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• 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

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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 multi-directionally. 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 widly 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 *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