•综述•

骨髓间充质干细胞改善肝纤维化机制的研究进展

丁 翼, 罗晓敏, 杨 斌, 张博宇, 顾 健*, 袁志翔*

(西南民族大学药学院, 四川 成都 610041)

摘要: 肝纤维化以肝脏组织瘢痕为特征, 是慢性肝脏疾病发展为肝癌的中间病理过程, 发生机制涉及多种信号通路, 其逆转性是目前的研究热点。骨髓间充质干细胞 (bone marrow mesenchymal stem cells, BMSCs) 是一种具有多向分化潜能的成体干细胞, 在体内和体外均具备分化为肝样细胞, 发挥正常肝细胞功能的能力。现代药理实验研究表明, 单独使用BMSCs或联合活性因子、中药或中药单体、基因修饰等方式可以促进其增殖、分化、迁移, 提高治疗效果, 发挥改善肝纤维化的作用。通过归纳总结现有文献, 从肝纤维化发病机制、肝纤维化改善机制、BMSCs生物学特性及其改善机制等方面对BMSCs改善肝纤维化疗效机制进行综述, 为后期发展BMSCs细胞疗法提供参考。

关键词: 肝纤维化; 改善机制; 骨髓间充质干细胞; 联合治疗; 作用机制研究; 应用前景

中图分类号: R966 **文献标识码**: A **文章编号**: 0513-4870(2022)04-0863-12

Research progress on the mechanism of bone marrow mesenchymal stem cells in improving liver fibrosis

DING Yi, LUO Xiao-min, YANG Bin, ZHANG Bo-yu, GU Jian*, YUAN Zhi-xiang*

(*College of Pharmacy, Southwest Minzu University, Chengdu 610041, China*)

Abstract: Liver fibrosis is characterized by scarring of liver tissue, which is an intermediate pathological process of chronic liver disease developing into liver cancer. Its mechanism involves multiple signal pathways, and its reversibility is a current research hotspot. Bone marrow mesenchymal stem cells (BMSCs) are adult stem cells with multi-differentiation potential. They have ability to differentiate into liver-like cells *in vivo* and *in vitro* to perform normal liver cell functions. Modern pharmacological experimental studies have shown that the use of BMSCs alone or in combination with active factors, Chinese medicine or Chinese medicine monomers, genetic modification and other methods can promote their proliferation, differentiation, and migration, improve the therapeutic effect, and play a role in improving liver fibrosis. By summarizing the existing literature, the therapeutic mechanism of BMSCs in improving liver fibrosis is reviewed from the aspects of the pathogenesis of liver fibrosis, the improvement mechanism of liver fibrosis, the biological characteristics of BMSCs and its improvement mechanism, so as to provide reference for the later development of BMSCs cell therapy.

Key words: liver fibrosis; improvement mechanism; bone marrow mesenchymal stem cell; combination therapy; mechanism study; application prospect

外界因素如酒精[1]、四氯化碳[2] (carbon tetrachloride, CCl_4)、高脂饮食[3]等多种致病因素会导致肝脏慢性炎症, 进而发展成肝脏损伤的另外一种形式, 即肝纤维化 (hepatic fibrosis, HF)。肝纤维化是一种可逆的病理生理过程[4], 是慢性肝病发展为肝硬化肝癌的必要途径, 其基本特征是细胞外基质 (extracellular matrix, ECM) 过度、异常沉积[5,6]。肝纤维化最终会发展为肝硬化等晚期肝病。鉴于现有医疗手段, 肝移植是治疗

收稿日期: 2021-08-23; 修回日期: 2021-09-28.

基金项目: 国家自然科学基金资助项目 (82174083); 西南民族大学中央高校基本科研业务费专项资金优秀学生培养工程项目 (2021NYYXS100).

*通讯作者 Tel: 86-28-85656463,

E-mail: gujiancd@163.com; Zhixiang-yuan@hotmail.com

DOI: 10.16438/j.0513-4870.2021-1220

晚期肝病的唯一可行治疗手段, 但因价格昂贵、供体资源稀缺、移植存在排斥反应等, 很难成为高效的治疗手段[7], 因此, 寻找另外一种有效且高效的方法用于治疗晚期肝病十分有意义。

间充质干细胞 (mesenchymal stem cells, MSCs) 是干细胞家族中重要一员, MSCs作为一种治疗疾病手段具有增殖、自我更新和免疫原性低, 可进行异体移植[8]。在骨髓中被发现分离的MSCs, 被称为骨髓间充质干细胞 (bone marrow mesenchymal stem cells, BMSCs)。BMSCs是一类具有自我更新和多向分化潜能的成体干细胞, 可分化为脂肪细胞、软骨细胞和成骨细胞[9]。因其特有的优势, 如增殖分化、免疫调节功能、分泌多种因子等[10], 在基础实验和临床研究中, BMSCs治疗不同外界因素导致的肝纤维化是研究热点, 其治疗机制也一直被深入研究。本文就BMSCs改善肝纤维化机制的研究进行综述。

1 肝纤维化成因

肝纤维化的成因复杂, 涉及多方面的因子和细胞因素。在外界不利因素的影响下, 肝实质细胞发生损伤和凋亡, 同时炎性细胞向损伤区聚集, 释放趋化因子, 引起肝星状细胞 (hepatic stellate cells, HSC) 激活, 激活的HSC通过增生和分泌细胞外基质参与肝纤维化的形成和肝内结构的重建, 通过分泌促进纤维细胞、成纤维细胞和骨髓衍生的髓纤维细胞来产生胶原蛋白, 奠定肝纤维化病理学的基础[11] (图1)。以下从因子、氧化应激等方面介绍肝纤维化成因机制及改善肝纤维化机制。

1.1 转化生长因子-β1 (transforming growth factor-β1, TGF-β1)

在肝纤维化的形成过程中, TGF-β1是HSC的关键活化剂, 也是致肝纤维化的重要细胞因子之一。在正常HSC中, TGF-β1几乎不表达。当肝损伤时, Kupffer细胞、肝窦内皮细胞和肝细胞分泌TGF-β1[12,13], TGF-β1上调与ECM合成相关的细胞受体, 增强了ECM组分表达[14], 促进产生肝纤维化。TGF-β1介导的信号通路抑制HSC的凋亡, 诱导其合成产生过量的基质蛋白, 影响基质降解蛋白酶的产生并上调蛋白酶抑制剂, 促进ECM的产生并抑制其降解[15]。Smad蛋白 (Smad proteins, Smad) 家族是TGF-β1下游的关键信号转导分子, TGF-β1/Smad信号通路在肝纤维化发展过程中发挥重要作用[16]。TGF-β1通过与TGF-β受体II (TGF-β receptor II, TβRII) 结合来引发细胞内信号传导, 激活TGF-β受体型I (TGF-β receptor I, TβRI) 激酶, 导致Smad2和Smad3的磷酸化[15]。此外, TGF-β1激活丝裂原活化蛋白激酶 (mitogen-activated protein kinase, MAPK)、核因子κB (nuclear factor-κB, NF-κB) 和磷脂酰肌醇-3-激酶 (phosphoinositide 3-kinase, PI3K) 通路使HSC活化造成肝纤维化。因此, 阻断TGF-β1的表达是抑制肝纤维化的重要途径。

1.2 血小板衍生生长因子 (platelet-derived growth factor, PDGF)

PDGF与HSC的增殖、分化、迁移有关[17], 促进胶原

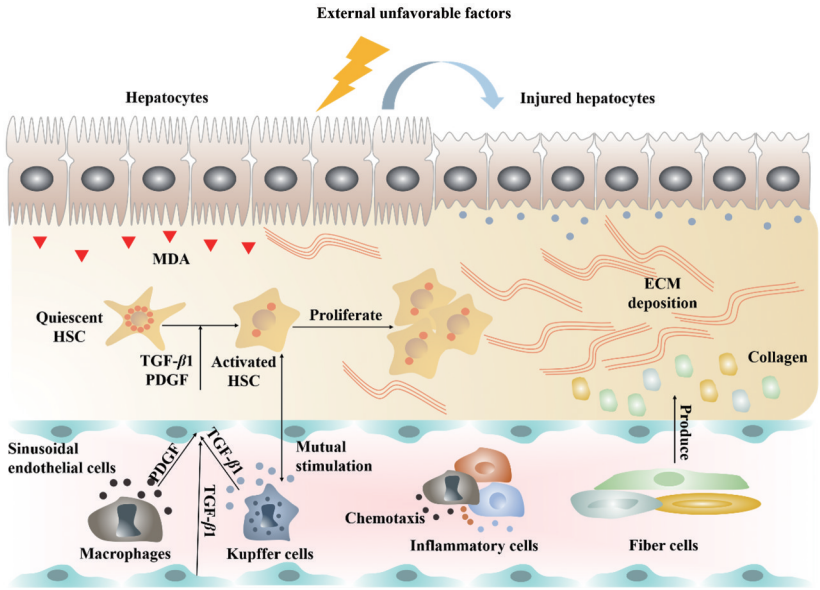

Figure 1 The mechanism of liver fibrosis includes injury of normal liver cells, activation and proliferation of hepatic stellate cells, release of active factors by Kupffer cells, macrophages, sinusoidal endothelial cells and inflammatory cells, collagen production by fibroblasts and extracellular matrix deposition. MDA: Malondialdehyde; HSC: Hepatic stellate cells; ECM: Extracellular matrix; TGF-β1: Transforming growth factor-β1; PDGF: Platelet-derived growth factor

蛋白的产生和沉积, 并将HSC转化为肌纤维细胞。正常生理情况下, PDGF在血小板α-粒子中表达, 当肝脏受损时, PDGF在受损内皮细胞、HSC中高度表达。血小板衍生生长因子受体 (platelet-derived growth factor receptor, PDGFR) 是PDGF蛋白质家族的受体, 属于酪胺酸激酶受体的一种, 主要位于血管内皮细胞、成纤维细胞和Kupffer细胞[18]。PDGF激活HSC并活化细胞膜上的PDGFR, 使PDGFR增加, 增强细胞趋化性, 参与ECM的生成[19]。PDGFR与配体结合后使受体各亚单位二聚化, 酪氨酸激酶区活化, 受体自身磷酸化, 引起不同信号转导通路级联反应的发生。PDGFR通路的激活涉及关键下游信号通路, 如肾素血管紧张素系统 (renin-angiotensin-system, RAS) 途径和PI3K途径。通过抑制PDGF的表达能够在一定程度上减缓肝纤维化的发展进程。

1.3　缺氧诱导因子-1α (hypoxia inducible factor 1α, HIF-1α)

HIF-1α是缺氧诱导因子-1 (hypoxia inducible factor 1, HIF-1) 的组成部分, 在正常氧浓度下其含量极低[20], 在缺氧状态下, HIF-1α降解受阻, 在细胞内堆积, 堆积的HIF-1α易位到细胞核内, 与核内的含β亚基的HIF-1结合, 形成二聚体, 进而与缺氧反应元件 (hypoxia-responsive elements, HRE) 相结合, 促进产生多种蛋白质, 调节机体以适应缺氧环境[21]。已有实验证明, HIF-1α与肝损伤密切相关[22-27]。细胞外调节蛋白激酶 (extracellular regulated protein kinases, ERK) 是一类丝/苏氨酸蛋白激酶, ERK/HIF-1α信号通路被认为在进行性纤维化的发生中起重要作用。有研究表明HIF-1α可能至少部分通过ERK通路促进肝纤维化, 这可能与抑制ERK通路来抑制HIF-1α活性或HIF-1α易位有关[28]。雷帕霉素靶蛋白 (mammalian target of rapamycin, mTOR) 参与细胞生长和增殖[29], HIF-1α活性升高会使mTOR的表达量增加, 表明HIF-1α/mTOR信号通路在肝纤维化发生和发展中起重要作用[30]。因此, 通过阻断ERK/HIF-1α、HIF-1α/mTOR信号通路及抑制HIF-1α的表达来减轻肝纤维化具有可行性。

1.4　氧化应激

氧化应激 (oxidative stress, OS) 是指体内氧化与抗氧化作用失衡的一种状态, 偏向于氧化状态, 导致中性粒细胞炎性浸润, 蛋白酶分泌增加, 大量氧化中间产物生成。在慢性肝损伤的过程中, 脂质过氧化是诱发肝纤维化的重要因素之一, 脂质过氧化会使丙二醛 (malondialdehyde, MDA) 含量升高, 刺激Kupffer细胞产生相关因子, 诱导肝细胞凋亡坏死, 胶原生成, 促进肝纤维化发展[31-34]。活性氧 (reactive oxygen species, ROS) 是体内一类氧的单电子还原产物, 其来源途径之一是线粒体。肝脏中富含大量线粒体, 当肝脏受损时, 酶活性降低, 产生的ROS大量堆积, 难以清除。ROS攻击细胞膜脂上的多不饱和脂肪酸, 诱导肝细胞产生MDA, 直接破坏组织细胞中的脂质分子、蛋白质及DNA, 进而发生脂质过氧化, 引起肝细胞的凋亡和其他生理病理反应, 这些与TGF-β、MAPK、PI3K、核因子E2相关因子2/血红素加氧酶-1 (nuclear factor E2 related factor 2/heme oxygenase-1, Nrf2/HO-1) 信号通路有关[35-43]。

2　肝纤维化改善机制

2.1　抑制HSC活化

HSC的活化受到肝实质细胞和非实质细胞相互作用、细胞质基质组成成分和整合素介导相互作用的影响, 活化HSC具有增殖、收缩、炎症和趋化作用[44,45]。TGF-β1是促使HSC活化、增殖的关键细胞因子, 被认为是最有效的纤维化因子之一。TGF-β1促进并维持胶原蛋白基因在自分泌环中的表达, 上调组织金属蛋白酶抑制剂 (tissue inhibitor of matrix metalloproteinases, TIMPs) 表达[46], TGF-β/Smad信号通路在肝纤维化发展过程中起着重要作用[16]。miRNA是真核生物体中约有22个核苷酸的内源非编码小RNA, 参与细胞的存活、增殖、自噬和凋亡等过程[47,48]。阻断β受体后miRNA-200a过表达, α-平滑肌肌动蛋白 (alpha-smooth muscle actin, α-SMA) 的表达水平下调, HSC上皮−间质转化 (epithelial-mesenchymal transition, EMT) 过程受到抑制[49]。通过抑制HSC活化相关基因表达和通路传导, 达到抑制HSC活化的目的, 能够改善肝纤维化。

2.2　抑制炎症反应

发生炎症时, 多种介质会损伤肝窦和血管内皮稳态, 引起肝脏微血栓形成, 促使肝细胞变性坏死, 受损肝细胞会分泌更多的炎性细胞因子, 诱发HSC活化, 促进肝纤维化发展[50]。脂多糖 (lipopolysaccharide, LPS) 攻击肝脏, 激活Kupffer细胞和HSC, 释放一系列炎性因子, 使肝组织中白介素-1 (interleukin-1, IL-1)、IL-6、肿瘤坏死因子α (tumor necrosis factor α, TNF-α) 明显升高, I型胶原表达合成升高, 炎症因子启动炎症的级联反应, 导致肝组织炎症反应和肝纤维化发展[51]。炎症因子分泌增加, 能够促进肝纤维化的发生, 反之, 肝纤维化发展受到抑制[52,53]。因此, 通过抑制炎症反应能够减缓肝纤维化的进展, 达到抵抗肝纤维化的作用。

2.3　促进HSC凋亡

凋亡是细胞程序性死亡的一种形式, 活化的HSC可以通过凋亡来减少[54]。肝纤维化的逆转伴随着炎症微环境的消退和活化的HSC数量的减少。活化的

HSC会激活NF-κB通路, 使抗凋亡蛋白如B淋巴细胞瘤-2基因 (B-cell lymphoma-2, Bcl-2) 的表达增强, 诱导其分泌来抵抗HSC凋亡[55]。阻断NF-κB通路可能促进HSC凋亡, 减缓或减轻肝纤维化程度。自然杀伤细胞 (natural killer cell, NK) 能够直接杀死活化的HSC并产生干扰素-γ (interferon gamma, IFN-γ) 促进该行为, 在抑制肝纤维化方面发挥作用[56]。因此, 通过促进HSC的凋亡能够直接减少活化的HSC数量, 抑制肝纤维化的发展。

2.4 调节胶原蛋白代谢

肝纤维化的特征是ECM的异常沉积, 胶原蛋白是ECM的主要成分之一[5]。ECM的稳态是依靠基质金属蛋白酶 (matrix metalloproteinases, MMPs) 和TIMPs的调控。TIMP-1可以抑制胶原酶的生物活性, 导致胶原沉积。抑制肝脏组织中TIMP-1和I型胶原的表达, 重建MMP-2/TIMP-2和MMP-9/TIMP-1的平衡, 可以减轻肝纤维化程度, 这提示促进胶原降解和抑制胶原表达能够改善肝纤维化[57,58]。调节胶原蛋白的代谢, 可能成为抗肝纤维化的一个策略。

3 BMSCs生物学特性及改善肝纤维化机制

3.1 MSCs生物学特性

MSCs是一类具有自我更新和多向分化潜能的成体干细胞。MSCs可以从骨髓、脂肪、牙髓、外周血等组织中获取。国际细胞治疗学会提出了以下定义MSCs的最低标准: ① 贴壁生长; ② 存在特定的细胞表面抗原标记物CD73、CD90、CD105, 同时不存在CD14、CD34、CD45和人类白细胞DR抗原 (human leukocyte antigen DR, HLA-DR) 等其他标记物; ③ 在体外有分化为脂肪细胞、软骨细胞和成骨细胞的能力。BMSCs是从骨髓中提取分离的, 其具有增殖分化能力强、免疫原性低、免疫调节和易获取等特点[59]。已有研究证明, BMSCs分化的肝细胞能够代替受损肝细胞并发挥作用, 分化过程中, BMSCs会分泌多种细胞因子, 发挥旁分泌作用。此外, 移植的BMSCs会降低氧化应激水平, 抑制HSC活化和促进HSC凋亡[60-62]。BMSCs凭借其特点优势和功能优势, 可能成为治疗肝纤维化的最有前途的候选细胞之一。

3.2 BMSCs改善肝纤维化机制

3.2.1 BMSCs归巢机制 干细胞与周围相邻的细胞、ECM以及多种细胞因子等构成微环境, 称为“干细胞巢” (niche), niche为干细胞提供养分, 决定干细胞分化方向, 指导干细胞行动。MSCs归巢可定义为: MSCs在目标组织的脉管系统里被捕获, 随后跨越血管内皮细胞迁移至目标组织的过程[63]。微环境的改变是使BMSCs发生归巢行为的重要因素之一。当机体组织受损, 组织会释放各种趋化因子、黏附因子以及生长因子等各种信号因子, 吸引内源性或外源性的BMSCs向受损的组织部位迁移且定向分布, 优势分布。趋化因子是一类小分子分泌蛋白, 能够趋化反应细胞发生迁移, 而趋化因子受体能够介导趋化因子的行动。一般而言, 趋化受体能够感受到外部环境中趋化因子浓度改变的信号, 并引导细胞向趋化因子浓度高的地方迁移[64]。基质衍生因子1α (stromal cell-derived factor 1α, SDF-1α) 是一种由骨髓内皮细胞和基质细胞表达的特异性趋化因子家族的化学引诱蛋白, 特异性趋化因子受体4 (CXC chemokine receptor 4, CXCR4) 是BMSCs的一种受体且能与SDF-1α结合。大量研究表明, SDF-1α/CXCR4轴在干细胞归巢、趋化、黏附因子表达、移植、增殖和存活等方面发挥重要作用[65,66]。当机体损伤, SDF-1α在损伤部位产生, 其浓度会高于骨髓处, 同时CXCR4表达增强, 促进骨髓处的MSCs迁移至损伤处[67]。但是, 归巢的具体机制尚不明确, 现认为某些特定的信号分子与细胞膜上的相应受体结合, 共同驱动其归巢行为。

3.2.2 BMSCs旁分泌机制及细胞−细胞接触 BMSCs的生物学特性使其具有异体移植的可能性, 归巢行为使其可能具有定向增殖分化的能力, 这提示BMSCs能够迁移至受损组织并分化为局部成分, BMSCs移植可能成为一种新型的治疗方式。

BMSCs可以通过多种途径改善肝纤维化。一方面, BMSCs可以通过减少炎症反应、胶原沉积以及重塑受损肝细胞来修复肝纤维化[68]。另一方面, BMSCs会分泌多种可溶性因子如TNF-3α、IL-10、肝细胞生长因子 (hepatocyte growth factor, HGF), 抑制胶原合成, 诱导HSC凋亡[69] (图2)。一般来说, MMPs和TIMP分别对肝纤维化起着抑制与促进作用。尾静脉注射BMSCs于肝纤维化小鼠体内, 使肝脏胶原蛋白和组织脂质过氧化降低, 上调MMP-9和MMP-13的表达, 下调TIMP-1的表达[70]。荧光标记BMSCs可以明显观察到受损肝组织中显现荧光, 血清中转氨酶活性降低[71]。BMSCs可以通过影响MMPs的表达来减少胶原沉积, 减轻肝纤维化。BMSCs激活Nrf2/HO-1, 抑制了MDA、NF-κB p65和炎性细胞因子的产生, 增强了抗氧化剂的表达, 抑制氧化应激, 减少炎症反应进而阻碍肝纤维化的发展[72]。MSCs通过旁分泌机制和细胞−细胞接触机制调节HSC活化和凋亡, 可溶性因子对肝细胞具有抗凋亡和促进分裂作用。共培养下的BMSCs能够直接抑制HSC活化能力, 减少ECM沉积[73], 全身灌注MSCs培养基能够抑制体内肝细胞死亡, 增强肝再生能力[74], 调节HSC活性的机制与TNF-α、IL-10、

HGF的分泌有关, 其中TNF-α和IL-10在抑制胶原产生和HSC增殖方面可能具有协同作用[75]。另有研究发现, MSC分泌的神经生长因子 (nerve growth factor, NGF) 能够促进HSC凋亡, NF-κB Bcl-xl参与了此过程[76]。细胞−细胞接触能够直接影响HSC, 共培养体系下, BMSCs部分通过Notch通路激活介导的细胞−细胞接触模式, 显著抑制HSC增殖, 并下调α-SMA表达[77]。BMSCs分泌HGF并介导Toll样受体4 (Toll-like receptor 4, TLR4)/骨髓分化因子88 (myeloid differentiation factor 88, MyD88)/NF-κB信号通路来影响细胞−细胞接触, 从而抑制HSC的增殖和活化[78]。总的来说, BMSCs能够改善肝纤维化主要归因于其归巢机制、具备多向分化潜能以及分泌多种影响周围组织的活性因子。

虽然目前BMSCs在临床上改善肝纤维化的研究还未大范围普及, 但是已有部分学者进行了肝硬化相关临床研究。兹通过https://clinicaltrials.gov/网站收集BMSCs治疗肝硬化的相关临床研究 (表1)。这类临床研究收治不同年龄段的患者进行长期治疗, 在治疗结束后检测生理病理指标变化, 评估BMSCs的安全性和有效性。中山大学学者针对乙型肝炎病毒 (hepatitis B virus, HBV) 相关的早中期肝硬化 (NCT00993941) 和慢性HBV肝硬化 (NCT01223664) 分别进行BMSCs自体移植和同种异体移植, 观察临床症状改变, 评估Child-Pugh得分以及考察肝功能。对于BMSCs移植的安全性和有效性研究, 空军军医大学学者对HBV相关肝硬化患者通过肝动脉自体移植BMSCs, 治疗一年后评价患者存活率 (NCT01724697)。南京解放军总医院学者收治难治性腹水肝硬化患者, 并对其进行肝动脉自体移植BMSCs, 治疗周期结束后检测肝功能、腹水变化和Child-Pugh评分 (NCT01854125)。

4　BMSCs联合用药改善肝纤维化

作为一种新型的治疗方式, 已有文献[79-81]报道BMSCs联合活性因子、中药或中药单体、基因修饰等方式能够改善肝脏指标, 改善肝纤维化。

4.1　BMSCs联合活性因子改善肝纤维化

有多种细胞生长因子对BMSCs向肝细胞分化有一

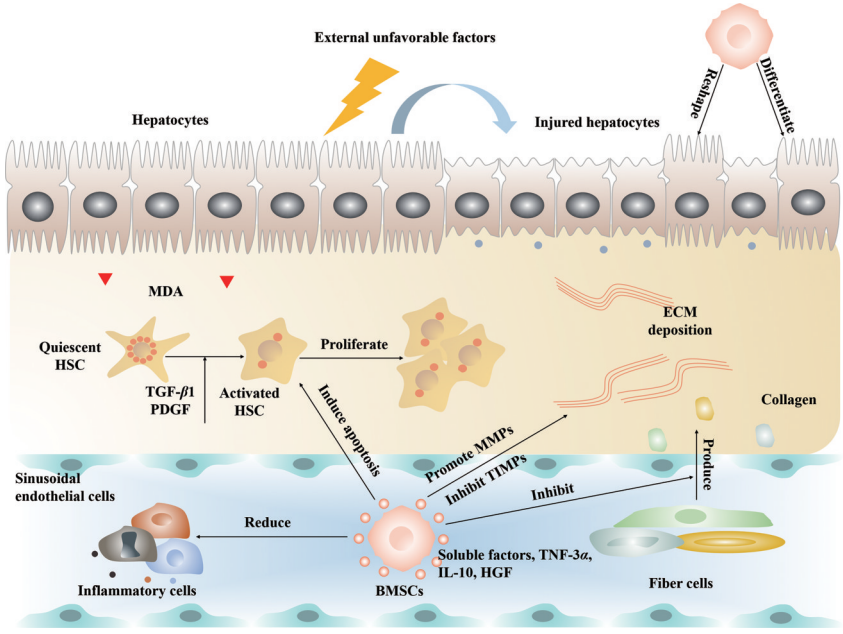

Figure 2　BMSCs improve the mechanism of liver fibrosis. BMSCs remodel damaged liver cells or differentiate into normal liver cells, release soluble factors to inhibit collagen synthesis, promote MMPs expression, inhibit TIMPs expression and inflammatory cytokine release, and induce apoptosis of activated HSCs, thereby reducing ECM deposition. BMSCs: Bone marrow mesenchymal stem cells; MMPs: Matrix metalloproteinases; TIMPs: Tissue inhibitor of matrix metalloproteinases; TNF-3α: Tumor necrosis factor 3α; IL-10: Interleukin-10; HGF: Hepatocyte growth factor

Table 1　Related research on clinical improvement of BMSCs in liver cirrhosis. HBV: Hepatitis B virus

Disease	BMSCs transplantation method	BMSCs transplantation route	Phase	ClinicalTrials.gov identifier
Early and middle stage of liver cirrhosis on the basis of HBV infection	Autologous BMSCs transplantation	Portal vein	Phase II	NCT00993941
HBV-related liver cirrhosis	Autologous BMSCs transplantation	Hepatic artery	Phase I/II	NCT01724697
Liver cirrhosis resulting from chronic HBV	Allogenic BMSCs transplantation	Portal vein or hepatic artery	Phase II	NCT01223664
Liver cirrhosis with refractory ascites	Autologous BMSCs transplantation	Liver artery	Phase III	NCT01854125

定的影响。其中HGF、成纤维细胞生长因子 (fibroblast growth factor, FGF) 已在体外实验被证明能够诱导BMSCs向肝细胞的分化, 分化得到的肝细胞能够表达肝脏特异性标志物白蛋白 (albumin, Alb)、角蛋白18 (keratin-18, CK-18), 行使肝细胞糖原储存、尿素产生等功能[82-84]。HGF能够促进细胞迁移、增殖, 调控干细胞不同生物反应, 同时在肝脏的发育与再生过程中起着重要作用。将BMSCs短期暴露于HGF中可以诱导Met受体 (一种具有细胞内酪氨酸激酶结构域的跨膜蛋白) 和下游效应子ERK1/2、p38MAPK和PI3K/Akt的激活, 而K252A酪氨酸激酶抑制剂可抑制HGF的诱导反应[85]。FGF可由内皮细胞分泌, 促进新血管形成, 修复受损的内皮细胞。在一项探究细胞因子对BMSCs分化的研究中, FGF能够诱导BMSCs向肝细胞分化, 并从内胚层模式的初始阶段起作用[86]。体外加入细胞因子来模拟因子分泌影响BMSCs向肝样细胞分化, 提示联合细胞因子有利于BMSCs向肝细胞分化, 有助于治疗肝纤维化。但各种因子的加入顺序可能会影响分化效果, 需要进一步探究。

4.2 BMSCs联合中药改善肝纤维化

目前中药广泛用于肝脏疾病的临床治疗, 具有毒副作用小、不良反应少以及改善患者症状等特点, 中医药的经络、活血、行气等在治疗肝脏疾病方面具有潜在的靶向作用[87]。BMSCs结合中医药或许能够为治疗肝纤维化提供新思路。BMSCs的增殖效率对改善肝纤维化有着重要作用, 而有些中药及其提取物能够促进其增殖。黄芪中的黄芪甲苷[88]和黄芪多糖[89]均能对BMSCs的增殖起促进作用, 可能机制与其促进BMSCs对干细胞因子 (stem cell factor, SCF)、血管内皮生长因子 (vascular endothelial growth factor, VEGF)、SDF-1 mRNA表达有关。中药提取物也可以提高BMSCs的抗凋亡能力, 提高其存活率。淫羊藿苷能够显著上调HGF水平, 并通过上调HGF/c-Met通路, 在BMSCs抗凋亡活性中起到关键作用[90]。通过促进BMSCs的增殖可以进一步提高向肝细胞分化的成功率。在虫草多糖体外诱导BMSCs分化研究中, 虫草多糖组甲胎蛋白 (alpha fetoprotein, AFP)、Alb、CK-18表达增加, 糖原染色呈阳性, 表明虫草多糖成功诱导BMSCs向肝样细胞分化[91]。20 μmol·L^{-1}姜黄素能够单独诱导BMSCs向肝细胞分化, 与FGF-4联合使用不仅可以降低姜黄素诱导浓度, 降低毒性, 还能提高诱导分化效率[92]。大黄提取物能促进BMSCs的增殖分化为正常肝细胞, 其中的微量物质可能促进肝细胞分泌活性物质及生长因子, 促进肝细胞的再生和修复[93]。此外, BMSCs联合中药复方也能促进其增殖分化为肝样细胞, 达到改善肝纤维化的目的。表2[94-97]总结了部分中药复方联合BMSCs改善肝纤维化的药理作用及机制研究。

4.3 基因修饰BMSCs改善肝脏指标

BMSCs同时也是理想的基因治疗细胞载体, 易被腺病毒、慢病毒、腺相关病毒以及逆转录病毒转染, 修饰后的BMSCs不仅能提高其归巢增殖分化的能力, 还能表达目的基因, 在组织再生、器官损伤修复和免疫调节等方面改善BMSCs功能[98], 使二者具有协同作用。SDF-1α/CXCR4轴参与MSCs的募集过程, 在MSCs归巢、趋化因子表达等方面有着重要作用, 趋化因子的表达有助于MSCs的归巢[65,66,99]。CXCR4修饰的人源骨髓MSCs (CXCR4-MSCs) 能够显著升高SDF-1α浓度并保持在相对稳定的水平, 在肝脏中能够检测到强烈的标记MSCs的荧光信号而在脾脏中不能检测到荧光信号, 这表明CXCR4-MSCs大量准确地迁移至受损肝脏, CXCR4促进MSCs的归巢效应, 改善肝功能[100]。TGF-β1是致肝纤维化的关键因素, 而Smad7是TGF-β1/Smad信号通路的负调节因子。采用慢病毒转染的方式成功构建能够稳定表达Smad7和增强型绿色荧光蛋白 (enhanced green fluorescent protein, EGFP)的BMSCs (Smad7-EGFP-BMSCs), 在与HSC共培养

Table 2 BMSCs combined with traditional Chinese formular to improve liver fibrosis indicators. EGF: Epidermal growth factor; TBIL: Total bilirubin; Jak-STAT: Janus kinase-signal transducer and activator of transcription; Wnt: Wingless/integrated; CSF3: Colony-stimulating factor 3; sFRP: Secreted frizzled-related protein; CSF2R: Colony-stimulating factor 2 receptor; CNTFR: Ciliary neurotrophic factor receptor; GSK3: Glycogen synthase kinase-3; Akt2: Protein kinase B2; PKCγ: Protein kinase C gamma; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase

Traditional Chinese formular	Change in related indicator	Possibly related mechanism or pathway
Yiguan Decoction[94]	Alb, AFP, HNF4α mRNA, CK-18 ↑; Wnt3α, β-catenin ↓	Down-regulate Wnt/β-catenin signaling pathway
Zuoguiwan[95]	Wnt1, EGF, FGF2, FGF16, MAPKK1, E2F, CSF3, Myd88, sFRP1, sFRP5, CSF2R, CNTFR, caspase12 ↑; MAPK9, Rac1, GSK3, Wnt10a, IL12a, Akt2, Activin AR, PKCγ ↓	Affect Wnt, MAPK, TGF-β, Jak-STAT and Toll-like receptor signaling pathways
Biejiajian Pills[96]	ALB ↑; ALT, AST, TBIL, SDF-1 ↓	Act on SDF-1/CRCX4
Ruanganjian[97]	ALB ↑; ALT, AST ↓	Improve the content of albumin in plasma

体系中, Smad7-EGFP-BMSCs促进HSC的早期凋亡, Smad7和MMP-1的mRNA水平和蛋白水平明显增加, TGF-β1、Smad2、Smad3和TIMP-1的mRNA水平和蛋白水平降低, 此外, α-SMA、I型胶原 (type I collagen, Col I) 和III型胶原 (type III collagen, Col III) 表达降低。Smad7-EGFP-BMSCs影响肝纤维化相关蛋白表达, 可能通过调节TGF-β1/Smad信号通路抑制HSCs的纤维化[101]。HGF是一种成纤维细胞、内皮细胞等间质细胞分泌的多功能细胞因子, 具有促进血管生成、修复受损组织以及抗纤维化的作用[102-104]。在大鼠肝纤维化模型中, HGF-BMSCs即基因修饰表达HGF的BMSCs, 通过调节HSC活性、促进大鼠内源性HGF表达, 并抑制TGF-β1表达来减少活体肝移植胶原沉积, 这提示HGF-BMSCs治疗可能成为一种预防活体肝移植致肝纤维化的新策略[105]。此外, 移植的HGF-MSCs促进MMP-9、MMP-13、MMP-14的表达, 抑制TIMP-1的表达, 表明HGF-MSCs可能是治疗肝纤维化的一种新的有效工具[106]。FGF能够调节细胞增殖、分化和迁移, 其中FGF-4是有丝分裂活性最高的FGF之一[107,108]。过表达FGF-4的BMSCs移植到肝硬化大鼠中, FGF-4能够在早期促进BMSCs定位于肝脏, 促进Jagged-1阳性干细胞增殖以及细胞核抗原 (proliferating cell nuclear antigen, PCNA) 和上皮黏附分子 (epithelial cell adhesion molecule, EpCAM) 的表达, 表明FGF-4转导的BMSCs可能通过移植的微环境促进肝脏再生[109]。

5　新型载药系统: MSCs及其外泌体

肝纤维化不加以控制很有可能发展为肝癌, MSCs可以作为一种载药系统治疗肝癌和其他癌症[110]。癌症化疗的主要目的是将药物递送至肿瘤微环境, 在杀死癌细胞的同时毒性最低。BMSCs能够融入肿瘤组织中, 过表达TNF相关凋亡诱导配体 (TNF-related apoptosis-inducing ligand, TRAIL) 的MSCs能够将产生的TRAIL递送至肿瘤组织中, 显著降低肿瘤生长[111]。有研究发现, 在含紫杉醇的培养基中, BMSCs与紫杉醇相互整合且以时间依赖的方式缓慢释放, 使得BMSCs获得有效的抗肿瘤活性, 这种活性呈剂量依赖性, 能够减缓肿瘤的发生生长[112]。但是, 载药MSCs在递送的过程中会对非靶向组织产生毒性, 导致其受损[113], 因此, 药物浓度、载药量、细胞数量等有待进一步研究。由于MSCs载药能力有限, 研究人员将研究热点转移至MSC衍生的外泌体 (MSC-exosomes, MSC-Exos)。MSC-Exos具有与MSCs相似的生物学功能, 但具有体积更小、可以穿透生物膜、免疫原性低等特点[114]。在治疗方面, MSC-Exos能够抑制细胞凋亡、促进细胞再生、血管生成和组织修复、调节免疫和炎症反应[115]。与MSCs相比, MSC-Exos具有其他独特的优势: ① 外泌体易于收集, 多种MSCs都能分泌外泌体且分泌量大; ② 外泌体体积小, 可以长期稳定储存; ③ 外泌体不会增殖, 在临床上不会致瘤, 安全性高于MSCs。葡萄糖调节蛋白/BiP (glucose regulated protein/BiP, GRP78) 在抗癌药物索拉非尼耐药的癌细胞中过表达, 在一项研究中, siGRP78修饰的MSC-Exos与索拉非尼结合, 靶向癌细胞中的GRP78并在体内体外抑制癌细胞的生长、侵袭和转移, 逆转癌细胞对索拉非尼的耐药性[116]。目前, MSCs作为载体实现对肿瘤靶向治疗具有可行性, 但由于MSCs的局限性, 外泌体作为药物或基因载体更具有发展前景。总的来说, MSCs及其外泌体作为一种载药系统在临床治疗肿瘤方面具有很大的应用前景, 但需要大量的研究来阐述其治疗机制, 发挥治疗潜力。

6　讨论与展望

肝纤维化作为由慢性肝病发展为肝癌的中间过程, 其可逆的特性成为抗肝纤维化、治疗肝脏疾病的研究热点。但目前临床并没有十分有效的手段来治疗肝纤维化。BMSCs自身具备分化为肝细胞潜能、免疫调节特性、分泌多种细胞因子以及抗氧化能力, 可能成为改善、治疗肝纤维化最有前途的细胞之一。除单独使用BMSCs治疗外, 也可以与各种活性因子、中药或中药单体以及基因修饰相结合使治疗更加有效。尽管已有不少体外和动物体内实验证实BMSCs能够分化为肝细胞, 并在一定程度上改善肝纤维化, 达到治疗的目的, 但是临床上对于BMSCs治疗肝纤维化的研究报道甚少。BMSCs疗法在应用于临床治疗肝纤维化之前, 需要解决以下问题来提高改善效果: ① 改进移植方式。目前在动物实验中, 多采用门静脉注射、肝动脉注射、外周静脉注射、腹腔注射以及局部注射的方式移植BMSCs, 临床上移植方式包括门静脉注射、肝动脉注射和外周静脉注射。不同的移植方式对BMSCs的存活率和归巢率有着很大影响, 需要确定最佳的植入方式; ② 提高归巢效率。移植的时间和数量影响MSCs的归巢效率, 早期细胞的归巢效率要好于经过培养的细胞, 原代MSC的归巢率可以达到55%～65%, 但培养24 h后归巢率仅有10%[117]。由于原代MSCs数量少, 需要经过体外培养扩增, 以增加细胞数量, 但会使其归巢能力降低, 故需要寻找合适的辅助方法在获取大量干细胞的同时提高其归巢效率; ③ 明确其治疗肝纤维化机制。肝纤维化是多种内外因素共同作用下的疾病, 多种信号通路与肝纤维化相关; BMSCs能够分泌多种活性因子以完成增殖、分化、迁移等生理过程。明确分泌的活性因子与致肝纤维化信号通路之间的关

联, 有助于提高治疗效果; ④ 降低致瘤性。由于MSCs能够产生广谱的细胞因子、趋化因子和生长因子, 因此被认为是可能促进肿瘤生长的关键细胞[118,119]。肿瘤衍生囊泡将蛋白质和核酸递送至MSCs, 并与MSCs表面受体相互作用, MSCs摄取蛋白质和核酸, 使自身分泌肿瘤生长所必需的因子。MSCs外泌体携带和传递mRNA、miRNA和蛋白质, 一方面直接作用于肿瘤细胞, 促进其生长, 另一方面改变肿瘤微环境中的非肿瘤细胞表型和功能特征, 增强其促肿瘤能力[120]。在治疗方面可考虑通过分子或基因调节沉默肿瘤微环境中由MSCs介导的促肿瘤效应来降低致瘤性。此外, 目前BMSCs改善肝纤维化、肝硬化的临床试验研究面临受试患者少、受试区域小的问题、临床试验个体差异大、缺乏可比性以及可重复性差等重难点, 未来的临床研究需要加入大量的对照实验、双盲实验以及扩大受试范围, 不断探究BMSCs临床改善肝纤维化、肝硬化的潜力。综上, BMSCs改善肝纤维化作为一种新型细胞疗法, 展现出巨大的治疗潜力, 如能顺利解决上述问题, 其应用于临床必将发挥出前所未有的治疗能力, 让数以百万的肝病患者受益。

作者贡献: 丁翼、罗晓敏、杨斌、张博宇、顾健和袁志翔共同进行文献收集与阅读; 丁翼负责撰写; 顾健和袁志翔负责思路设计和文稿校审。

利益冲突: 所有作者均认可文章无相关利益冲突。

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