REVIEW ARTICLE

Current Understanding of Stem Cell and Secretome Therapies in Liver Diseases

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Abstract Liver failure is one of the main risks of death worldwide, and it originates from repetitive injuries and inflammations of liver tissues, which finally leads to the liver cirrhosis or cancer. Currently, liver transplantation is the only effective treatment for the liver diseases although it has a limitation due to donor scarcity. Alternatively, cell therapy to regenerate and reconstruct the damaged liver has been suggested to overcome the current limitation of liver disease cures. Several transplantable cell types could be utilized for recovering liver functions in injured liver, including bone marrow cells, mesenchymal stem cells, hematopoietic stem cells, macrophages, and stem cell-derived hepatocytes. Furthermore, paracrine effects of transplanted cells have been suggested as a new paradigm for liver disease cures, and this application would be a new strategy to cure liver failures. Therefore, here we reviewed the current status and challenges of therapy using stem cells for liver disease treatments.

Keywords Liver failure · Liver regeneration · Stem cell transplantation · Secretome

Abbreviations

NPCs	Non-parenchymal cells
HSCs	Hepatic stellate cells
PH	Partial hepatectomy
TNF- α	Tumor necrosis factor- α
IL-6	Interleukin-6

HGF	Hepatocyte growth factor
EGF	Epidermal growth factor
FGF	Fibroblast growth factor
VEGF	Vascular endothelial growth factor
IGF	Insulin-like growth factor
TGF- β 1	Transforming growth factor-beta 1
ECM	Extra cellular matrix
PDGF-B	Platelet-derived growth factor-B
TIMP	Tissue inhibitors of metalloproteinases
BMCs	Bone marrow cells
MSCs	Mesenchymal stem cells
PSCs	Pluripotent stem cells
ESCs	Embryonic stem cells
iPSCs	Induced pluripotent stem cells
CM	Conditioned medium
MFG-E8	Milk fat globule-epidermal growth factor 8

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1 Introduction

The liver is one of the important organ that has diverse functions such as digestion, metabolism, detoxification, storage, production, and so on [1, 2]. Since the liver is responsible for many vital roles in maintaining life, liver failure is closely related to the patient mortality.

The liver has strong regenerative capacity, however, repetitive damages in the liver can cause malfunction of hepatic repair. Virus infections, alcohol abuse, drug-induced hepatotoxicity, and many other factors induce damage of liver cells by mediating inflammations and tissue injuries [3, 4]. When the liver is exposed to these harmful conditions persistently, the regenerative capacity of the liver is progressively lost and fibrosis occurs, then, the liver cirrhosis develops which there is irreversible scarring of the liver. Currently, liver transplantation is the only reliable and effective treatment for this irreversible damage of the liver, however, this approach is hampered by the limited supply of donor and complications of long-term immunosuppression [5].

To overcome donor scarcity of liver transplantation, researchers have begun to develop the cell transplantation strategy using stem cell and stem cell-derived hepatocytes [6–8]. Many research groups have demonstrated the therapeutic potentials of various cell types such as bone marrow cells, mesenchymal stem cells, hematopoietic stem cells, macrophages, and *in vitro* differentiated hepatocytes [9–12]. Although each cell type has eminent strengths and weak points to be improved, the transplanted cells have therapeutic effects on the improving host liver functions and inducing liver regeneration by both replacing host tissues and improving host environment [13, 14]. Interestingly, it is also believed that the beneficial effects of transplanted cells for liver disease cure come from the paracrine effects by secreted molecules from transplanted cells [14, 15]. Therefore, unravelling the action of stem cells for liver regeneration would be an invaluable study to develop the liver disease cure.

In this review, we focus on the current strategy to overcome liver failure and the role of stem cells and its therapeutic use.

2 The liver

Liver architecture involves main five components: vascular system, hepatocytes, hepatic sinusoidal cells, biliary system, and stroma [2]. Among the various components of the liver, cell types that are responsible for the main functions of the liver are hepatocytes, which perform most of metabolic, synthetic, and detoxifying functions and

occupies the majority of total liver mass. The remaining parts of the liver are composed of the non-parenchymal cells (NPCs) such as sinusoidal endothelial cells, hepatic stellate cells (HSCs), kupffer cells, cholangiocytes, and the others [16]. Hepatocytes have well-organized polarity with the adjacent NPCs and the maintenance of structural polarity directly contribute to the functional polarity [17].

The liver is the primary site of detoxification and plays a pivotal role as the safety guard to defense and detoxify any toxic and harmful materials such as metabolic wastes, drugs, alcohol and chemicals. The liver also processes the nutrient absorption and secretes a variety of proteins such as the plasma protein, albumin, glucose, cholesterol, and many specialized proteins. Especially, the liver secretes bile, which helps the digestion and absorption of fats. Further, the liver functions as a storage reservoir of glucose and regulates the ammonia level in the body thereby maintaining homeostasis.

3 Liver regeneration

Damages such as viral infection, chemical toxicity and metabolic overload cause hepatic cell death, and it activates the series of molecular events to repair and regenerate damaged tissue. Regeneration process is controlled by the action of various cytokines and growth factors [18], and the factors involved in the liver regeneration are well studied in animal model of partial hepatectomy (PH), a surgical method to remove the injured liver (Table 1). Upon PH, kupffer cells activate tumor necrosis factor- α (TNF- α) [19, 20], and this cellular event is mediated by the induction of NF- κ B, and functions as an important regulator of the early stage of regeneration. Simultaneously, kupffer cells upregulate the expression of interleukin-6 (IL-6) by the action of secreted TNF- α [21, 22]. IL-6 exerts its function through the downstream signaling pathways and mediates mitotic activation and recovery of liver tissue [23, 24]. Furthermore, hepatocyte growth factor (HGF) is dramatically increased after PH, and enhances hepatocyte proliferation and regeneration [25, 26]. Other growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF) are involved in liver regeneration [27–33].

During liver regeneration process, many signaling cascades are also involved. β -catenin is predominantly stabilized in hepatocytes and contributes to proliferation and regeneration [34, 35]. The main components of Notch pathways are upregulated after PH, and genetic study revealed that the Notch pathway is involved in the regeneration of injured tissue and liver vasculature [36, 37]. Transforming growth factor-beta 1 (TGF- β 1) expression is

Table 1 Key factors involved in liver regeneration

Factors	Major sources	Phase	Effects on liver growth	References
TNF- α	Kupffer cell	Priming phase	Proliferative	[19, 20]
NF- κ B	Kupffer cell	Priming phase	Proliferative	[19, 20]
IL-6	Kupffer cell	Priming phase	Proliferative	[20–22]
HGF	Stellate cell	Proliferation phase	Proliferative	[7, 19, 31]
	Endothelial cell			
EGF	Duodenum	Proliferation phase	Proliferative	[7, 31]
	Salivary gland			
FGF	Stellate cell	Proliferation phase	Proliferative	[31, 32]
	Hepatocyte			
VEGF	Hepatocyte	Proliferation phase	Proliferative	[31, 32]
	Biliary cell			
IGF	Stellate cell	Proliferation phase	Proliferative	[16, 33]
	Hepatocyte			
β -Catenin	Hepatocyte	Proliferation phase	Proliferative	[34, 35]
Notch	Hepatocyte	Proliferation phase	Proliferative	[36, 37]
TFG- β 1	Stellate cell	Termination phase	Anti-proliferative	[31, 38, 39]
	Kupffer cell			
	Endothelial cell			
SMAD	Hepatocyte	Termination phase	Anti-proliferative	[40, 41]

transiently peaked after PH and genetic study shows that TGF- β 1 interrupts hepatocyte proliferation during regeneration phase [38, 39]. Activation of downstream molecule, SMAD mechanistically explains the role of TGF- β 1 in regeneration. Considering the working mechanism of TGF- β 1, it might be involved in the termination of liver regeneration [40, 41].

4 Liver fibrosis

Although the liver is a remarkable organ playing diverse roles with excellent regenerative ability [6], subsequent and repeated damages on liver tissue cause severe inflammations and attenuate the ability to regenerate and repair the damaged parts of the liver. In other words, hepatic tissue proceeds to the next stage, called liver fibrosis. When the fibrosis get worse, liver cirrhosis occurs which accelerate hepatocellular dysfunction and liver failure.

Liver fibrosis is characterized by altering the hepatic architecture caused by aberrant accumulation of extracellular matrix (ECM) proteins [42, 43]. The main reason of the accumulation of ECM is HSCs. Continuous damages to the liver such as inflammation result in the activation of quiescent HSCs, and increase the expression of ECM proteins. Excessive ECM interrupts with normal physiology and function of the liver. Among various cytokines involved in fibrogenesis, TGF- β 1 plays an important role in the progression of fibrosis. TGF- β 1 stimulates the conversion of HSC into fibroblast-like cell and mediates

abundant fibrillar collagen deposition [44, 45]. Platelet-derived growth factor-B (PDGF-B) is also overexpressed during hepatic fibrogenesis and functions as a mitogen for HSCs [46]. Angiotensin II which raises the blood pressure, induces hepatic inflammation and mediates the serial action of fibrogenesis by acting through angiotensin II receptor type 1 receptors [47, 48]. One of the major adipokines, Leptin is also known to be required in hepatic fibrogenesis and ECM remodeling [49]. Fibrotic ECM is mainly regulated by the expression level of matrix metalloproteinases and tissue inhibitors of metalloproteinases (TIMP) [50, 51]. When TIMP-1 is overexpressed by activated HSCs, ECM degradation is strongly inhibited and the liver becomes stiffer. If liver fibrosis is continuously progressing, it leads to hepatic cirrhosis or liver cancer, which lowers the quality of life (Fig. 1).

5 Source of transplantable cells for liver failure

To treat severe liver failure, liver transplantation is the only effective cure for the patients. However, it is limited by the lack of healthy organ donation [13]. Therefore, studies on cell therapy and cell transplantation have developed to overcome the facing limitation. Among the transplantable cell sources, primary adult human hepatocytes (PHHs) acquired from donors could provide a great source for transplantation. However, the PHHs are not suitable for transplantation due to prolonged ischemia and

Fig. 1 Mechanism for development and progression of liver fibrosis. Continuous damages on liver tissue result in the secretion of various pro-fibrotic factors such as TGF- β , PDGF-B, angiotensin II, and leptin. These pro-fibrotic factors activates hepatic stellate cells (HSCs), then, the activated HSCs produce extracellular matrix

(ECM) and further developed into myofibroblast-like cells in the process of liver fibrosis. Furthermore, overexpression of TIMP-1 by activated HSCs in the liver inhibits ECM degradation, then, finally accelerate the progression of liver fibrosis

contamination of fat tissue. Moreover, proliferation ability and survival rate of the PHHs *in vitro* are still questionable [52].

Another possible cell type for cell transplantation could be fetal liver cells because of their proliferative capacity. Although fetal liver cells derived from aborted fetuses are more proliferative than adult hepatocytes, maturity of the fetal liver cells is an issue for use in disease cure. Recently, the existence of hepatic stem cells (oval cells) in the adult liver has been identified [52, 53], and the oval cells are considered as a good transplantable source for liver disease treatment because of their bi-potentiality. However, the numbers of the oval cells in the liver are very limited and technical development for the isolation, purification, and expansion of the oval cells is a challenging task [54, 55]. Therefore, stem cells are proposed as a new source of cell transplantation for liver disease cure because of their proliferation and pluripotency (Table 2).

6 Stem cell therapy for liver diseases

To improve the prognosis of liver failure, many researchers have studied on the therapeutic potential of stem cell transplantation (Table 3).

Bone marrow cells (BMCs) have been studied for a long time to treat liver diseases [56–58]. BMCs are multipotent cells which have the differentiation potential into liver cells and secrete beneficial molecules such as matrix metalloproteinase to reduce liver fibrosis [9, 59, 60]. Although therapeutic effect of BMCs is promising, cell preparation step requires quite invasive procedure and the complex and heterogeneous components of BMCs need to be identified.

Mesenchymal stem cells (MSCs) are one of the great cell sources for alternative therapeutic options to treat liver disease [10, 61, 62]. MSCs have attractive features such as proliferation ability, differentiation capability, and immune-evasive property. Many different types of MSCs have been proven to be effective transplantable sources in liver failures by differentiating into hepatocytes, and there

are several reports about therapeutic effects of MSCs from various stem cell niches. MSCs taken from bone marrow showed improving liver functions when injected into patients suffered from different types of liver disease such as hepatitis and alcohol abuse [63–68]. Liver-derived MSCs also could be a novel therapeutic source for liver disease because they are able to differentiate into mature hepatocytes and contribute to liver regeneration in acute liver injury model [69–71]. Amniotic fluid-derived MSCs and its hepatic progenitor cells showed recovering liver injuries in the mouse model of acute hepatic failure [72]. MSCs collected from other sources such as umbilical cord or cord blood also exhibited clinical therapeutic effect in patients with liver cirrhosis [73]. Recently, transplantation of hepatic cell sheets derived from MSCs also accelerated liver regeneration in mouse models, raising the possibility of an alternative therapy of liver transplantation [74, 75]. MSCs are an excellent therapeutic candidate that are free from ethical concerns and could be isolated from various sources [76, 77]. However, major hurdle for use of MSCs in clinical applications is variability in cell quality depending on donors, sources, and preparation techniques [78].

Other cell types also have been considered as a transplantable source for liver disease treatment. Administration of hematopoietic stem cells into patients with chronic liver disease also showed clinical and biochemical improvement of the patients [79, 80]. However, although hepatocyte differentiation potential of hematopoietic stem cells reported, exact therapeutic mechanism is still controversial [11, 81]. Injection of macrophages derived from bone marrow also improves clinically relevant parameters in liver fibrosis animal model [12]. Interestingly, in the study of transgenic mouse model (CD11b-DTR) that could selectively deplete macrophage, it was found that macrophage has opposing effects depending on the stage of progression, and beneficial effect of macrophage was prominent in recovery stage not injury stage [82].

Otherwise, human pluripotent stem cells (PSCs) including embryonic stem cells (ESCs) and induced

Table 2 Potential sources of transplantable cells for liver failure

Cell types	Advantages	Disadvantages
Primary adult hepatocytes	Fully differentiated hepatocytes	Lack of donor Prolonged ischemia Contamination of other tissue Low proliferation ability
Fetal liver cells	High proliferation ability	Lack of donor Lack of maturity
Hepatic stem cells (oval cells)	Hepatocyte differentiation potential	Limited numbers of cells Lack of culture methods
Stem cell-derived hepatocytes	Unlimited source of hepatocytes Well established culture methods	Lack of maturity

Table 3 Studies on the liver failure treatment using stem cells or stem cell-derived factors

Injected cell type or secretome	Cell origin	Amount of injection	Injection route	Animal model	Mouse	Chemical dose	References
BM cell	Mouse	1×10^5 cells	iv (tail vein)	CCl4-injured mice	6 weeks	0.5 ml/kg (ip, 4 weeks, twice a week)	[9, 60]
BM-derived MSC and its derived hepatocyte	Human	1.4×10^6 cells	iv (tail vein) and ip	CCl4-injured NOD/SCID mice	–	0.28 ml/kg (by gavage, single injection)	[10]
BM-derived MSC	Human	2×10^6 cells	Direct injection (twice, right lobe of liver)	Thioacetamide-induced Sprague–Dawley rat	7 weeks	300 mg/kg (ip, 12 weeks, twice a week)	[68]
Liver-derived MSC and BM-derived MSC	Human	1×10^6 cells	Intrasplenic injection	CCl4-injured NOD/SCID mice	6–8 weeks	5 ml/kg (ip, single injection)	[69]
Liver-derived MSC	Human	2×10^5 cells	iv	<i>N</i> -acetyl- <i>p</i> -aminophen-injured NOD/SCID mice	6–8 weeks	150 mg/kg (ip, single injection)	[70]
Amniotic fluid–MSC and its derived HPL cell	Human	1.5×10^6 cells	iv (tail vein)	CCl4-injured NOD/SCID mice	6–8 weeks	1.63 ml/kg (ip, single injection)	[72]
MSC-derived Hepatic cell sheet	Human	–	Transplantation onto liver surface	CCl4-injured NOD/SCID mice	8–9 weeks	0.2 ml/kg (by oral gavage, single injection)	[74]
Human iPSC-derived hepatic cell sheet	Human	8×10^5 cells	Transplantation onto liver surface	CCl4-injured Rag2 ^{−/−} /Il2rg ^{−/−} mice w/partial hepatectomy	8–9 weeks	3 ml/kg (ip, single injection)	[75]
Hematopoietic stem cell	Mouse	1×10^5 cells	iv (tail vein)	CCl4-injured mice	6–10 weeks	10 ml/kg (ip, single injection)	[11]
BM-derived macrophage	Mouse	1×10^6 cells	iv (hepatic portal vein)	CCl4-injured mice	Adult	0.75 ml/kg (ip, 10 weeks, twice a week)	[12]
Human ESC-derived hepatocyte	Human	1×10^6 cells	Intrasplenic injection	retrotransposon-treated NOD/SCID mice w/partial hepatectomy	–	70 mg/kg (ip, twice separated by 2 weeks)	[86]
Human iPSC-derived hepatic cell	Human	2×10^6 cells	iv (tail vein)	DMN-injured NSG mice	6–9 weeks	5 mg/kg (ip, 4 weeks, weekly)	[87]
Human ESC-derived hepatocyte-like cell	Human	2×10^6 cells	Intrasplenic injection	CCl4-injured BALB/c nude mice	5–6 weeks	5 ml/kg (ip, single injection)	[88]
Human ESC-derived hepatocyte	Human	1×10^6 cells	Intrasplenic injection	Acetaminophen-injured NOD/SCID mice	4–6 weeks	300 mg/kg (single injection)	[89]
BM-derived MSC	Human	5×10^5 cells	iv (tail vein)	NOD.CB17-Prkdc ^{scid} /J mice	6 weeks	0.5/1.0/1.5 ml/kg (ip, 6 weeks, twice a week)	[94]
Amniotic membrane-derived MSCs	Human	1×10^5 cells	Intrasplenic injection	CCl4-injured C57Bl/6 J mice	–	1 ml/kg (ip, 8 weeks, twice a week)	[95]
UCMSC secretome	–	500 µg	ip	Thioacetamide-injured C57BL/6 mice	5–6 weeks	200 mg/kg (ip, 8 weeks, three times a week)	[93]
hpUCMSC secretome	–	500 µg	ip	CCl4-injured BALB/c mice	6 weeks	0.5 ml/kg (ip, 6 weeks, twice a week)	[93]

Table 3 continued

Injected cell type or secretome	Cell origin	Amount of injection	Injection route	Animal model	Mouse	Chemical dose	References
Conditioned medium from MSC	–	0.9 ml	iv (penile vein)	D-Galactosamine-induced Sprague–Dawley rat	–	0.6 g/kg (ip, single injection)	[101]
Conditioned medium from liver-MSC	–	250 μ l (concentrated)	ip	GalN/LPS-induced SCID mice	7–8 weeks	600 mg/kg-GalN 125 ng-LPS (ip, single injection)	[102]
Conditioned medium from liver-MSC	–	100 μ l (concentrated)	ip	70% partial hepatectomy in C57BL/6 mice	13–18 weeks	–	[103]
Exosome from umbilical cord-MSC	–	250 μ g	Direct injection into liver	CCl ₄ -injured mice	4–5 weeks	0.6 ml/kg (ip, 6 weeks twice a week)	[105]
Exosome from menstrual blood-MSC	–	50 μ g	iv (tail vein)	D-GalN/LPS-induced C57BL/6 mice	6–8 weeks	800 mg/kg-GalN 50 μ g/kg-LPS (ip, single injection)	[106]

MSC mesenchymal stem cell, HPL hepatic progenitor-like, BM bone marrow, iPSC induced pluripotent stem cell, ESC embryonic stem cell, UCMSC umbilical cord-derived MSC, hpUCMSC UCMSC-derived hepatocyte-like cell, ip Intraperitoneal injection, iv intravenous injection, CCl₄ carbon tetrachloride, DMN dimethylnitrosamine, GalN/LPS D-galactosamine N/lipopolysaccharide

pluripotent stem cells (iPSCs) are also valuable sources in tissue regeneration [83]. Since the human PSCs could proliferate indefinitely and differentiate into all functional cell types in our body, they could provide stable transplantable hepatocytes for liver disease cure [84, 85]. In that reason, recently, many research groups have demonstrated that hepatocyte-like cells derived from human PSCs successfully engrafted into the liver tissue and contributed to recovery of injured liver [86–89]. Upon transplantation of human PSC-derived hepatocytes, they could integrate into the host liver and rescue the liver disease in the animal model. However, the cell therapy using human PSC-derived hepatocytes still have limitations including risk of teratoma formation [90], and the maturity of differentiated hepatocytes from PSCs is also still on issue [91, 92].

More recently, many evidences indicate that transplanted cells could integrate into the host liver, as well as the cells could influence the host regenerative system by paracrine effects [72, 88, 93]. These potent paracrine effects of stem cell-derived factors could provide “cell-free” therapeutic options in liver disease cure.

7 Stem cell-derived factors for liver disease cure

Many researches indicate that stem cell therapy using MSCs shows significant improvements in liver functions [14, 94, 95]. However, other studies questioned beneficial effects of MSC transplantation [96–98]. Depending on

methodological differences including cell types, injection routes, and phase of liver fibrosis, it is difficult to interpret the discrepancy observed in previous studies. Therefore, soluble factors secreted by MSCs have been suggested to play an important role in liver regeneration [14]. In the context of beneficial paracrine effects of MSCs, it has been demonstrated that bone marrow-derived MSC conditioned medium (CM) showed anti-apoptotic and pro-mitotic effects on cultured hepatocytes by increasing hepatocyte protective cytokine IL-6 [99, 100]. Moreover, systemic injection of MSC-CM could inhibit hepatic cell death and improve liver regeneration in D-galactosamine-induced acute liver injury model [101]. Specific molecules responsible for MSC-CM therapy are expected to be VEGF, HGF, IGFBP, IL-6, and other cytokines related with liver regeneration. In fact, CM obtained from MSC marker expressing-human liver stem cells isolated from human cryopreserved normal hepatocytes contains many paracrine factors such as IL-10, VEGF, IL-8, HGF, IGF-1, and IL-6 [102]. Therefore, administration of MSC-CM induces expression of genes relevant for hepatocyte proliferation, anti-inflammatory responses, and pro-angiogenic factors after partial hepatectomy [103]. Further, human umbilical cord-derived matrix stem cells (hUCMSCs) significantly increased the survival of acute liver failure in mice by stimulating endogenous liver regeneration [104]. Study from co-culture with hUCMSCs indicates that paracrine effects play key roles in stimulation of endogenous liver regeneration.

In addition to liver regeneration by stem cell-derived factors, the MSC exosomes have also been described to have anti-fibrotic effects by inhibiting TGF- β 1/Smad signaling pathway and suppressing epithelial mesenchymal transition. [105]. Exosomes are considered as intracellular compartments that manage protein wastes, however, recent studies indicate that exosome contains various microRNAs, mRNAs and proteins, which are involved in intracellular communication. Exosomes derived from human menstrual blood-derived stem cell expressed cytokines such as IL-6, IL-8, insulin-like growth factor-binding protein-6 and improved survival rates and recovered liver functions when the exosomes were injected in D-galactosamine and lipopolysaccharide induced mouse model [106]. Since exosomes are known to modulate progression of hepatic fibrosis, the exact mode of actions and responsible molecules need to be explained in more detail [107–109].

However, the secretome contains various factors including molecules that may result negative effect on liver regeneration. Therefore, to discover key therapeutic factors in secretome from stem cells and their differentiated hepatocyte-like cells, proteomic analysis was performed by researchers and several identified putative molecules were reported (Table 4). Among various candidates, milk fat globule-epidermal growth factor 8 (MFG-E8) was identified as a key molecule for rescuing liver fibrosis [93]. In fact, injection of human MFG-E8 instead of whole secretome showed comparable anti-fibrotic effects to the whole secretomes [93]. Further, MFG-E8 truly decreased the ECM accumulation in the liver fibrosis model and inactivated HSCs by inhibiting TGF- β signaling pathways, one of the major mechanistic regulators of liver fibrosis [93].

Therapeutic use of stem cells in paracrine manner is also well documented in other disease models. Neurodegenerative disease such as Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, and many others, are characterized by structural and functional destruction of neurons [110–113]. Recent studies on stem cell therapy indicate that the beneficial effect mainly rely on auto/paracrine activities. A variety of cytokines and growth

factors that secreted by injected stem cells regulate rearrangement of the microenvironment, and mediate neural protection and repair processes. Similar approach confirmed the phenotypical improvement of cardiovascular disease such as myocardial infarction [114, 115]. In mice model of ischemia, MSC secretome mediated neovascularization and induced myocardial protection by preventing apoptosis, inflammation, and fibrosis. As mentioned above, finding out the therapeutic molecules secreted from stem cells is the current trend in research and clinical fields (Fig. 2). Therefore, further research would be promising to find out the other key molecules in secretome and its downstream signaling pathways that are also involved in therapeutic improvements in liver failure.

8 Conclusion and future perspectives

As the liver is essential for maintaining life, liver regeneration should be finely coordinated. Many efforts have been made to find out the best way for the treatment of liver diseases. Up to date, organ transplantation is the widely accepted option to treat the patient with liver failures or liver diseases. However, there are several limitations such as an excessively deficient number of organ donation and possible complications that include immune rejection, risk of infection, and graft failure. From these circumstances, therapeutic use of stem cells has emerged as an alternative treatment for the liver diseases.

Transplantation of stem cell such as BMCs, MSCs, hematopoietic stem cells, macrophages truly shows beneficial effects in the patient with liver failure. Therapeutic improvement after stem cell treatment is mainly mediated by replacing damaged tissues and secreting soluble factors. BMCs are multipotent progenitors that could attenuate liver fibrosis. MSCs are the most frequently studied cell types that have many advantages on the clinical applications including differentiation capabilities, regenerative potential, immune modulation, and paracrine activity. Hematopoietic stem cells are versatile cells harboring hepatocyte differentiation

Table 4 Identified key factors from stem cell-derived secretome

Gene ID	Full name	Roles in liver regeneration	References
GAS6	Growth arrest specific 6	Activating wound healing response after liver injury	[120]
C3	Complement C3	Mediating key signaling networks to promote hepatocyte proliferation	[121]
VTN	Vitronectin	Enhancing tissue remodeling by regulating cell adhesion and cellular motility	[122]
MMP2	Matrix metalloproteinase 2	Digesting the extracellular matrix in the early stages of liver regeneration	[123]
QSOX1	Quiescin sulfhydryl oxidase 1	Promoting hepatocyte proliferation.	[124]
MFG-E8	Milk fat globule-EGF factor 8 protein	Inhibiting TGF- β signaling and reducing extracellular matrix deposition	[93]

Fig. 2 Beneficial effects of stem cells and stem cell-derived factors for liver therapy. Transplantation of hepatocytes derived from stem cells rescues liver diseases by replacing damaged cells and activating host liver regeneration by paracrine manner. Further studies on the

paracrine effects revealed that beneficial secretory molecules accelerate the regeneration of host liver and prevent liver fibrosis progression without hepatocyte transplantation. These applications would be a promising therapeutic option for liver disease treatment

potential. Macrophages have clinical potential by reducing fibrosis and stimulating liver regeneration. However, in most cases, stem cell-derived population is heterogeneous and preparation step requires invasive surgery. In addition,

maintaining good quality of cells for therapeutic application needs to be finely regulated.

Recently, another trial using human PSCs has been very actively ongoing. Human PSCs are versatile cell types,

which have much higher potential to differentiate. Since human PSCs could proliferate indefinitely without losing their characteristics, it is feasible preparing large quantities of cells needed for treatment. When differentiation technique is combined with reprogramming strategy, also known as induced pluripotent stem cell generation, it is possible to generate transplantable liver cells without the concern for immune rejection [116]. Cellular approaches that are mentioned above, could integrate with other techniques such as gene therapy, bioartificial liver devices, or bioengineered organs to improve therapeutic applications [117]. However, there are still challenges to be solved. *In vitro* differentiated hepatocytes have similar properties of primary hepatocytes but they are considered to be fetal-like cells in terms of maturation [118]. For therapeutic applications, upregulating the levels of albumin production, urea production, cytochrome P450 activity, and mitochondrial function need to be achieved.

Recently, paracrine effects of transplanted cells are actively under investigations. For example, MSCs are known to secrete various cytokines and molecules, which are secreted in response to environmental stimuli upon transplantation. These paracrine effects include anti-inflammatory, immune modulating, anti-tumor, and regenerative potentials, and many others [119]. Further researches on bioactive molecules secreted from stem cells will lead to the development of efficient and effective therapies without applying cell injection which is still on controversial issues. Collectively, therapeutic use of stem cells holds promising potentials due to their capability of replacing damaged tissue and exerting beneficial effects in a paracrine manner. Even though stem cell transplantation for liver failure is still on the stage of technology development, research progresses in this field are very rapid and active. Currently, optimizing experimental protocols for qualified stem cells would be the first priority. Unraveling the role of important paracrine factors responsible for curing liver failures needs to be explained in detail. In addition, larger clinical studies are essential to prove the clinical efficacy of stem cells. However, above all, it should be followed by understanding the molecular basis and underlying mechanism of liver regeneration to fully overcome and manage the liver failure.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no competing interests.

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