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-Title: Application of Modified Mesenchymal Stem Cells Transplantation in the Treatment of Liver Injury

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Application of Modified Mesenchymal Stem Cells Transplantation in the Treatment of Liver Injury

Summary

Acute and chronic hepatitis, cirrhosis, and other liver diseases pose a serious threat to human health; however, liver transplantation is the only reliable treatment for the terminal stage of liver diseases. Previous researchers have shown that mesenchymal stem cells (MSCs) are characterized by differentiation and paracrine effects, as well as anti-oxidative stress and immune regulation functions. When MSCs are transplanted into animals, they migrate to the injured liver tissue along with the circulation, to protect the liver and alleviate the injury through the paracrine, immune regulation and other characteristics, making mesenchymal stem cell transplantation a promising alternative therapy for liver diseases. Although the efficacy of MSCs transplantation has been confirmed in various animal models of liver injury, many researchers have also proposed various pretreatment methods to improve the efficacy of mesenchymal stem cell transplantation, but there is still lack a set of scientific methods system aimed at improving the efficacy of transplantation therapy in scientific research and clinical practice. In this review, we summarize the possible mechanisms of MSCs therapy and compare the existing methods of MSCs modification corresponding to the treatment mechanism, hoping to provide as a reference to help future researchers explore a safe and simple transplantation strategy.

Key words: Mesenchymal Stem Cells; Liver Diseases; Gene Expression Regulation; Regeneration; Immunologic Factors;

Introduction

The liver is the primary organ involved in metabolism, and as such, is the main target of toxic substances. Long-term repeated or short-term exposure to large doses of toxic substances can cause a variety of liver diseases, such as alcoholic hepatitis, drug-induced hepatitis, viral hepatitis, liver fibrosis, liver cirrhosis, that seriously affect the quality of life (Hu *et al.* 2020, Huang *et al.* 2016, Lu *et al.* 2020, Wan *et al.* 2020). Around the world, liver transplantation is still the most often used and effective treatment for acute liver failure and advanced liver diseases (Xu *et al.* 2017) . However, due to shortage of donors, the high cost, and the allografts rejection affecting the prognosis and quality of life of patients, this therapy is not widely available (Puglisi *et al.* 2011). MSCs are derived from various tissues such as bone marrow, umbilical cord, placenta, amniotic fluid, gums, and fat (Lou *et al.* 2017),

have the characteristics of self-renewal, proliferation and differentiation, and immune regulation. These cells have the potential to undergo multidirectional differentiation into osteocytes, chondrocytes, adipocytes, neuron-like cells and hepatocyte-like cells (Boyd *et al.* 2019, Kladnická *et al.* 2019, Miao *et al.* 2016). Therefore, stem cells transplantation can be used to repair damaged tissues through their ability of homing to the liver, where they undergo proliferation and differentiation, and secrete cytokines to regulate the liver humoral environment to reduce injury (Eom *et al.* 2015, Hu *et al.* 2019, Yan *et al.* 2009). Vascularisation promotion also serve as an explanation of MSCs function (Pytlík *et al.* 2017). In addition, MSCs can regulate immune responses and induce tolerance to antigens, with the ability to regulate the activities of various immune cells, such as inhibiting the proliferation and function of T cells, B cells and NK cells (Gazdic *et al.* 2017). The ability of MSCs to proliferate, differentiate and their effect on immune regulation highlights the potential of these cells for the treatment acute and chronic liver injury (Eom *et al.* 2015, Fathi *et al.* 2019, Gazdic *et al.* 2017).

It has been found that MSCs transplantation is a safe and highly feasible option for the treatment of liver diseases; however, further studies are required to clarify many aspects of the application of MSCs transplantation, such as the underlying mechanism of MSCs treatments and strategies to improve the therapeutic effect. In fact, different attempts have been made to improve the efficacy of MSCs in the treatment of liver diseases (Table 1). Understanding various methods to improve the efficacy of MSCs transplantation in liver diseases is of great significance for the further improvement and clinical application of MSCs therapy.

Underlying mechanisms of MSCs therapy

Trans-differentiation into liver tissue-like cells

MSCs are malleable and have the potential of trans-differentiation or de-differentiation in response to suitable conditions. Several previous studies have also verified the potential of bone marrow mesenchymal stem cells (BM-MSCs) to differentiate into bone tissue or dedifferentiate and reprogram (Chen *et al.* 2017, Rui *et al.* 2015). In addition, several studies have provided evidences that MSCs have the potential to differentiate into hepatocyte-like cells. In 2002, Schwartz et al. firstly discovered that pluripotent adult progenitor cells from humans, rats and mice could be in vitro induced to differentiate into hepatoid cells with functions such as albumin (ALB) secretion, cytochrome P450 production, low-density lipoprotein absorption

and glycogen storage (Schwartz *et al.* 2002). Later, Lee et al. discovered the potential of BM-MSCs to differentiate into hepatocyte-like cells in vitro (Lee *et al.* 2004). Thus, the differentiation potential of MSCs to various cell types was discovered (Chen *et al.* 2016, Xu *et al.* 2017, Yu *et al.* 2018). Recently, the efficacy of artificially induced stem cells transplantation has been confirmed, and the trans-differentiation ability of MSCs directly transplanted in animal models of acute liver failure, liver fibrosis, liver cirrhosis as well (Banas *et al.* 2009, Bruckner *et al.* 2017, Chen *et al.* 2016, El Baz *et al.* 2020, Sato *et al.* 2005, Zhou *et al.* 2017). From the results of these systematic studies, we speculate that MSCs can protect the liver through differentiation into hepatocytes. However, some studies have shown that only a small proportion of stem cells transplanted into animals have undergone trans-differentiation into hepatocytes, suggesting that differentiation of MSCs may not be the main mechanism of their therapy effects (Chen *et al.* 2017, Dai *et al.* 2009).

Anti-oxidant stress and tissue damage reduction

Drugs such as carbon tetrachloride (CCl_4), dimethylnitrosamine (DMN), and thioacetamide (TAA) are commonly used to construct animal models of liver injury in the laboratory (Cho *et al.* 2012, Moon *et al.* 2019). Ischemia-reperfusion injury is also a stable model (Jiao *et al.* 2020). Exposure of animals to these types of harmful factors stimulated the production a large amount of reactive oxygen species (ROS), which cannot be cleared rapidly, thus inducing oxidative stress. Such conditions lead to tissue necrosis induced acute liver failure, liver fibrosis, liver cirrhosis and other liver pathological changes (Cash *et al.* 2010, Jiao *et al.* 2020, Moon *et al.* 2019, Sabry *et al.* 2019, Zhang *et al.* 2020). However, studies have shown that transplantation of MSCs in animal models reduces liver cells injury by increasing superoxide dismutase (SOD) activity and reducing malondialdehyde (MDA) levels (Zheng *et al.* 2020). Another study by Wan et al. also showed that MSCs exert an antioxidant stress effect in a mouse model of alcoholic hepatitis, resulting in a significant decrease in MDA and a significant increase in glutathione (GSH) in the treatment group (Wan *et al.* 2020). In addition, Jiao et al. found that the MSCs derived from adipose tissue reduced the endoplasmic reticulum (ER) stress response in Bama miniature pigs and down-regulated the expression of ER stress-related proteins, thereby reducing the tissue injury in the liver (Jiao *et al.* 2020). These findings provide evidence that MSCs can reduce oxidative stress and reduce tissue damage.

Exosome-mediated cellular regulation

Cells can communicate with extracellular vesicles (EVs) through membrane fusion, and the substances contained in these vesicles usually have certain physiological characteristics. The most extensively studied type of vesicles to date is exosome with diameters of 30-100 nm (Gould and Raposo 2013, Raposo and Stoorvogel 2013). Exosomes bud from the endosome system (Cheng *et al.* 2020) and contain the non-protein coding miRNAs and DNA. When vesicles fuse with the target cell membrane, they may play a protective role in liver tissue by regulating gene expression (Han *et al.* 2020, Lelek and Zuba-Surma 2020, Valadi *et al.* 2007, Zhang *et al.* 2020). In animal models, this process has been shown to suppress immune cells activation and inflammatory factors releasing, promote angiogenesis, reduce liver fibrosis as well as alleviate liver injury (Anger *et al.* 2019, Fiore *et al.* 2020, Sabry *et al.* 2019, Yang *et al.* 2020). For example, Mardpour et al. investigated the role of exosomes from embryonic MSCs in a rat model of liver cirrhosis. They found that exosome transplantation significantly inhibited the proliferation of mononuclear cells in peripheral blood. Furthermore, the levels of the anti-inflammatory factors transforming growth factor-β (TGF-β) and Interleukin-10 (IL-10) were significantly increased, while Interferon-γ (IFN-γ)levels decreased, suggesting that exosome transplantation plays a role in inhibiting the inflammatory response. In addition, the changes in the expression of major apoptosis-related genes, caspase-3, B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein (BAX); and major fibrosis-related genes, such as α smooth muscle actin (α -SMA), tissue inhibitor of matrix metalloproteinases 1 (TIMP1), matrix metalloprotein 9 (MMP9), matrix metalloprotein 13 (MMP13). These results confirmed the anti-fibrosis and anti-apoptosis effects of exosomes (Mardpour *et al.* 2018).

Exosomes also contain bioactive substances such as proteins, hormones and cytokines, which can also be transferred between MSCs and target cells to exert regulatory effects (Akyurekli *et al.* 2015, Baglio *et al.* 2012).However, exosomes have some disadvantages that limit their clinical application prospects. For example, it has been reported that exosomes may mediate the spread and metastasis of cancer cells and viruses, which requires extra caution in clinical usage (Boelens *et al.* 2014).

Paracrine and immune regulation

MSCs secrete many types of soluble factors into the intercellular stroma, and have different effects on target cells. The two main types are nutritional factors, which

are involved in the repair and protection of liver tissue from damage, and inflammatory factors, which are involved in the initiation and development of inflammatory responses (Gazdic *et al.* 2017, Volarevic *et al.* 2014). However, paracrine regulation is often considered to be the main mechanism responsible for liver regeneration and protection from inflammatory responses. The nutritional factors secreted by MSCs play an important role in the liver regeneration. Compared with MSCs derived from other tissues, umbilical cord mesenchymal stem cells (UC-MSCs) have significant paracrine characteristics. In a study on the protein expression profile of UC-MSCs, Wang et al. identified 236 molecules, including cytokines, receptors, and transporters, that are involved in biological processes, such as proliferation, differentiation and apoptosis (Hu *et al.* 2013).

Numerous studies have shown that MSCs secrete a variety of cytokines with multiple functions that form a network (Bai *et al.* 2016). For example, acid fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and Placental growth factor (PLGF), promote angiogenesis (Amable *et al.* 2014, Liang *et al.* 2014, Samsonraj *et al.* 2015). Stromal cell-derived factor-1(SDF-1) and C-X-C chemokine receptor-1 (CXCR-1) mediate the migration and homing of MSCs to target tissues (Kim *et al.* 2011, Samsonraj *et al.* 2015). Growth factors and nerve growth factors such as hepatocyte growth factor (HGF), granulocyte Colony-Stimulating Factor (G-CSF), platelet derived growth factor-AA (PDGF-AA), TGF-β, and neurotrophin are involved in liver tissue repair (Amable *et al.* 2014, Banas *et al.* 2008, Hsieh *et al.* 2013, Miranda *et al.* 2015, Shen *et al.* 2015). In addition, bFGF, HGF, and VEGF showed anti-apoptotic and anti-fibrotic effects (Samsonraj *et al.* 2015). Thus, it is not difficult to see that the paracrine effect plays an important role in improving liver injury. In addition to nutritional factors that promote liver repair and regeneration, MSCs also secrete cytokines such as TNF-α and other inflammatory factors, which participate in immune regulation and reduce inflammation (Hu and Li 2019).

MSCs have been shown to reduce inflammatory cell infiltration in model animal liver tissues (Figure 1). In a mouse model of alcoholic hepatitis, Wan et al. found that BM-MSCs reduced the infiltration of neutrophils and macrophages into liver tissues, and decreased the activity of the neutrophil marker myeloperoxidase (MPO) (Wan *et*

al. 2020). Huang et al. obtained similar results in other animal models. In models of fulminant liver failure caused by thioacetamide and liver fibrosis caused by carbon tetrachloride, they found that MSCs and their secreted exosomes reduced the infiltration of hepatic cells. In addition, MSCs also induced the $CD4^+$ T cell system to transform towards an anti-inflammatory phenotype (Huang *et al.* 2016).

In addition to reducing macrophage infiltration, MSCs can also inhibit the activation of dendritic cells (DCs), helper T cells, natural killer cells, Kupffer cells and other immune cells by secreting soluble cytokines to avoid the activation of a large-scale inflammatory response when liver tissues are damaged (Hu *et al.* 2020). Zhang et al. co-cultured TNF-α-pretreated MSCs derived exosomes with the RAW264.7 monocytes cell line in vitro and then transplanted the cells into C57BL/6 male mice with acute liver failure. By inhibiting the activation of the NACHT, LRR, and NOD-like receptor pyrin domain-containing protein 3 (NLRP3) inflammasome, the exosomes alleviated the inflammatory damage induced by macrophages and promoted the repair of liver tissue(Zhang *et al.* 2020).

Prostaglandin E2 (PGE_2) derived from MSCs plays an important role in its immune regulation function. It has been reported that PGE_2 can reduce the serum levels of the pro-inflammatory cytokines TNF-α, Interleukin-12 (IL-12) and Interleukin-4 (IL-4), and promote the release of the anti-inflammatory cytokine IL-10, thus changing the polarization of helper T cells and differentiating into anti-inflammatory direction (Sharma *et al.* 2014). PGE_2 also increases the differentiation of primitive T cells into $CD4^+CD25^+Foxp3^+$ regulatory T cells, thereby reducing the total number of helper T cells (Volarevic *et al.* 2011). Studies have also shown that PGE_2 may change the microenvironment of liver tissue by stimulating the activation of macrophages into the anti-inflammatory M2 type (Kojima *et al.* 2019). PGE_2 secreted by MSCs can also affect other immune cells, for example, through expressing TNF receptor superfamily, member 6 (Fas) to recruit Monocyte chemoattractant protein-1(MCP-1), a monocyte chemokine, to recruit T cells, and then promote T cell apoptosis via Fas ligand (FasL) and inhibit T cell proliferation (Akiyama *et al.* 2012). Inhibition of both DC differentiation and the related cytokine secretion leads to T cell inactivation (Parekkadan *et al.* 2007, Volarevic *et al.* 2010, Volarevic *et al.* 2011).

Strategies to improve the efficacy of MSCs treatment

Promote homing

Directional migration to lesions after transplantation is one of mechanisms by which stem cells exert their protective effects on the liver. Therefore, strategies that enhance the recruitment of stem cells and promote their homing to the lesion are potential approaches to improving the efficacy of stem cell transplantation.

Shams et al. showed that HGF and fibro-blast growth factor 4 (FGF4) pretreatment promoted the homing efficiency of MSCs in a carbon tetrachloride mouse model of acute liver fibrosis (Shams *et al.* 2015). Liu et al. showed that c-Met overexpression in stem cells further reduced the level of serum transaminase, while HGF increased the expression of c-Met and phosphorylated Met, thereby improving the homing efficiency . This finding suggests that the HGF/c-Met signaling pathway plays an important role in promoting the directional migration of MSCs to the liver (Liu *et al.* 2014). This study further explored the effect of HGF on the homing of MSCs to the liver (Liu *et al.* 2014). In addition, it has been reported that glycyl tRNA synthetase is released when tissues or cells respond to damage signals, and may have the various beneficial effects, including enhanced the migration of MSCs through its receptor cadherin-6 (CDH-6) (Park *et al.* 2018).

In addition to the HGF/c-Met pathway, the chemokine C-X-C motif receptor4/ chemokine C-X-C motif ligand12 (CXCR4/ CXCL12) axis is also worthy of our attention in promoting stem cell homing. Stromal cell-derived factor-1, also known as CXCR12, is a cytokine that is believed to have a chemotactic effect on lymphocytes and MSCs (Hajinejad *et al.* 2018, Jin *et al.* 2018).Wang Jin et al. reported that SDF-1 level was significantly increased in a mouse liver ischemia-reperfusion model. Furthermore, the homing efficiency of MSCs over-expressed SDF-1 was improved under hypoxic pretreatment (Jin *et al.* 2018). It has also been reported that resveratrol enhanced the homing of BM-MSCs pretreated with SDF-1α in the rat liver cirrhosis model (Hajinejad *et al.* 2018).

In a study of a mouse liver ischemia-reperfusion model, Zheng and Li et al. found that rapamycin-induced autophagy enhanced the migration of UC-MSCs to hepatic ischemic areas by upregulating the expression of CXCR4 and CXCR12 in MSCs, while MSCs migration was reduced following inhibition of CXCR4 activation. This finding confirms that CXCR4 plays an important role in the homing process of MSCs from both positive and negative aspects (Zheng *et al.* 2019).

The migration of stem cells to the lesion is a prerequisite for exerting their various functions and therapeutic effects. However, after transplantation, most stem cells do not migrate in a directional manner, which restricts their efficacy (Rustad and Gurtner 2012). Therefore, these methods of improving the directional migration of MSCs is reliable ways to improve their therapeutic efficacy.

Promote stem cell-dependent liver regeneration

Stem cell-dependent liver regeneration is not only reflected in the proliferation of MSCs, but also in the MSCs differentiation to hepatic pluripotent stem cells (Kamel *et al.* 2018, Katselis *et al.* 2015). Therefore, strategies to promote the differentiation of MSCs into hepatocytes or enhance their ability to induce other stem cells to differentiate into hepatocytes is the key to promoting stem cell-dependent liver regeneration.

Yu et al. demonstrated that human umbilical cord blood-derived mesenchymal stem cells (HUCB-MSCs) have the potential to differentiate into hepatocyte-like cells in D-galactosamine/lipopolysaccharide (GalN/LPS) induced liver injury mice and can alleviate liver injury (Yu *et al.* 2012). Similar results were obtained in by transplantation of HUCB-MSCs in an animal model of liver injury induced by thioamide (KIM *et al.* 2011).

Yu et al. made a comparison of the hepatocyte differentiation ability between UC-MSCs and BM-MSCs. RT-qPCR and Western blot analysis showed that the expression levels of hepatocyte specific genes glucose-6phosphate(G-6P), ALB, cytochrome P450 3A4(CYP3A4), tryosine-aminotransferase(TAT), α 1 antitrypsin (α 1AT) in UC-MSCs differentiated cells were higher than BM-MSCs differentiated cells. However, the expression levels of alpha fetoprotein (AFP) were lower. These results suggest that UC-MSCs have better differentiation potential. In addition, in the same study, ELISA test showed that within 2-5 weeks, the secretion of ALB and blood urea nitrogen by UC-MSCs was significantly higher than that of BM-MSCs. These results also suggest that UC-MSCs have better differentiation potential (Yu *et al.* 2018).

HGF is the cytokine that mediates anti-apoptotic effects and promotes mitosis in liver cells, thereby playing an important role in the regeneration and reconstruction of the damaged liver. HGF over-expressed BM-MSCs protected and promoted the

proliferation of graft liver in small-for-size liver transplantation model (Yu *et al.* 2007). Seo et al. reported that UCB-MSCs over-expressing HGF were more effective in treating liver fibrosis in rats than untreated MSCs (Seo *et al.* 2014). In another study, Chen et al. found that microRNA-26a-5p(miR-26a-5p) inhibits HGF expression. Furthermore, they inhibited miR-26a-5p to promote HGF synthesis and improve the efficacy of BM-MSCs transplantation. This study echoes the observations of Seo et al. on the expression of HGF the transcriptional level (Chen *et al.* 2017). Previous studies have also shown that HGF plays an important role in the differentiation of MSCs into hepatocytes. Considering the role of HGF in inducing the differentiation of MSCs into hepatocytes and inhibiting hepatocyte apoptosis to promote hepatocyte proliferation, we speculate that treatments or modifications related to high expression of HGF before transplantation of MSCs may be an effective way to promote stem-cell-dependent liver regeneration, and further improve the curative effect on the basis of direct transplantation of MSCs.

Jung et al. found that chorionic-derived MSCs downregulate the methylation of the Interleukin-6/signal transduction and activator of transcription 3 (IL-6/STAT3) promoter region in liver tissue through a paracrine pathway, which promotes activation of IL-6/STAT3 signaling pathway and induces hepatocyte proliferation. However, the paracrine pathway by which MSCs mediates this effect has not yet been elucidated, which suggests that the efficacy of MSCs transplantation can be improved by promoting the paracrine process (Jung *et al.* 2015).

In summary, the potential of MSCs to differentiate into hepatocytes is dependent on various sources, and the properties of MSCs to promote hepatocyte maturation of other stem cells or de-differentiated cells (Lysy *et al.* 2007). MSCs can be induced to differentiate or modified to influence their paracrine regulation pathway or expression of key genes related to liver protection. In this way, the ability of MSCs to promote liver regeneration can be enhanced to improve the therapeutic effect.

Strengthen the immune regulation ability

The efficacy of MSC transplantation has been widely confirmed in various animal models. For example, we have previously shown that UC-MSCs can be reprogrammed via in vitro neuronal differentiation and de-differentiation reprogrammed human UC-MSCs, this modification offered therapeutic advantages in inflammatory bowel disease (IBD) treatment via PGE_2-dependent T lymphocyte

suppression. thereby improving the immunosuppressive ability and transplantation efficacy of MSCs (Yang *et al.* 2018).

Based on the unique immunomodulatory characteristics of MSCs, immune regulation has become the most likely potential mechanism by which MSCs alleviate liver injury and improve liver function. MSCs secrete a variety of cytokines that regulate cytotoxic T cells, helper T cells, natural killer cells and NKT cells, as well as the function of DCs and other immune effector cells, which in turn, regulate the damage local microenvironment, relieve acute liver failure and control the processes of liver fibrosis and cirrhosis (Fikry *et al.* 2016, Gazdic *et al.* 2018, Gazdic *et al.* 2018, Liang *et al.* 2018, Qu *et al.* 2015, Zhang *et al.* 2014).

DCs are professional antigen presenting cells, which can also affect neighboring lymphocytes through paracrine regulation. Therefore, DCs play a pivotal role in the immune response process. Zhang et al. reported that MSCs successfully recruited DC precursors to the liver, and activated their receptors E-prostanoid 2 (EP2) and E-prostanoid 4 (EP4) through paracrine regulation by PGE_2 to phosphorylate downstream phosphoinositide 3-kinase (PI3K) and extracellular signal-regulated kinase 1/2 (ERK1/2), which then induces the differentiation of precursor DCs into regulatory DCs through EP4 and the related PI3K and ERK1/2 signaling pathways (Zhang *et al.* 2014).

In addition, in this report, MSCs inhibited the activation and migration of $CD4^+$ T cells to the liver, resulting in decreased expression of $CD4^+$ cell-transport-related chemokines and their receptors, reduced the proportion of helper T cells, while promoted the differentiation of naïve T cells into $CD4^+CD25^+Foxp3^+$ regulatory T cells, which act as anti-inflammatory T cells against helper T cells to reduce the intensity of the regulatory immune response, the degree of inflammation and liver injury in the animal model (Zhang *et al.* 2014). Inflammatory responses can be reduced by promoting regulatory T cell differentiation and the TGF-β and IL-10 secretion, reducing the content of pro-inflammatory factors such as TNF-α and IFN-γ in the serum. These findings were in accordance with several other reports showing that MSCs inhibit the function of the different immune cells by activation cytokine secretion (cellular level), the levels of mRNAs (transcription), and concentration of cytokines in liver tissue and serum (translation) to reduce the inflammatory response and improve liver function in model animals (De Luna-Saldivar *et al.* 2019, Duman *et al.* 2019, Miao *et al.* 2016, Qu *et al.* 2015).

Alternatively, Gazdic et al. showed that MSCs decreased the activity of inducible nitric oxide synthase (iNOS) and indoleamine 2,3-dioxygenase (IDO) in NKT cells to decrease their cytotoxicity and improve liver injury (Gazdic *et al.* 2018). It is interesting to note that other researchers investigated cytotoxic T cells, Volarevic et al. reported that the loss of cytotoxic T cells by promoting their apoptosis, inhibiting their proliferation and decreasing the expression of CD80 and CD86 molecules enhanced the inhibition of T cell immune response by reducing the toxicity of cytotoxic T cells (Volarevic *et al.* 2010, Volarevic *et al.* 2011). In addition, MSCs can inhibit the polarization of macrophages into the M1-type, increase the M2-type differentiation, and inhibit the release of pro-inflammatory factors, thereby inhibiting the inflammatory response to reduce liver injury (Liang *et al.* 2018, Miao *et al.* 2016).

The immunomodulatory effect of MSCs is not only reflected in the inhibition of the inflammatory response and the reduction of damage, but also in the involvement of MSCs in slowing fibrosis and preventing cirrhosis through the secretion of interleukin-17 (IL-17), TGF-β and other factors. Farouk et al. reported that MSCs down-regulated IL-17A of the IL-6/STAT3 signaling pathway, thus playing an immunomodulatory role in preventing liver fibrosis (Farouk *et al.* 2018). TGF-β not only acts as an anti-inflammatory factor, its role in animal liver fibrosis is also recognized and supported by research findings (De Luna-Saldivar *et al.* 2019, Radwan and Mohamed 2018).

These studies demonstrated the important role of the immunomodulatory ability of MSCs in protecting the liver and improving liver function. Therefore, pretreatment or modification of MSCs to enhance their immunomodulatory ability represents an potential strategy to improve the efficacy of MSC transplantation in liver injury.

Gu et al. showed that the deubiquitination enzyme Ubiquitin C-terminal hydrolase 1(UCHL1) has a negative regulatory effect on the survival and secretion capacity of MSCs. High expression of UCHL1 increased TNF-α and IFN-γ while inhibited the secretion of iNOS and IDO. Inhibition of UCHL1 expression improved the therapeutic effect of MSCs in a Concanavalin A (ConA)-induced model of liver injury through activation of the nuclear factor kappa-B (NF-κB) signal transduction and activator of transcription 1(STAT1) pathways, enhanced the viability of MSCs via the Bcl-2 gene (Gu *et al.* 2018). In another study, black algae oil improved the efficacy of MSCs alleviate radiation-induced liver damage. The evaluation indexes of

ALT, MDA and SOD in the combined treatment group were all improved when compared with the effects of using black algae oil or MSCs alone. The combination group had the lowest proportion of cells producing TNF-α, INF-γ and IL-6, and the highest proportion of cells producing IL-10. The trends in the levels of TNF-α, INF-γ, IL-6, IL-10, TGF-β and other cytokines were consistent with the trends in the proportion of cells producing these factors. This finding confirmed that the treatment with the combination of black algae oil and MSCs improved the inflammatory regulation and liver protection capacities of MSCs (Radwan and Mohamed 2018).

In addition to pretreatment and combined transplantation therapy, MSCs can also be modified to induce over-expression or silencing of certain genes by genetic modification and gene editing. Wang et al. reported that transplantation of stem cells genetically modified to over-express interleukin-35 (IL-35) significantly reduced the expression of IFN-γ via the Janus kinase 1-signal transduction and activator of transcription 1/signal transduction and activator of transcription 4 (JAK1-STAT1/STAT4) signaling pathway, and strengthened the immune regulation ability of MSCs. This approach also reduced hepatocyte apoptosis by reducing the expression of FasL on monocytes, which improved the therapeutic efficacy (Wang *et al.* 2018). In another study, Ye et al. reported that compared with unmodified MSCs, MSCs over-expressing hepatocyte nuclear factor-4α (HNF-4α) increased the expression of iNOS via the signal transduction and activation of transcription NF-κB signaling pathway, thereby enhancing the anti-inflammatory effect, and further reducing the expression of IL-6, TNF-α, and IFN-γ. At the same time, HNF-4α over-expression also enhanced the homing of MSCs to the liver, liver regeneration, inhibited the immune response related to $CD68^+$ Kupffer cells (Ye *et al.* 2019). In addition, Choi et al. applied transcription activator-like effector nucleases (TALENS) gene editing to generate MSCs produce IL-10 and these cells showed significantly improved anti-fibrotic and anti-inflammatory abilities, as well as decreased levels of TGF-β, type I collagen, and matrix metalloprotein 2 (MMP 2) related to liver fibrosis in animal model. The proportion of $CD68^+$ Kupffer cells decreased from 50% to 25%, and the levels of IL-1, TNF-α and INF-γ were further decreased. It was shown that the MSCs expressing IL-10 further reduced the severe inflammatory response, prevented liver fibrosis, and improved liver function (Choi *et al.* 2019).

By releasing PGE_2, IDO and increasing iNOS activity, MSCs can reduce mononuclear macrophage infiltration and inhibit the release of pro-inflammatory

factors, such as TNF-α, INF-γ, IL-1 and IL-6, meanwhile promote the release of IL-10 and TGF-β. These factors produced by MSCs can also reduce the cytotoxic effect of cytotoxic T cells, NKT cells and inhibit the function and maturation of DCs to inhibit the inflammatory response and reduce liver injury. Therefore, pretreatment, combined treatment with drugs, gene modification and editing can be applied to promote the immune regulation ability of MSCs, then further enhance their capacity to protect and improve liver function.

Anti-oxidant stress and anti-apoptosis

Oxidative stress caused by exposure to a large amount of ROS, such as peroxide, superoxide, ozone ions, and oxygen free radicals, is an important mechanism by which acute liver injury is induced. Carbon tetrachloride is a stimulator of oxidative stress in animal model of liver injury induction (Chen *et al.* 2014). Since MSCs have both anti-oxidative and anti-apoptotic effects, these cells represent a simple and direct approach to reduce apoptosis and protect hepatocytes (Francois *et al.* 2013, Qi *et al.* 2018).

The anti-oxidant effects of MSCs on reduction of hepatocyte apoptosis and resistance to liver injury have been investigated in two animal models induced by radiation and carbon tetrachloride (Farouk *et al.* 2018, Francois *et al.* 2013, Jiang *et al.* 2018). Jiang et al. found that transplantation of exosomes from human UC-MSCs improved liver function through their antioxidant potential in animal model. Furthermore, it was shown that exosomes from human UC-MSCs had better effects than the commonly used drug biphenyl diester (Jiang *et al.* 2018). In the same animal model of liver fibrosis caused by carbon tetrachloride-induced oxidative stress, Shams et al. found that transplantation of MSCs pretreated with FGF and FGF4 resulted in recovery of glycogen storage capacity, and significantly reduced collagen, alkaline phosphatase and bilirubin levels compared with animals that received untreated MSCs. Furthermore, the liver function was restored, the degree of fibrosis decreased, and the level of oxidative stress decreased. In addition, Terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) staining revealed the stronger anti-apoptotic ability of the pretreated MSCs. This conclusion was confirmed by qPCR analysis of BAX, BCL-xl and Caspase-3 at the mRNA expression level (Shams *et al.* 2015). In a similar carbon tetrachloride-induced model of liver injury, Jieun Jung promoted IL-6/STAT3 signal transduction by reducing the methylation of the IL-

6/SATA3 promoter in MSCs, thus promoting liver cell proliferation and improving the efficacy (Jung *et al.* 2015).

Conclusion

Stem cells transplantation has shown promise as an alternative to organ transplantation. MSCs have a wide range of applications in the treatment of liver injury. Definite therapeutic effects have been obtained in animal models of acute liver injury mimicking improper drug use, toxic exposure, chronic persistent hepatitis and fibrosis caused by various pathogen infections and repeated stimulation by adverse factors. However, improvements in the efficacy of MSCs transplantation are required to ensure this approach can be widely applied in clinical practice. Modification of MSCs is a simple and feasible approach to enhancing their therapeutic potential prior to transplantation.

At present, the modifications to enhance the therapeutic effect of MSCs in liver diseases have four major purposes: strengthening homing ability, promoting regeneration, improving immune regulation ability and protecting liver cells. These strategies have yielded some promising results in animal models. In particular, the immune regulation ability of MSCs to protect liver tissue and inhibit the progression of fibrosis has received most attention. However, we have not yet defined a complete and accurate scheme that can be used to precisely correspond with the mechanism by which the therapeutic effect of MSCs can be improved. Moreover, the clinical effects of MSCs after in vitro stimulation or genetic modification have not yet been systematic identified, and the safety of their usage must be confirmed. Therefore, further investigations are required not only to improve the efficacy of MSCs, but also to translate the modified cells into clinical applications.

Author Contributions

LY Liu collected literature and wrote the manuscript. FY Yang designed, wrote, edited and prepared the manuscript for submission. All authors read and approved the final manuscript.

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MSCs source	Modification	Dose	Route	Animal	Cause	Effect	Mechanisms	References
Adipose	HGF and FGF4 pretreated MSCs	1×10^6	Intrahepatic	Mice	Ccl4	restored glycogen storage↑, collagen↓, ALP↓,bilirubin↓ ,LDH↓,anti-apoptotic，greater homing of MSCs in liver	Transdifferentiation of MSCs into hepatocytes-like cells, improvement of liver function and in reduction of fibrosis	Shams S *et al.* 2015
Bone marrow	pretreated with HGF or SU11274 (c-Met inhibitor)	3×10^6	Tail vein	Rats	I/R injury	ALT↓， AST ↓， C-met↓， hepatic histopathological change	Upregulated c-Met and phosphorylated Met expression	Liu J *et al.* 2014
Umbilical cord	rapamycin or 3 - methyladenine	1×10^6	Intravenous	Mice	I/R injury	ALT↓， AST ↓,Caspase-3↓,hepatic histopathological change	Preconditioning with rapamycin enhances the ability of UC-MSCs to home towards ischaemic livertissue by increasing the expression of CXCR4	Zheng J *et al.* 2018
Bone marrow	cultured under hypoxic	1×10^6, 2×10^5, 4×10^4	Tail vein	Mice	Ccl4	RNA expression levels of Ptges and microRNA210↑,ALT↓, oxidative stress↓, and fibrosis↓,hepatocyte apoptosis ↓,changed macrophage polarity to an anti-inflammatory.	Upregulated PGE_2 and miR210 expression	Kojima Y *et al.* 2019
Human umbilical cord blood	transfected with the pMEX-HGF plasmid	2×10^6	Tail vein	Rats	Ccl4	ALT↓、 AST↓、 ALP↓,collagen fibres↓	HGFoverexpressing in HumanUCB-MSCs	Kim S *et al.* 2011
Bone marrow	Expression of antisense of microRNA-26a-5p to increase HGF production	1×10^6	N/A	Mice	Ccl4	HGF↑,fibrotic area↓, portal pressure ↓,sodium excretion↑	Suppression of microRNA-26a-5p to increase HGF production	Seo KW *et al.* 2014
Bone marrow	Nigella sativa oil	1×10^6	Tail vein	Rats	exposure to γ radiation	ALT↓ AST↓ MDA↓ SOD↑ IL-6↓ TNF-α↓ IL-10↑TGF-β↑	Improvement of liver function and reduction of oxidative stress, inflammatory and fibrogenic	Farouk S *et al.* 2018
Bone marrow	intervening UCHL1 by shRNA knockdown or its inhibitor LDN57444 or overexpression	1×10^6	Tail vein	Mice	Con-A	iNOS↑,Percentages of necrosis area↓,mononuclear cells↓, CD4+ and CD8+ T cells↓	UCHL1 inhibition promotes the activation of NF-κB and STAT1 signaling and suppressed apoptosis of MSCs via upregulation of Bcl-2.	Radwan and Mohamed 2018
Bone marrow	recombinant HNF-4α overexpression adenovirus transfection	1×10^6	Tail vein	Mice	Ccl4	ALT↓， AST↓， TNF-α ↓ ， INF-γ ↓， IL-6 ↓， CD68+ kupffer cells↓	Activation of NF-κB signaling pathway increases iNOS release	Ye Z *et al.* 2019
Adipose	transfected with the IL-35 plasmid	N/A	Tail vein	Mice	Con-A	ALT↓， AST↓， IL-17↓,hepatocytes necrosis and apoptosis↓	MSCs expressed IL-35, activated Jak1 - STAT1 / STAT4 pathway, and reduced the secretion of IFN- γ by monocytes	Wang W *et al.* 2018

Table 1, MSCs modification strategies and effects.

List of abbreviations

aFGF	acid fibroblast growth factor	IL-6	Interleukin-6
ALB	albumin	JAK1	Janus kinase 1
AFP	alpha fetoprotein	LPS	lipopolysaccharide
bFGF	basic fibroblast growth factor	MDA	malondialdehyde
Bcl-2	B-cell lymphoma 2	MMP13	matrix metalloprotein 13
BAX	Bcl-2-associated X protein	MMP 2	matrix metalloprotein 2
BM-MSCs	bone marrow mesenchymal stem cells	MMP9	matrix metalloprotein 9
CDH-6	cadherin-6	MCP-1	Monocyte chemoattractant protein-1
ConA	Concanavalin A	MPO	myeloperoxidase
CXCR-1	C-X-C chemokine receptor-1	MSCs	mesenchymal stem cells
CXCL12	C-X-C motif ligand12	NKT	Natural killer T cell
CXCR4	C-X-C motif receptor4	NLRP3	NOD-like receptor pyrin domain-containing protein 3
CYP3A4	cytochrome P450 3A4	NF-κB	nuclear factor kappa-B
DCs	dendritic cells	PI3K	phosphorylate downstream phosphoinositide 3-kinase
GalN	D-galactosamine	PLGF	Placental growth factor
DMN	dimethylnitrosamine	PDGF-AA	platelet derived growth factor-AA
ER	endoplasmic reticulum	PGE_2	Prostaglandin E2
ERK1/2	extracellular signal-regulated kinase 1/2	ROS	reactive oxygen species
EVs	extracellular vesicles	STAT1	signal transduction and activator of transcription 1
FasL	Fas ligand	STAT3	signal transduction and activator of transcription 3
FGF4	fibro-blast growth factor 4	STAT4	signal transduction and activator of transcription 4
G-6P	glucose-6phosphate	SDF-1	Stromal cell-derived factor-1
GSH	glutathione	SOD	superoxide dismutase
G-CSF	granulocyte Colony-Stimulating Factor	TUNEL	Terminal deoxynucleotidyl transferase-mediated nick end labeling
HGF	hepatocyte growth factor	TAA	thioacetamide
HNF-4α	hepatocyte nuclear factor-4α	TIMP1	tissue inhibitor of matrix metalloproteinases 1
HUCB-MSCs	human umbilical cord blood-derived mesenchymal stem cells	Fas	TNF receptor superfamily, member 6
IDO	indoleamine 2,3-dioxygenase	TALENS	transcription activator-like effector nucleases
iNOS	inducible nitric oxide synthase	TGF-β	transforming growth factor-β
IBD	inflammatory bowel disease	TAT	tryosine-aminotransferase
IFN-γ	Interferon-γ	TNF-α	tumor necrosis factor α
IL-1	interleukin-1	UCHL1	Ubiquitin C-terminal hydrolase 1
IL-10	Interleukin-10	UC-MSCs	umbilical cord mesenchymal stem cells
IL-12	Interleukin-12	VEGF	vascular endothelial growth factor
IL-17	interleukin-17	α -SMA	α smooth muscle actin
IL-35	interleukin-35	α 1AT	α 1 antitrypsin
IL-4	Interleukin-4		

Table 2, List of abbreviations.

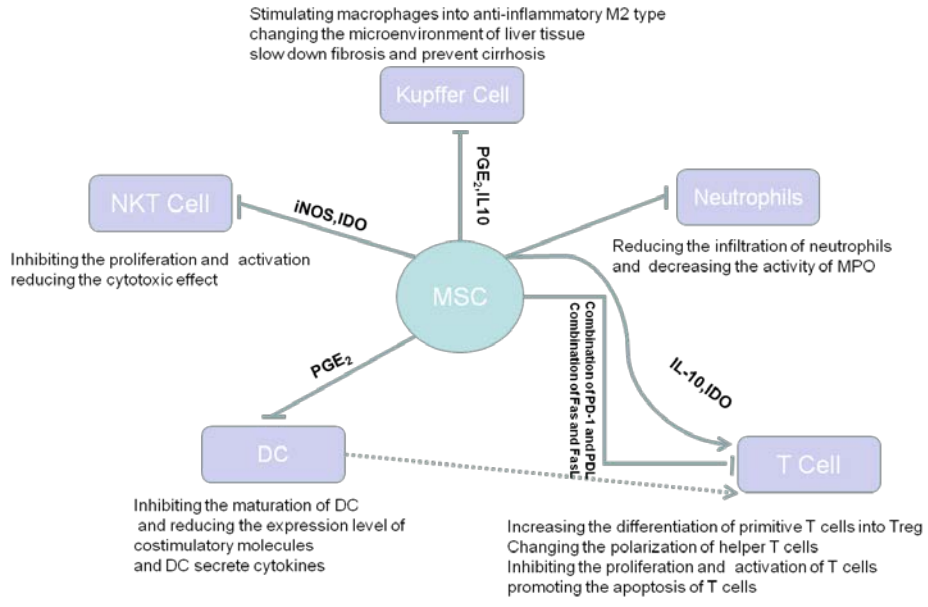

Figure 1, Mechanisms of MSCs Immune regulation functions