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Regenerative Medicine

Review

Treating end-stage liver diseases with mesenchymal stem cells: an oak is not felled at one stroke

Z Li[1], C He[1], J Xiao[1,2,*], ZY Chen[1,*]

Abstract

Introduction

Currently, orthotropic liver transplantation is the only effective therapy for end-stage liver diseases, including acute liver failure, cirrhosis and liver cancer. However, the shortage of donor liver severely limits its application. The advent of the technology of turning mesenchymal stem cells from adult tissues into liver cells has opened the possibility of obtaining transplantable hepatocytes without donor livers and raised much interest in the field of hepatology. In this review, we summarized recent advances in using mesenchymal stem cells as a therapeutic strategy for treating liver diseases.

Conclusion

For the acute liver failure, many studies have demonstrated promising results. However, for the treatment of cirrhosis and liver cancer, the results do vary. Therefore, further studies are warranted before considering mesenchymal stem cells for active use in clinical applications.

Introduction

The discovery of stem cells and their disease-treatment potential have provided hope for many patients with 'incurable disease'. Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), both pluripotent cells and capable of maturing into all types of human cells, promise great potential in the treatment of human diseases. However, there have always been ethical and tumorigenic issues associated with their use. Recently, therapeutic applications of mesenchymal stem cells (MSCs) has become a hot topic in translational medicine with the emergence of technology to convert them directly into a variety of cell types without a pluripotent stem cell in the intermediate stage. Compared to ESCs and iPSCs, MSCs have the beauty of simplicity and may be more suited to clinical application. Some specific advantages of MSCs, also referred to as stromal stem cells or adult stem cells or somatic stem cells, include the following: (a) both autologous and allogeneic MSCs can be obtained conveniently. These cells are heterogeneous populations residing in almost all tissues, including bone marrow, umbilical cord, placenta, adipose tissue, peripheral blood, muscle and so on. (b) They are multipotent cells and have the capacity of directly maturing into many cell types, including hepatocytes, osteocytes, chondrocytes, adipocytes, myocytes and nerve cells[1]. (c) They are highly proliferative and self-renewable so that can be easily expanded *in vitro*. (d) There are ethical issues associated with their use unlike the case of embryo-derived ESCs. (e) These differ from iPSCs, the generation of which requires intensive manipulation *in vitro*, a procedure inherent with risks resulting in harmful genetic and epigenetic alterations. MSCs are much safer and cheaper because they can be induced to become specific cell types without much treatment before application and, in specific circumstances, even untreated MSCs can mature to compensate for lost cells at the damaged sites of tissues[2]. (f) Derived from the patient's own body, autologous MSCs can avoid the graft-versus-host diseases, a major concern in ESCs and iPSCs therapy. Even in allogeneic MSCs settings, the immune incompatibility may not be a serious problem. It has been shown that MSCs are low in expressing major histocompatibility complex (MHC)-I, the molecule presenting the cellular antigen to mediate the immune rejection reaction of allogeneic transplants. Furthermore, the expression levels of co-stimulatory molecules of T-cell-mediated graft rejection—the CD40, CD80 and CD86—are extremely low on the surface of MSCs. Consequently, the immunogenicity of MSCs is quite low, making them easier to evade the host immune surveillance[3].

Liver is one of the most important organs in the human body. It performs a series of vital physiological functions, including lipid storage, digestion, detoxification and innate immunity regulation. Currently, the only effective clinical therapy for end-stage liver diseases (e.g. acute liver failure, cirrhosis and liver cancer) is orthotropic liver transplantation. However, several defects, such as surgical complexity, immunological rejection, low survival rate and particularly the shortage of donor liver, preclude it from active use in clinics[4]. MSCs provide a new and convenient solution. They have been used in treating diseases in cardiovascular systems, liver, bone, cartilage and skin in a series of preclinical and clinical studies[5]. In the current review, we mainly focus on the recent progress in the use of MSCs to treat end-stage liver diseases along with discussion in mechanisms, problems and possible solutions.

*Corresponding author
Email: tjxiao@jnu.edu.cn; zy.chen1@siat.ac.cn

[1]Centre for Gene and Cell Engineering, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China
[2]Department of Immunobiology, Jinan University, Guangzhou 510632, China

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Discussion

Use of MSCs in treating acute hepatic failure

Liver failure is a common and life-threatening disease, usually caused by virus infection or toxic chemical ingestion. Acute liver failure is characterized by the death of a large number of parenchymal hepatocytes that occurs in a short period of time and rapid disease progression. The rate of mortality due to acute liver failure is very high, ranging from 60% to 80%[6]. Multiple pre-clinical studies have shown that MSCs have a clear therapeutic effect in acute liver failure. Using a carbon tetrachloride (CCl_4)-induced lethal fulminant hepatic failure mice model, for example, it was repeatedly shown that transplantation of MSCs could improve hepatic histology, promote liver regeneration, and significantly increase survival rate[7]. MSCs have the tendency to accumulate in the damaged area. In a CCl_4-induced mouse acute liver injury model, Cho *et al.* found that almost all of the delivered MSCs were recruited to the damaged areas of the liver with few seen in other organs, suggesting that host cells in the damaged areas were able to produce the recruiting signals[8]. Hong *et al.* observed a similar phenomenon[9]. MSCs may play a therapeutic role via two mechanisms: (a) to differentiate directly into parenchymal hepatocytes to compensate for the loss of their counterparts, and (b) to secrete protective and nourished factors that prevent progressive apoptosis of functional cells and stimulate replication of host cells[9]. The direct conversion of MSCs into hepatocyte-like cells has been repeatedly demonstrated, but there is evidence suggesting that this does not account for all the therapeutic effects of MSCs. Kuo *et al*., for example, showed that the quantity of the combination of both engrafted MSCs and MSC-derived hepatocytes accounted for only about 1% to 3% of total liver cells in the damaged areas, suggesting that MSCs might also play therapeutic roles through alternative mechanisms, for example, generating cellular factors that are capable of speeding up the liver recovery[10].

MSCs therapy in liver cirrhosis

Chronic liver injury often causes liver fibrosis, cirrhosis and cancer. Liver cirrhosis has been considered an irreversible disease since it is nearly impossible to reverse the fibrotic changes with conventional treatments[11]. In recent times, research has witnessed a marked change in this perspective. There are evidences suggesting that the transplantation of stem cell is able to reduce the fibrotic index, a consequence of dissolving the existing fibrosis, and improve liver function. In a clinical study, for example, Peterson *et al.* demonstrated that bone-marrow-derived MSCs could migrate into the cirrhotic liver and differentiate into hepatocytes[12]. In another clinical study, Terai *et al.* reported that 24 weeks after receiving therapy of bone-marrow-derived MSCs a group of nine patients with liver cirrhosis demonstrated significant improvement in serum albumin production and cirrhosis severity, as measured by child score[13].

Currently, the mechanism of MSC-mediated improvement in liver function and cirrhosis severity is not fully understood. The following mechanisms are proposed: (a) MSCs differentiate into parenchymal hepatocytes to improve liver function. It is estimated that approximately 2-3 × 10^{10} healthy parenchymal hepatocytes are needed to maintain normal function of an adult liver. In severe fibrotic or cirrhotic livers, the number of hepatocytes is significantly reduced. Trans-differentiation of donor MSCs to become parenchymal hepatocytes has been repeatedly demonstrated[2,14,15]. In an attempt to reveal the underlying mechanism, Prosser *et al.* demonstrated that MSCs were able to produce hepatocyte growth factor (HGF), which in turn could promote the MSCs to undergo the process of trans-differentiation into parenchymal hepatocyte[16]. (b) MSCs prevent the liver from undergoing fibrogenesis via secreting a variety of cytokines, such as HGF, interleukin (IL)-6 and -10 and so on. Hepatic stellate cells (HSCs) play a key role in hepatic fibrosis, for they are the major cells that produce various collagens and are major components of the intracellular matrix. These cellular factors may inhibit hepatic fibrosis through multiple mechanisms. It is suggested that, for example, in addition to its capacity to promote transition of MSCs into hepatocytes, HGF can suppress HSCs by blocking the phosphorylation of extracellular signal-regulated kinase (ERK) and inhibiting the proliferation of α-smooth muscle actin (α-SMA) positive cells[17]. HGF may also play a beneficial role in suppressing production of transforming growth factor (TGF)-β, the main player in the pathway activating the HSCs[16,17]. IL-6 and IL-10 may attenuate hepatic fibrosis through suppression of HSCs[18–20]. (c) MSCs may dissolve fibrosis directly. There was evidence suggesting that MSCs were able to produce the matrix metalloproteinase (MMPs), an enzyme capable of degrading the extracellular matrix, which alleviates hepatic cirrhosis directly[21].

However, there is still a long way to go before MSC therapy can achieve significant improvement in both function and histology of liver cirrhosis. As pointed out in a recent Hepatology review article, cirrhotic environment is not conducive for maturation of MSCs into hepatocytes and this, together with the presence of excessive fibre tissue, may become a serious obstacle that may extract a lot of effort from researchers involved and potentially thwart successful outcomes in stem cell therapy[22].

MSCs therapy in liver cancer

Liver cancer, the most frequent form of which is hepatocellular carcinoma (HCC), is the end-state of many chronic liver diseases. Till date, there

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are only a limited number of trials that used MSCs to treat HCC and the results are mixed. Some studies showed that MSCs could directly suppress tumour cell proliferation and reduce tumour growth. In contrast, others found that MSCs also have tumour-promoting properties.

There are evidences suggesting that MSCs have the tendency to accumulate in tumour tissues. In a melanoma model, Studeny *et al.* demonstrated that donor MSCs preferentially accumulated in tumour sites[23]. In another study, Beckermann *et al.* found that MSCs dominantly resided in the pancreatic tumour tissue[24]. MSCs may inhibit tumour growth through multiple mechanisms. It has been shown that MSCs could inhibit leukemic cell proliferation by secreting dickkopf-1, which acts as a negative regulator of the WNT signalling pathway to inhibit the proliferation of cancer cells[25]. In a Kaposi's sarcoma experiment, it was demonstrated that MSCs could inhibit the PI3K/Akt pathway, resulting in the suppression of tumour cell proliferation[26]. Results from a set of *in vitro* experiments demonstrated that MSCs could inhibit tumour cell proliferation and increase their apoptosis. Qiao *et al.* reported that, in the presence of MSCs, the expression of c-myc, Bcl-2 and proliferating cell nuclear antigens (PCNA) of hepatoma cells was downregulated, leading to a reduced tumour cell proliferation rate and increased apoptosis[27]. MSCs also induce production of cell-cycle negative-regulator protein p21 and apoptotic executioner enzyme caspase-3, which results, in tumour cells' arrest at the G0/G1 stage and their apoptosis subsequently[28]. In a rat HCC model, it was shown that administration of MSCs inhibited tumour growth, with improved liver histology and function[29].

In contrast to the beneficial effects, however, adverse effects of MSCs on multiple tumour models have also been reported. It has been found that in tumour tissues MSCs could differentiate into myofibroblasts-like cells, which have been proven to play a crucial role in tumour promotion through multiple mechanisms, including promotion of tumour stroma remodelling and tumour invasiveness[30]. It was demonstrated that when exposed to a tumour-conditioned medium over a long period MSCs gained a myofibroblastic phenotype as evidenced by an expression of carcinoma-associated markers, including stroma-derived factor-1 (SDF-1), α-SMA and fibroblast surface protein[31]. In addition to the myofibroblast pathway, MSCs may also promote tumour growth via immunosuppressive effects. In a sarcoma model, it was found that the host anti-tumour functions were suppressed by expanded clones of $CD4^+$ $CD25^+$ T-regulatory lymphocytes derived from the bone-marrow-derived donor MSCs[32]. In an engraft melanoma tumour model, it was found that B16 melanoma cells in allogeneic recipients grew only when MSCs were co-injected, which did not happen in the absence of co-injection. These effects were primarily attributed to the immunosuppressive effects of allogeneic T-regulatory lymphocytes derived from donor MSCs[33]. In a clinical study on liver cancer, it was found that application of MSCs enhanced tumour growth but significantly inhibited the invasiveness and metastasis of HCC cells, indicating the effects of MSCs in controlling the metastatic recurrence but not the growth of primary HCC[34].

Conclusion

The capability of MSCs from multiple sources to undergo trans-differentiation and maturation into hepatocyte-like cells has laid down the solid foundation for application of MSCs as an alternative to donor liver in order to meet the high demand in transplantable hepatocytes. Many clinical and preclinical studies have been conducted in the use of MSCs for treating end-stage liver diseases with some staged successes. Promising results were seen in treatment of acute liver failure and cirrhosis, although optimization in the selection of MSCs sources and culture conditions (e.g. method of administration and dosage) is needed. In the treatment of liver cancer, however, mixed results were seen. Upon application of MSCs, both inhibition and promotion of tumour growth and invasion and metastasis were seen. A possible solution may be the combination of MSCs and gene therapy. By exploiting the feature of their intendance to accumulate at the tumour site, it may be possible to use MSCs to express anti-tumour gene products inside tumours and cure them.

Abbreviations list

CCl_4, carbon tetrachloride; ERK, extracellular signal-regulated kinase; HCC, hepatocellular carcinoma; HEF, hepatocyte growth factor; HSCs, hepatic stellate cells; IL, interleukin; MHC, major histocompatibility complex; MSCs, mesenchymal stem cells; PCNA, proliferating cell nuclear antigen; SDF, stromal-derived factor; SMA, smooth muscle actin; TGF, transforming growth factor; TNF, tumour necrosis factor.

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