• Review

Advances in mesenchymal stem cells combined with traditional Chinese medicine therapy for liver fibrosis

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ABSTRACT: Liver fibrosis is a primary cause of liver cirrhosis, and even hepatocarcinoma. Recently, the usage of mesenchymal stem cells (MSCs) has been investigated to improve liver fibrosis. It has been reported that the differentiation, proliferation and migration of MSCs can be regulated by traditional Chinese medicine treatment; however, the mechanisms are still unclear. In this article, the authors review the characteristics of MSCs such as multidirectional differentiation and homing, and its application in animal experiments and clinical trials. The authors also list areas that need further investigation, and look at the future prospects of clinical application of MSCs.

KEYWORDS: mesenchymal stem cells; liver fibrosis; liver cirrhosis; medicine, Chinese traditional; therapy; reviews

1 Introduction

Liver fibrosis can result from chronic liver injuries of any etiology, and presents with extensive deposition of extracellular matrix proteins[1]. Chronic liver diseases can lead to severe hepatic dysfunctions and even life-threatening conditions such as liver cirrhosis and hepatocarcinoma[2]. Globally, 1.4 million deaths occur annually as a result of chronic liver diseases[3]. Given the above, effective medical treatment for liver fibrosis is much needed.

Currently, orthotopic liver transplantation is the only definitive therapeutic option for terminal liver diseases. However, its clinical application is limited due to poor long-term graft survival, shortage of donor organs, and high costs associated with the procedure[4]. Western medicine also lacks targeted drugs that can reverse liver fibrosis and repair injured livers in any meaningful way[5]. Therefore, other treatment options that focus on improving liver function, alleviating symptoms, and supporting nutritional status would be clinically useful alternatives[6].

Following the development of tissue engineering and cell therapy, mesenchymal stem cells (MSCs) are emerging as a research hotspot for their therapeutic potential in a variety of diseases. MSCs are derived from the mesoderm, exist in connective tissue and interstitial organs, and are especially rich in bone marrow. In this review, bone-derived MSCs (BMSCs) are discussed. MSCs are capable of self-replication and multidirectional differentiation[7]. Recent technological advances include convenient tissue sampling, minimal trauma, little or no immune rejection, high transfection rate, and stable exogenous gene expression. Procedures involving MSCs are being given ethical and regulatory approval[8]. MSCs make it possible to supply oneself with the needed tissue and are easy to be separated; they can be genetically modified without the potential risk of spreading diseases[9]. All these properties make MSCs an attractive new approach for treating diseases, including liver fibrosis.

Previous research has reported that MSCs can be transplanted...
into an injured liver to rebuild liver structure and improve liver function, which is a great advancement in the field of treating liver fibrosis\(^{[10]}\). This review will briefly introduce the significance and reality of using MSCs for liver fibrosis, as well as a summary of the potential role and application of traditional Chinese medicine (TCM) in combining with MSCs.

### 2 Characteristics of MSCs

MSCs are non-hematopoietic stem cells, which can be separated and identified both in vivo and in vitro, and have the potential to renew, proliferate, and differentiate multidirectionally. There are some ways to obtain relatively pure MSCs, such as separation by density gradient centrifugation, adherent cell separation, separation by flow cytometry, and immune magnetic-bead separation. Among them, density gradient centrifugation and adherent cell separation are the most widely applied methods because they are simple procedures that have little impact on activation of MSCs\(^{[11]}\).

It is generally believed that MSCs cultured in vitro are easy to grow sticking on wall and arrange in a radial of spindle with a big ratio in plasma. MSCs have a great expansion ability. For one single cycle, one cell can proliferate up to 15-70 cells. Furthermore, even after many generations of culturing, MSCs still exhibit their normal karyotype and telomerase activity\(^{[12]}\).

Under different culturing conditions, MSCs can be induced to differentiate into mesoderm cells such as osteoblasts, fat cells, skeletal muscle cells and ectoderm cells, as well as neurons, endothelial cells, and cardiomyocytes. The extensive plasticity and strong potential of differentiation make MSCs good candidates for tissue repair\(^{[13]}\).

### 3 Differentiation of MSCs

The chief characteristic of MSCs is multidirectional differentiation, meaning they can be induced to differentiate into a variety of cells under certain conditions\(^{[14]}\). To understand the possible mechanisms involved, Perterson et al\(^{[15]}\) conducted a study on differentiation of MSCs in 1999 and showed that MSCs originate from bone marrow. From that point on, many studies have been conducted to explore the underlying mechanisms of MSCs in differentiating into various cell types, including hepatocytes. Current research projects are mainly focusing on the mechanism of horizontal differentiation, cell fusion and related cytokine research\(^{[14]}\). The differentiation of MSCs has been seen in two main routes: (1) directly into hepatocytes; (2) induced by cytokines such as hepatocyte growth factor (HGF) that is secreted by MSCs and injured liver tissue\(^{[16]}\).

#### 3.1 Differentiation of MSCs in vitro

The mechanism of MSC differentiation is still not clearly understood. Most researchers consider microenvironment to be a main factor. One study showed that when MSCs were cultured with hepatocytes in a co-culture system and a direct cell-cell contact culture system, they could be induced to turn into hepatocytes by a local microenvironment that was formed by hepatocytes, and this was verified by testing the expression of albumin\(^{[17]}\). Another study cultured BMSCs in a man-made microenvironment consisting of HGF, fibroblast growth factor 4 (FGF4), and basement membrane matrix. The BMSCs differentiated into hepatocyte-like cells, which expressed surface marker α-fetoprotein and albumin; results were verified by the characteristic uptake and shedding of indocyanine green\(^{[18]}\).

The correlation between MSCs and a variety of cytokines in the microenvironment has also been investigated\(^{[19]}\). MSCs differentiating into hepatocytes appear to need some cytokines such as FGF4\(^{[20]}\), epidermal growth factor (EGF)\(^{[21]}\) and oncostatin M (OSM)\(^{[22]}\) under the induction of HGF. With the presence of HGF, MSCs were induced into hepatocytes successfully\(^{[23]}\). Without HGF, the combination of acidic fibroblast growth factor (AFGF) and OSM could not induce the differentiation of BMSCs into hepatocytes. It was suggested that HGF may play an important role in the process of MSC differentiation.

#### 3.2 Differentiation of MSCs in vivo

Cultured with HGF, MSCs can be induced to differentiate into hepatocytes in vitro\(^{[24]}\). Different animal models have been used to further study MSC differentiation into hepatocytes in vivo experiments.

The allotype BMSCs transplanted by punctiform injecting can differentiate to albumin-positive hepatocytes in mice with allyl alcohol-induced liver damage\(^{[25]}\). The differentiation of MSCs into hepatocytes also has been found in the model of partially hepatectomized female mouse\(^{[26]}\).

The experiments that showed MSCs have the ability to improve liver function also indicated that MSCs can differentiate into hepatocytes. A more detailed description of those processes is in Part 5.2.1.

### 4 Homing of MSCs

MSCs have an affinity for sites of tissue damage\(^{[27]}\). Fibrotic microenvironment produced by hepatic stellate cells (HSCs)\(^{[28]}\) efficiently induces the migration of MSCs. Additionally, the number of MSCs migrating to the liver is related to the extent of liver injury\(^{[29]}\).

In cellular activity, genes play an important role. Some studies on inducing pluripotent stem cells have also examined the genes regulating the process of MSC therapy\(^{[30]}\). Previous research has indicated that the forkhead box A2 gene, a liver transcription factor that regulates the development of liver organogenesis, efficiently promotes the incorporation of MSCs into the liver\(^{[31]}\). BMSCs have also been shown...
to express a variety of integrins, such as β1 and α4, which mediate the interaction of cell-to-cell and cell-to-extracellular matrix through the adhesion of vascular cell adhesion molecule-1 (VCAM-1) and fibronectin. In addition, cytokines such as sphingosine 1-phosphate receptor type 3 and stromal-derived factor-1 are also involved in the induction of BMSC migration.

5 Applications of MSCs for liver fibrosis therapy

Effective transplantation of MSCs into injured livers to rebuild liver structure and improve liver function is significant for liver fibrosis therapy. The following sections summarize the application of MSCs for liver fibrosis therapy, which includes methods of transplantation, therapeutic effect evaluation, and mechanism of cell therapy.

5.1 Transplantation of MSCs

To explore the effects of MSCs on liver fibrosis, the transplantation of MSCs is the first step in animal experiments. The experiments are mainly conducted through the portal vein, femoral artery, spleen, abdominal cavity, and tail vein. With magnetic resonance imaging, MSCs infused into liver tissues through the mesenteric vein can be detected to be active in the fibrotic liver of rats 12 d after injection. However, in human subjects, MSCs are mainly infused through veins. A study on the distribution of MSCs after infusion was conducted in 4 patients. Results of the planar whole-body acquisitions (anterior and posterior projections) acquired after cell infusion showed that the infusion of MSCs through a peripheral vein is safe in patients with liver cirrhosis.

The technique of transplanting MSCs into animals has been studied extensively, but it still has low applicability in human due to the limited research and participants.

5.2 Cell therapy of MSCs

Research on MSC therapy has been conducted in both animal experiments and clinical trials. MSCs effectively improved the liver condition of animals with liver fibrosis, non-alcoholic steatohepatitis (NASH), as well as acute hepatic failure. Compared to animal experiments, the number of relevant clinical trials was limited. Among these reports, an overwhelming majority showed positive outcomes.

5.2.1 Animal experiments

To clarify the effects of MSCs on CCl4-induced liver fibrosis models, studies have shown that the hepatocyte-like cells differentiated from MSCs can engraft into fibrotic livers, localize in the collection tube and liver sinus regions, and substitute for partial liver function in rats. When cultured with HGF or pretreated with injured liver issue, BMSCs were shown to be much more effective in homing and hepatic differentiation abilities. The transplantation of human MSCs into CCl4-induced rats and small-for-size liver transplantation rats demonstrated their potential for repairing liver damage.

In other liver disease models, administration of MSCs prevented the onset of NASH in obese mice through the preclusion of the inflammatory process. MSCs also showed an effect on Schistosoma japonicum-induced liver injury model and fibrosis mice. Additionally, BMSCs were shown to ameliorate hepatic function through implantation of groin vein and intrasplenic transplantation in the acute hepatic failure model.

Some studies have investigated how to optimize the function and longevity of MSCs in liver fibrosis regions. Nitric oxide (NO) has been used to effectively augment MSCs’ ability to repair liver fibrosis. Piryaei et al[46] showed that the topographic properties of nanofibers enhance the differentiation of MSCs into hepatocyte-like cells and also maintain their function in long-term culture. However, not all research has given positive outcomes. In an experiment using the cirrhotic rat model, the infusion of human cord-blood-derived MSCs was shown to exhibit pro-fibrogenic potential instead of improving liver function and liver fibrosis.

5.2.2 Clinical trials

The potential application of MSCs in liver fibrosis, cirrhosis, as well as hepatocellular carcinoma (HCC) is proposed in view of available evidence from not only animal experiments and cell research, but also clinical trials.

In patients compromised by liver cirrhosis, the infusion of a higher number of MSCs may have beneficial effects. A clinical trial was conducted in 39 patients with end-stage liver diseases, who had infusion of autologous MSCs through hepatic artery. The results showed that all participants had symptom alleviation; 37 exhibited improved liver function; and no one experienced serious adverse reaction and complications. In Sweden, 8 patients with end-stage liver disease were treated with autologous MSCs, which were taken from the iliac crest. They all had improved liver function and clinical features at baseline and 1, 2, 4, 8, and 24 weeks after injection.

MSCs possess higher proliferative capacity, are less immunogenic and more resistant to cryopreservation and ischemic injury; these are properties that can enhance their engraftment within the recipient liver. The autologous BMSC transplantation in patients with cirrhosis has shown promising results, but some other studies have demonstrated that the therapeutic use of autologous MSCs from patients with chronic hepatitis B and liver cirrhosis may not be a good choice because they exhibit unbalanced cytokine production.

Considering that not all the animal experiments and clinical trials could demonstrate the effectiveness of MSCs without side effects, more experiments are needed.
to determine which type of liver diseases and which grade of liver lesions are indications of MSCs.

### 5.3 Mechanisms of MSC therapy

Previous research has reported extensively on the mechanism of MSC therapy. Collectively, MSCs have been shown to have anti-inflammatory and anti-fibrotic effects, which are related to healing the injured or fibrotic tissue through several manners as follows: (1) besides directly differentiating into hepatic cells to replace the injured tissue \[55\], MSCs can improve insulin resistance \[56\]; (2) mediating the activation and apoptosis of HSCs partially by Notch pathway activation \[57\] and a restricted repertoire of cytokines secreted by paracrine mechanism \[58\]; (3) stimulating interleukin-10 (IL-10) release, which can modulate the host immune response and homeostasis between tissue inhibitor of metalloproteinase and matrix metalloproteinase (MMP) \[10,59\]; (4) decreasing levels of inflammatory cytokines, the concentration of transforming growth factor \[60\] and minimizing collagen deposition by MMP manner \[61\]; (5) stimulating the regeneration of endogenous parenchymal cells \[16\], and enhancing fibrous matrix degradation \[62\].

### 6 Research on MSCs with TCM

TCM has a long history in the treatment of liver diseases. Recently TCM therapy has been reported to improve liver fibrosis \[63\]. Research on TCM and MSCs has increased in the last decade (Figure 1A), including the effects of TCM on liver fibrosis therapy via regulation of MSCs. According to present studies, the effects of TCM on MSCs mainly include inducing differentiation, promoting proliferation, homing of MSCs and treatment collaboration (Figure 1B).

#### 6.1 Inducing differentiation

To induce differentiation in MSCs, the effects of TCM including herbs and their isolated components, herbal formulas, and acupuncture have been investigated. It has been reported that MSCs can be induced to differentiate into cardiomyocyte-like cells by *Astragalus* root, *Astragalus* glycosides, salvianolic acid B \[64\], *Epimedium* glycoside, *Rehmannia* glutinosa oligosaccharide, and ligustrazine \[65\], into nerve-like cells by *Astragalus* injection through Wnt signaling pathways \[66\]; into endothelial-like cells by total saponins of *Panax notoginseng* \[67\]; and into osteoblasts, probably through Notch signaling pathway, by oleanolic acid \[68\], cistanche \[69,70\], naringin \[71\], catechin \[72\] and *Fructus ligustri* \[73\].

Furthermore, it has been reported that the flavonoids of *Herba Epimedii* are able to induce osteogenic differentiation of MSCs through bone morphogenetic protein and Wnt/β-catenin signaling pathway \[74\]. *Rehmannia* glutinosa oligosaccharides increase the viability and proliferative capacity of MSCs \[75\], naringin enhances the proliferation of MSCs \[71\]; and catechin enhances protein phosphatase activity in MSCs \[72\].

The differentiation of MSCs can also be induced by needle stimulation. It has been reported that low-frequency electro-acupuncture can promote the differentiation of MSCs into chondrocytes, which was a novel non-drug-inducing method for the differentiation of MSCs \[76\].

#### 6.2 Proliferation and homing promotion

TCM has been shown to influence MSCs in a few other ways. It has been reported that tanshinone IIA and astragaloiside IV regulate MSC mobilization and migration, partially via modulation of CXC chemokine receptor 4 (CXCR4) expression, which is regulated by Notch signaling \[77\]. *Plastrum testudinis* and isopsoralen have been shown to promote proliferation in rat MSCs. Ginseng polysaccharide \[78,79\] and ginsenoside have an enhancing effect on mRNA expression of HGFs in rat MSCs \[80\], which in turn indicates they promote

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**Figure 1** Literature summary of TCM on MSCs research

A: Trend of TCM research on MSCs. B: Main aspects of TCM research on MSCs. The publications on MSCs study in PubMed databases from 2004 to November, 2013. All results were screened manually with the MeSH terms “marrow mesenchymal stem cells” combined with “traditional Chinese medicine”. MSCs: marrow mesenchymal stem cells; TCM: traditional Chinese medicine.
the activation of MSCs.

Components of some Chinese herbal formulas, namely those of Siwu Decoction\cite{81} and Buzhong Yiqi Decoction\cite{82}, have also been shown to stimulate MSCs proliferation.

6.3 Collaboration treatment

Studies examining TCM and MSCs indicate their combination has promising future applications. Most studies show that transplantation of MSCs alone is able to promote partial recovery of liver function and suppress liver inflammation, but has little effect in reducing the fibrotic area. Baicalin can promote differentiation of BMSCs into hepatocytes in vitro, so the combination may serve as an effective therapeutic regimen for severe liver diseases\cite{83}. Salidroside can promote differentiation of rat MSCs towards hepatocytes\cite{84}. In the process, e-cadherin and β-catenin are involved in hepatic differentiation of rat MSCs. Synergistic effects of MSCs and salidroside on experimental hepatic fibrosis have been exerted via suppressing the expression of transforming growth factor-β1.

Looking beyond the field of liver diseases, it has been also reported that Buyang Huanwu Decoction combined with MSC transplantation repairs injured blood vessels and tissue lesions, possibly by up-regulation of vascular EGF and Ki-67 expression\cite{85}. In addition, a combined therapeutic strategy of governor vessel electro-acupuncture and adult stem cell transplantation was shown to be an improved method in the treatment of spinal cord injury\cite{86}.

7 Problems and outlook

In this review, we summarized the characteristics of MSCs and the current reality of cell therapy using MSCs alone or in combination with TCM treatment for liver fibrosis. The main areas of research include the differentiation, proliferation, and homing of MSCs, and collaboration treatment with some cytokines or Chinese herbal formulas and their components. Cell therapy research with MSCs is shown in Figure 2.

The studies on MSC application are necessary for safety reasons as well as clinical and regulatory requirements. Various experiments have shown that transplantation of MSCs is not difficult, but the specific techniques of each step and infusion conditions should always be taken into consideration. The application of MSCs in human patients still needs further research in order to be applied safely and effectively, as the incorrect infusion of MSCs may cause injury or have serious side effects in both animal and human subjects.

In animal experiments, one area in particular needs further investigation, which is how to acquire a large number of very pure MSCs for therapy. This requires improved isolation, amplification and purification techniques, because MSCs are of limited quantity and are naturally present as a mixture with other cells. Other areas of further study include how to determine the indications of MSC usage; how to control the differentiation of MSCs after infusion without causing cancer; and how to mediate the patients’ immune systems to tolerate the treatments.

In preclinical settings, the main animal models are informative and easy to use; however, their results are limited in human body\cite{87}. Different experiments used MSCs from humans or animals, which have different physiological characteristics. Whatever the model is, MSCs seem to be initially trapped in the lungs; after this lung embolization, a low number of MSCs are in recirculation, and secondary homing occurs at the liver, spleen, and inflammatory or injured sites\cite{88}. This information is indicative that the treatments need to take into consideration the recirculation process of MSCs in vivo.

According to recent research, the differentiation of MSCs is related to their microenvironment, and HGF plays an important role in inducing MSCs to differentiate specifically into hepatocytes. After infusion into animals or humans via the portal vein and other routes, MSCs play a role in anti-fibrosis while under the regulation of a variety of cytokines. However, the particular mechanisms of how MSCs engraft into injured liver tissue, differentiate into hepatocytes, and improve overall liver function are still unclear.

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Figure 2 Main mechanisms of cell therapy with MSCs

MSCs are induced to differentiate into hepatocytes under the mediation of HGF and other cytokines. Then, MSCs migrate to sites of injured liver tissue and repair liver fibrosis through several different pathways. MSCs: mesenchymal stem cells; HGF: hepatocyte growth factor; FGF4: fibroblast growth factor 4; EGF: epidermal growth factor; OSM: oncostatin M; AFGF: acidic fibroblast growth factor; VCAM-1: vascular cell adhesion molecule-1; MMP: matrix metalloproteinase; HSCs: hepatic stellate cells.
Research regarding the application of TCM on MSCs already exists, even though only a few are specific for liver fibrosis therapy. In this review, we gave a general introduction of multiple effects of MSCs by TCM treatment, such as promoting differentiation, proliferation, and homing of MSCs. Hopefully, future studies will look into liver fibrosis therapy, frequency and route of administration of treatment, and the supplemental role of TCM that improves the effects of cell therapy.

MSCs are an exciting new field that brings the potential to cure various diseases, including liver fibrosis and cirrhosis. Even though there are many questions that need further study, further understanding the particulars of cell therapy with MSCs, especially combined with TCM treatment for liver fibrosis, may be of great benefit for liver fibrosis therapy in the future.

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9 Conflicts of interests

The authors declare that they have no competing interests.

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