系统,诱导铁死亡的发生,从而发挥对终末期肝病的治疗作用;并且FGFR4已被证实能够保护肝脏免受纤维化,仑伐替尼在纤维化期的作用有待进一步研究^[53,54]。

在胆管结扎(bile duct ligation, BDL)小鼠肝纤维化模型中,sorafenib可减少胆汁酸蓄积引起的肝内细胞毒损伤,包括肝细胞坏死、脂肪变性和炎症,降低肝纤维化相关基因的表达,包括肌动蛋白alpha 2、I型胶原蛋白alpha 1链、基质金属蛋白酶(matrix metalloproteinases, MMPs)和TIMP金属肽酶抑制剂1(timp metalloproteinase inhibitor 1, TIMP1),缓解肝损伤发生和ECM积累^[51,69]。Sorafenib作用与HSCs铁死亡导致的肝内羟脯氨酸积累和胶原沉积的显著减少有关,还与其能够抑

制转化生长因子- β 1(transforming growth factor β 1, TGF- β 1)在肝细胞中的促凋亡和促纤维化作用有关^[48,52]。在临床实验中,索拉非尼单药治疗显著降低了纤维化患者肝内纤维化标志物的水平,并上调了患者HSCs中BRD7的水平,导致纤维化患者肝内HSCs的铁死亡,从而发挥抗纤维化作用^[45]。

异甘草酸镁(magnesium isoglycyrrhizinate, MgIG)注射液在病毒性肝炎中发挥改善肝功能作用。研究发现,MgIG通过上调HSCs中HO-1的表达,抑制其下游靶基因转铁蛋白、转铁蛋白受体1和铁蛋白重链1(ferritin heavy chain 1, FTH1)生成,促进 Fe^{2+} 富集;还能够抑制GPX4表达和增加转铁蛋白受体和二价金属离子转运体1(divalent metal-ion transporter-1, DMT1)的表达,诱导ROS积累,引起HSCs铁死亡,从而改善肝纤维化^[14,70]。

在BDL小鼠模型中,铁死亡抑制剂erastin显示出改善肝纤维化的作用,进一步从纤维化肝脏中分离出原代肝细胞、巨噬细胞、肝窦内皮细胞(liver sinusoidal endothelial cells, LSECs)和HSCs,发现erastin治疗明显增加了原代HSCs中铁死亡标志物PTGS2的表达,但在原代肝细胞、LSECs和巨噬细胞中却没有,表明erastin可通过特异性诱导HSCs铁死亡来改善肝纤维化^[45]。同时sorafenib、erastin和RSL3诱导的泛素降解也可以下调ZFP36的转录,进而促进HSCs中的铁蛋白自噬,增加其铁死亡,从而改善肝纤维化^[48]。

2.3 中药及其活性成分

中药及其活性成分在预防和治疗肝纤维化方面有较好的疗效,部分被报道通过铁死亡来发挥作用^[13,14,70]。青蒿琥酯(artesunate)作为一种抗纤维化药物,利用原代小鼠HSC细胞、人肝星状细胞系等细胞模型发现,其通过消耗GSH、诱导铁死亡标志物GPX4和PTGS2与纤维化标志物 α -SMA共定位,引起 Fe^{2+} 释放、ROS积累和抗氧化能力丧失,导致脂质过氧化产生、HSCs死亡^[13,71]。在小鼠CCL4肝纤维化模型中,腹腔注射青蒿琥酯靶向小鼠肝内激活的HSCs铁死亡,显著降低了肝纤维化水平^[13]。此外,青蒿琥酯可引起HSCs中微管相关蛋白轻链3、自噬相关蛋白3(autophagy related 3, ATG3)、ATG5、ATG6/beclin1、ATG的上调和

p62、FTH1、核受体共激活因子4(nuclear receptor coactivator 4, NCOA4)的下调,从而触发铁蛋白自噬;通过溶酶体腔碱化剂氯喹和铁螯合剂DFO特异性抑制铁蛋白自噬,消除了青蒿琥酯的抗纤维化功能,表明青蒿琥酯介导的HSCs铁死亡是抗纤维化所必需的^[13]。

青蒿素的衍生物双氢青蒿素(dihydroartemisinin, DHA)可抑制血小板生长因子-BB(platelet-derived growth factor BB, PDGF-BB)诱导的体外HSCs活化,上调NCOA4的表达,引起铁死亡的发生从而改善肝纤维化^[55];腹腔注射DHA保护大鼠肝脏免受BDL引起的损伤和炎症,DHA治疗显著改善了肝脏组织学病理变化,降低了肝脏和血清中TNF- α 和IL-6的水平,抑制了胶原蛋白在肝内的严重沉积,延缓肝纤维化的进程^[72]。蒿甲醚(artemether, ART)通过抑制铁反应元件结合蛋白2(iron responsive element binding protein 2, IRP2)与含STIP1同源物U-框蛋白1(stip1 homology and U-box containing protein 1, STUB1)的结合来降低IRP2的泛素化,促进细胞内IRP2蛋白的积累,从而增加铁的流入,减少铁的储存和输出,导致细胞内铁的积累和过多的ROS累积,诱导HSC铁死亡,在体内外实验中均发挥良好的抗纤维化作用^[56]。此外,ART还可以促进p53诱导的铁死亡来抑制HSCs活化,使肝纤维化标志基因表达明显降低^[57]。此外,在CCL4小鼠模型中,DHA和ART通过铁死亡缓解了肝纤维化^[55,57]。

南蛇藤醇(celestrol)是一种从雷公藤中提取的天然化合物,其活性半胱氨酸位点能够直接与过氧化物(peroxiredoxins, PRDXs)结合,抑制PRDXs的抗氧化活性并增加ROS的产生,同时能够特异性上调活化HSCs中HO-1的表达,引起HSCs铁死亡的发生,为肝纤维化提供了一种有前景的治疗策略^[58]。鞣花酸(ellagic acid, EA)可增强囊泡相关膜蛋白2(vesicle associated membrane protein 2, VAMP2)在蛋白酶体依赖性途径中的降解,抑制铁泵蛋白(ferroportin, FPN)依赖性囊泡运输到质膜,阻断SNARE复合物的形成,导致FPN易位和细胞内铁过载,引起HSCs铁死亡,阻止纤维化和瘢痕形成^[59]。

小檗碱(berberine, BBR)通过抑制HSCs中的自

噬-溶酶体途径,导致细胞内蛋白质和细胞器质量控制的失调,增加细胞内ROS的产生,并通过泛素-蛋白酶体途径增加HSCs中铁蛋白的水解,从而诱导肝细胞内亚铁离子过载,进一步引起ROS介导的HSCs铁死亡来缓解肝纤维化,Fer-1治疗显著降低了BBR对肝纤维化的药理作用^[60]。姜黄素(curcumin)能够促进HSCs的自噬,介导NCOA4和FTH1复合物的降解,释放铁离子,导致HSCs铁含量增加,诱导铁死亡,并且可以通过抑制肝纤维化中的趋化因子CXC配体12(C-X-C motif chemokine ligand 12, CXCL12)/趋化因子受体4(C-X-C motif chemokine receptor 4, CXCR4)生物轴来防止HSCs活化和迁移,继而发挥抗肝纤维化的作用^[61,62]。研究还报道,茺菀素(decursin)通过降低GPX4水平、多索茶碱(doxofylline, DOX)通过抑制TGF- β /Smad信号通路等途径特异性诱导HSCs中的铁死亡,并减少ECM的产生来缓解肝纤维化^[63,64]。

3 总结与展望

从NAFL到终末期肝病进展缓慢,早期症状不明显,若未积极治疗,胞内脂肪酸聚集会引发炎症和氧化应激,炎性介质释放进而刺激肝内HSCs激活,导致肝内纤维化水平增加形成肝纤维化和肝硬化,目前尚无针对肝硬化经济有效的治疗手段。HSCs激活向成纤维细胞的转化已被证实是纤维化的主要驱动因素,因此探究有效的药物或方法逆转这一过程将为肝纤维化的治疗提供新的靶点或方向。虽然铁死亡导致的过度细胞死亡参与了肝纤维化的致病过程,但铁死亡作为一类程序性细胞死亡方式,对于多细胞生物体内的稳态维持至关重要,其功能也并非单一,正如最近的研究显示,部分药物和基因可通过特异性诱导HSCs的铁死亡,抑制HSCs向成纤维细胞的转化,从而抑制肝纤维化的生成和加重。目前,铁死亡参与肝纤维化的研究尚处于初步探索阶段,仍有很多未开发的领域亟待研究,已发现的治疗肝纤维化基因靶点和分子的具体机制也较为模糊,具有重要的研究价值。此外,铁死亡参与纤维化的加重或缓解,很大程度上取决于作用的细胞类型以及调控的靶蛋白负荷;进一步探究分子靶点及药物

对铁死亡的具体调控作用, 绘制其细胞作用与分子机制图谱, 将为肝纤维化甚至其他器官的纤维化治疗提供重要的参考。因此, 充分评估铁死亡在NAFLD中的病理生理作用、进一步判断铁死亡作用于NAFLD纤维化的不同节点、明确不同的分子靶点和药物对肝纤维化的具体功能, 将为NAFLD药物研发和临床治疗提供新的视角。

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