**REVIEW**

**Open Access**

CrossMark

# Liver-derived human mesenchymal stem cells: a novel therapeutic source for liver diseases

Yini Wang, Xiaopeng Yu, Ermei Chen and Lanuan Li[*]

## Abstract

Mesenchymal stem cells (MSCs) represent an attractive cell type for research and therapy due to their ability to proliferate, differentiate, modulate immune reactions, and secrete trophic factors. MSCs exist in a multitude of tissues, including bone marrow, umbilical cord, and adipose tissues. Moreover, MSCs have recently been isolated from the liver. Compared with other MSC types, liver-derived human MSCs (LHMSCs) possess general morphologies, immune functions, and differentiation capacities. Interestingly, LHMCSs produce higher levels of pro-angiogenic, anti-inflammatory, and anti-apoptotic cytokines than those of bone marrow-derived MSCs. Thus, these cells may be a promising therapeutic source for liver diseases. This paper summarizes the biological characteristics of LHMSCs and their potential benefits and risks for the treatment of liver diseases.

**Keywords:** Mesenchymal stem cells, Cell therapy, Hepatic differentiation, Liver-derived mesenchymal stem cells

## Background

The liver is involved in regulation of several major physiological processes, such as glycogen storage, lipid metabolism, plasma protein secretion, and xenobiotic detoxification [1]. Liver dysfunction and failure can have diverse etiologies. Orthotropic liver transplantation (OLT) is considered the most suitable therapeutic option for patients with liver failure. However, it is severely limited by organ shortages, high expense, graft rejection, and the requirement for long-term immunosuppression.

Cell-based therapy has been proposed as a potential alternative to OLT [2–4]. Over the past decade, mesenchymal stem cells (MSCs) have attracted considerable attention. MSCs are defined as adherent multipotent fibroblast-type stem cells with the ability to differentiate into mesodermal and ectodermal cells [5, 6]. Unlike other types of stem cells (such as embryonic stem cells and induced pluripotent stem cells), MSCs have low immunogenicity and marked immunomodulatory effects, which reduce the probability of immune rejection [7–9]. Moreover, MSCs are resistant to reactive oxygen species in vitro, reduce oxidative stress in recipient mice, and accelerate repopulation of hepatocytes after liver damage [10]. Therefore, pre-clinical and clinical trials have been performed to determine the therapeutic potential of MSCs [11, 12].

MSCs are distributed extensively and were initially identified in bone marrow [13] and then in various tissues, including the lung, umbilical cord, and adipose tissue [14, 15]. The liver is a novel reservoir of MSCs. Liver-derived human MSCs (LHMSCs) possess properties similar to those of MSCs from other tissues, including proliferative, differentiation, and immunomodulatory capacities. However, LHMSCs are different in certain respects, particularly in terms of their biomarkers and biological functions. This review focuses on hepatic differentiation of LHMSCs and their application in liver disorders, opening a new path toward further studies.

## Isolation and culture of LHMSCs

LHMSCs were first isolated from first-trimester fetal livers [16] and later from second-trimester fetal livers [17]. To ensure the safety, quality, and identity of cell products, a standardized procedure in compliance with current Good Manufacturing Practices has been formulated [18]. Briefly, disrupted liver tissue is harvested

* Correspondence: ljli@zju.edu.cn
State Key Laboratory for the Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for the Diagnosis and Treatment of Infectious Diseases, First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China

Wang et al. Stem Cell Research & Therapy (2016) 7:71

Page 2 of 8

using a homogenizer following removal of adjacent tissues. Then, mononuclear cells are isolated by density-gradient centrifugation and cultured in Dulbecco's modified Eagle's medium with 15 % fetal bovine serum.

The fetal origin of MSCs raises both ethical and safety issues. Thus, there was much enthusiasm over the isolation of MSCs from adult tissues, which develop and maintain their own stem cell pools. Evidence for the presence of MSCs in the adult liver has accumulated. Najimi et al. [19] successfully obtained adult liver-derived human MSCs by enzymatic disaggregation of adult human liver and the elimination of hepatocytes and other liver cell types. Moreover, Pan et al. [20] reported that these cells are likely a resident population rather than bone marrow-derived cells.

## Characterization of LHMSCs

In terms of morphology, cultured LHMSCs exhibit an elongated spindle shape with ovoid nuclei (Fig. 1), as well as stem cell properties, including positivity for stem cell markers (vimentin and nestin) and MSC markers (CD29, CD73, CD44, CD90, CD105, and CD166) [21]. However, LHMSCs are negative for hematopoietic stem cell markers (CD34, CD45, CD117), suggesting that these cells are not of hematopoietic origin [19, 22]. Compared with bone marrow-derived MSCs (BMMSCs), the expression of CD105, a marker used to evaluate the differentiation status of MSCs [23], is lower in LHMSCs. This observation suggests that LHMSCs may be at a more advanced stage of differentiation. Interestingly, LHMSCs express CD26, albumin, CK8, and CK18, indicating a partial commitment toward hepatic cell differentiation [21, 22, 24]. Similar to other MSCs, LHMSCs have low immunogenicity due to the absence of major histocompatibility complex (MHC) class II (human leukocyte antigen (HLA)-DP, -DQ, and -DR) antigens, FAS ligand or costimulatory molecules with the exception of MHC class I antigens [25, 26].

Similar to MSCs from other tissues, LHMSCs have the capacity for self-renewal, multipotent differentiation, and immunosuppression. LHMSCs exhibit high proliferative ability in long-term culture, and Wnt signaling has been shown to modulate their growth [20]. In conditioned media, LHMSCs are able to undergo osteogenic, chondrogenic, and endothelial but not adipogenic differentiation. Moreover, LHMSCs can differentiate into hepatocyte-like cells, gaining hepatic functions such as production of cytochrome P450, albumin, and urea. This implies the potential of LHMSCs in cell therapy and pharmacotoxicological testing [22, 27]. It is noteworthy that the proliferative and differentiation capacities of MSCs decrease with age [28, 29]. Thus, fetal LHMSCs may be superior. With regard to their immune effects, LHMSCs express HLA-G [30] and CD90 [22, 29], which regulate immune responses by inhibiting T-cell proliferation [31]. Furthermore, CD90 is stably expressed in LHMSCs, suggesting that such cells exert immunosuppressive effects [32, 33].

LHMSCs differ from other resident hepatic stem/progenitor cells. Dormant liver progenitor cells are periportally located in the healthy liver and actively proliferate after chronic liver injury or sub-massive liver cell loss [34]. LHMSCs are spindle-shaped, whereas liver progenitor cells are oval. Moreover, LHMSCs are negative for CD117, CD34, and CK19, markers of liver progenitor cells [21]. Hepatic stellate cells (HSCs) are another type of stem/progenitor cell in the liver that acquire a myofibroblast-like phenotype when activated. As is true of LHMSCs, activated HSCs express CD133, a molecular maker of stem/progenitor cells [35]. However, HSCs are positive for NCAM, CK19 and HLA-class II membrane markers [36], for which LHMSCs are negative. Moreover, chemokine levels differ markedly between LHMSCs and HSCs: the former cells secrete higher levels of therapeutic and immune-modulatory cytokines, including hepatocyte growth factor (HGF), interferon (IFN)-γ and interleukin (IL)-10 [37].
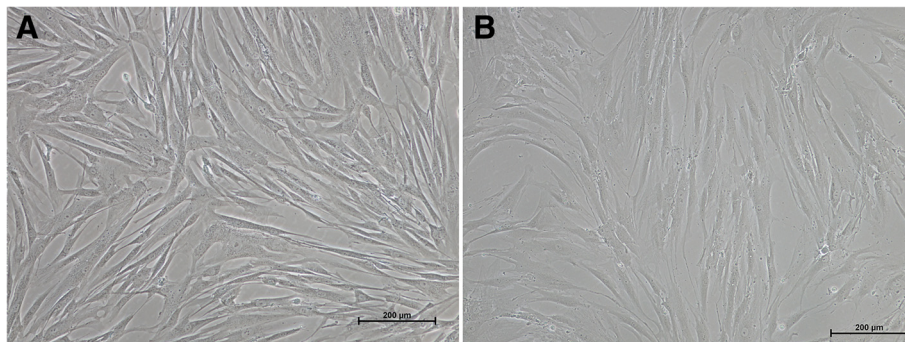


**Fig. 1** Comparison of the morphology of LHMSCs (**a**) and BMMSCs (**b**). Similar to BMMSCs, LHMSCs are spindle-shaped with ovoid nuclei. Both cells are at passage 3. Original magnification: 100×

Wang et al. Stem Cell Research & Therapy (2016) 7:71

Page 3 of 8

## Hepatic differentiation protocol

Iscove's modified Dulbecco's medium with sequential cytokine supplements (Fig. 2) is the most frequently used hepatic differentiation procedure.
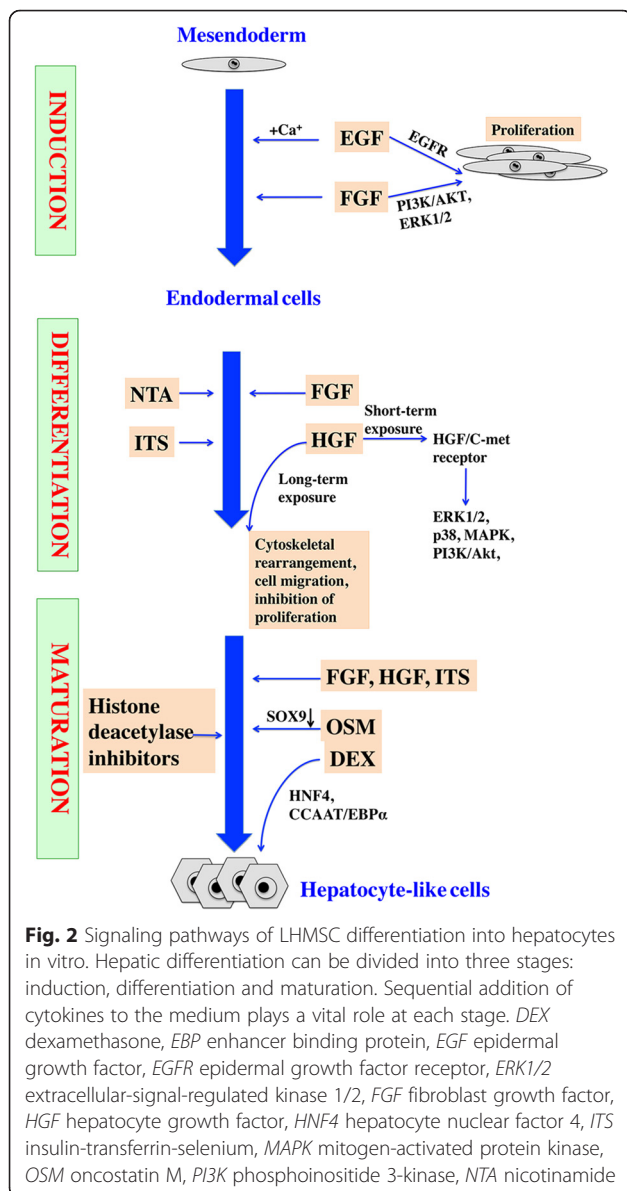
In the initial induction step, MSCs are induced into endodermal cells by epidermal growth factor (EGF) and fibroblast growth factor (FGF). EGF stimulates proliferation of MSCs by binding to EGF receptor (EGFR) [38]. MSCs transfected with an EGF vector and stimulated by $Ca^+$ can differentiate into epithelial-like cells [39]. FGF, which constitutes a family of at least seven closely related polypeptides with heparin-binding properties, plays a pivotal role during the initial stage of endodermal

patterning [40]. Among these, FGF-4 and basic FGF are used conventionally. Similar to EGF, FGF also increases the proliferation rate of MSCs [41–43].

FGF, HGF, nicotinamide (NTA) and insulin-transferrin-selenium (ITS) are commonly added to cultures to trigger cell differentiation. HGF is a pleiotropic cytokine of mesenchymal origin involved in the regulation of proliferation, differentiation, and chemotactic migration of MSCs [44, 45]. Ghaedi et al. [46] cultured adipose stem cells on HGF/collagen I spots for 2 weeks and found increased expression of hepatocyte-specific genes, indicating hepatic induction of HGF. Interestingly, Forte et al. [45] showed that short-term exposure of MSCs to HGF results in activation of the c-met receptor and the downstream effectors, ERK1/2, p38, MAPK, and PI3K/Akt, while long-term exposure resulted in cytoskeletal rearrangement, cell migration, and marked inhibition of proliferation. ITS and NTA promote the proliferation and survival of primary hepatocytes [47, 48]. Chivu et al. [49] compared the differentiation efficacy of various cytokines, including HGF, ITS, dexamethasone and NTA, and reported HGF and NTA to be the most potent inducers.

To induce further maturation, oncostatin M (OSM) and dexamethasone are required, together with the addition of FGF, ITS, and HGF. Zhou et al. [50] demonstrated that HGF promoted a mid/late hepatic phenotype but failed to induce functional hepatocyte maturation. Thus, a further maturation procedure is needed. OSM is a member of the IL-6 subfamily that plays an important role in progression from hepatocyte development to liver maturation [51, 52]. A recent study indicated that the hepatic induction effects of OSM might be correlated with downregulation of sox 9 [53], which enforces proliferation and maintains the pluripotency of stem/progenitor cells [54, 55]. Dexamethasone induces the expression of both HNF4 and CCAAT/EBPα. Both transcription factors are essential for hepatocyte differentiation [56]. Histone deacetylase inhibitors, such as trichostatin A and sodium butyrate, contribute to hepatic differentiation of stem cells [57–60]. Notably, histone deacetylase inhibitors enhance the expression of hepatocyte-specific genes and functions [61] but decrease the adipogenic, chondrogenic, and neurogenic differentiation potential of MSCs [62].

Although several hundred studies have demonstrated the generation of hepatocyte-like cells, the procedure for identifying a differentiated cell as a hepatocyte has not been standardized. The following sequence of tests is recommended to determine the generation of hepatocytes: 1) quantitative reverse transcription polymerase chain reaction (PCR); 2) protein expression evaluation; 3) ultrastructural evaluation; 4) functional analysis; and 5) engraftment, differentiation, and functional repopulation in vivo [63].



**Fig. 2** Signaling pathways of LHMSC differentiation into hepatocytes in vitro. Hepatic differentiation can be divided into three stages: induction, differentiation and maturation. Sequential addition of cytokines to the medium plays a vital role at each stage. *DEX* dexamethasone, *EBP* enhancer binding protein, *EGF* epidermal growth factor, *EGFR* epidermal growth factor receptor, *ERK1/2* extracellular-signal-regulated kinase 1/2, *FGF* fibroblast growth factor, *HGF* hepatocyte growth factor, *HNF4* hepatocyte nuclear factor 4, *ITS* insulin-transferrin-selenium, *MAPK* mitogen-activated protein kinase, *OSM* oncostatin M, *PI3K* phosphoinositide 3-kinase, *NTA* nicotinamide

Wang *et al. Stem Cell Research & Therapy* (2016) 7:71

Page 4 of 8

## Cell therapy for liver disorders

MSCs are considered ideal candidates for cell transplantation due to their immunosuppressive, angiogenic, and anti-inflammatory activities. We discuss the use of LHMSCs and LHMSC conditioned medium (LHMSC-CM) in animal models (Table 1) and the clinic.

### Metabolic disorders

The liver has a capacity for biotransformation. Khuu et al. [27] evaluated the abilities of differentiated LHMSCs to synthesize glucose and to metabolize ammonia as well as xenobiotics. These functions were enhanced after hepatic induction, suggesting the feasibility of using these cells as alternatives to mature hepatocytes for in vitro toxicopharmacological screening. With respect to the activity of phase II drug-metabolizing enzymes, LHMSCs were transplanted into rodents with Crigler-Najjar syndrome to correct hyperbilirubinemic conditions [64]. Furthermore, Baruteau et al. [65] demonstrated high L-phenylalanine hydroxylase (PAH) expression and a marked increase in PAH activity in differentiated LHMSCs, suggesting therapeutic potential for phenylketonuria.

Pre-clinical safety experiments have not shown any increasing risk of tumorigenicity, either in vitro or in vivo [66]. Thus, these cells have been used in clinical trials. Although LHMSCs exhibit immunosuppressive activities, immunosuppressants cannot be ignored. A patient with glycogenosis type 1A was intraportally injected with 3 billion $^{111}$InDTPA-labeled cells and no signal was observed in organs other than the liver, suggesting the feasibility of the therapeutic use of LHMSCs [67]. The engraftment potential of LHMSCs has been explored in patients with ornithine carbamoyltransferase deficiency [68] (clinicaltrials.gov identifier NCT01765283, NCT02489292, NCT02051049).

### Liver regeneration

The liver is unique in terms of its regeneration ability. Depending on the proliferation of residual mature hepatocytes that re-enter the cell cycle and proliferate, normal liver weight can be re-established within 8–15 days in humans (5–7 days in rodents) [69]. The two-thirds partial hepatectomy rodent model is the classic model used to study liver regeneration. Although LHMSCs have been demonstrated to participate in liver regeneration [19, 70], the mechanisms involved have not been clearly elucidated (Fig. 3).
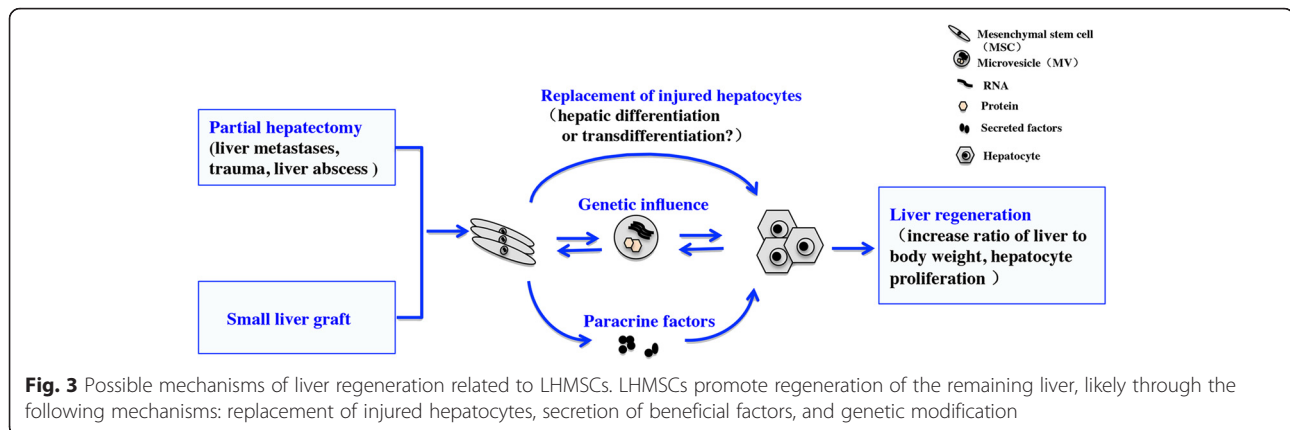
The effect might be due to hepatic differentiation of multipotent stem cells. In situ hepatic differentiation was observed in transplanted mice, which supports this hypothesis. Nevertheless, other data indicate that cells with donor markers and liver-specific markers in the recipient liver are a product of cell fusion rather than a real transdifferentiation [71, 72].

Paracrine signaling is another possible explanation. MSCs secrete various growth factors, cytokines, and chemokines, of which CCL7, vascular endothelial growth factor (VEGF), and CXC family members are involved in anti-inflammatory responses, apoptosis prevention, and angiogenesis [37]. Because secreted factors have no risk of rejection or malignant transformation, LHMSC-CM

**Table 1** Preclinical studies using HLMSCs or HLMSC-CM to treat liver diseases

| Cell type | Number of cells infused | Model | Animal | Administration route | Follow-up period | Efficacy | Ref. |
|---|---|---|---|---|---|---|---|
| LHMSCs | $2.5 \times 10^6$ | Rigler-Najjar type I syndrome | Gunn rats | iv (portal vein) | 6 months | Decrease in bilirubin level | [64] |
| LHMSCs | $1 \times 10^6$ | 20 % hepatectomy | SCID mice | ip | 60 days | Proliferation and differentiation of LHMSCs in vivo | [66] |
| LHMSCs | $1 \times 10^6$ | 70 % partial hepatectomy | uPA+/+-SCID, SCID mice | ip | 56 days | Proliferation and differentiation of LHMSCs in vivo | [19] |
| LHMSC-CM | – | 70 % partial hepatectomy | C57BL/6 mice | Not mentioned | 2 days | Enhanced liver regenerative responses | [73] |
| LHMSCs | $5 \times 10^5$ | Liver fibrosis | NOD/SCID/IL-2Rγ (null) mice | iv (tail vein) | 8 weeks | No benefits observed | [87] |
| LHMSCs | $2 \times 10^5$ | Acute liver injury | SCID mice | iv | 30 days | Proliferation of LHMSCs in vivo | [22] |
| LHMSCs from liver graft preservation fluids | $1 \times 10^6$ | Acute liver injury | NOD/SCID mice | ip | 4 weeks | Differentiation of LHMSCs in vivo | [20] |
| MSCs, LHMSC-CM | $2 \times 10^6$ (iv), $3 \times 10^7$ (ip), $5 \times 10^5$ or $2 \times 10^5$ (LP) | Acute liver failure | SCID mice | iv, ip, LP | 21 days | Increased survival rates, decrease in liver metabolic enzymes and ammonium | [81] |

*ip* intrasplenic injection, *iv* intravenous injection, *LHMSC* liver-derived human mesenchymal stem cell, *LHMSC-CM* liver-derived human mesenchymal stem cell conditioned medium, *LP* injection via liver parenchyma, *MSC* mesenchymal stem cell

Wang et al. Stem Cell Research & Therapy (2016) 7:71

Page 5 of 8



**Fig. 3** Possible mechanisms of liver regeneration related to LHMSCs. LHMSCs promote regeneration of the remaining liver, likely through the following mechanisms: replacement of injured hepatocytes, secretion of beneficial factors, and genetic modification

might be more beneficial than MSCs. In pre-clinical experiments, the efficacy of application of LHMSC-secreted factors in 70 % hepatectomized mice suggests their potential use in patients undergoing extensive liver resection or transplantation of small liver grafts [73].

The genetic influence of microvesicles (MVs) has also been investigated. MVs are heterogeneous circular membrane fragments that comprise two major populations: exosomes and microparticles. Exosomes originate from the exosomal compartment and are 30–100 nm in diameter. In contrast, microparticles are released directly from budding of the plasma membrane surface and are 100 nm to 1 μm in diameter. The biological significance of MVs was largely overlooked for many years. Recently, they have been recognized to carry proteins, micro-RNAs, and mRNAs, and to play important roles in cell-to-cell communication [74]. Quesenberry et al. [75] proposed a novel concept known as areas of influence, which refers to the influence of complex effectors on the lability of the stem cell phenotype. Areas of influence include cell cycle passage, complex interactions with stromal cells, and MV-mediated cell-to-cell transfer of genetic information. Stem cells undergo functional and phenotypic changes after receiving genetic information, transferred by MVs, from injured cells. Moreover, feedback from stem cells alters the functions of target cells, suggesting that stem cells repair damaged tissues without directly replacing parenchymal cells [76, 77]. The protective effect of MVs from LHMSCs was confirmed by Herrera et al., who reported that MVs shuttled mRNAs into hepatocytes of hepatectomized rats and accelerated hepatic regeneration [78].

### Acute liver injury

Acute liver failure, a lethal clinical syndrome, is characterized by rapid development of hepatocellular dysfunction with diffuse intrahepatic infiltration of inflammatory cells and massive multilobular necrosis. Based on their release of trophic and immunomodulatory factors, MSCs

are commonly used in the treatment of acute liver injury. Parekkadan et al. [79, 80] postulated that soluble factors present in MSC-conditioned medium (IL-6, VEGF, and HGF) were responsible for both local and systemic therapeutic effects. Herrera et al. [81] reported that LHMSCs also significantly prevented death in a fatal model of fulminant liver failure. They further stated that the therapeutic effect was due to a paracrine mechanism.

### Liver cirrhosis

Liver cirrhosis, the most advanced stage of fibrosis, connotes not only more scarring than that from fibrosis alone, but also distortion of the liver parenchyma associated with septae and nodule formation, altered blood flow, and risk of liver failure [82]. The activation of HSCs is a pivotal event in the development of liver cirrhosis [12]. It seems that the anti-fibrotic effects of MSCs in liver cirrhosis are based on the release of factors that alter the function of HSCs. HGF is expressed highly in LHMSCs. Overexpression of HGF promotes HSC apoptosis [83, 84]. Moreover, HGF is associated with hepatogenesis. The plasma HGF level increases considerably after partial hepatectomy [85]. Compared with BMMSC conditioned medium, the HGF level was ~50-fold higher in LHMSC-CM [81]. IL-10 and tumor necrosis factor (TNF)-α also reduce the proliferation of HSCs and synthesis of collagen type I [37, 79]. Moreover, LHMSCs can secrete IFN-γ, inducing anti-fibrotic effects [86].

However, in a murine model of CCl4-induced liver fibrosis, intravenous administration of LHMSCs failed to improve liver function [87] and the injected cells propagated in various tissues; less effective transplantation may explain the failure. To promote MSCs homing to the liver, the following approaches can be employed: 1) direct injection, such as injection via the portal vein, spleen, and liver parenchyma; and 2) use of MSCs modified by liver-specific receptors.

Wang *et al. Stem Cell Research & Therapy* (2016) 7:71

Page 6 of 8

## Conclusions

As a novel type of MSC, LHMSCs exhibit general properties of MSCs, including self-renewal, multipotent differentiation and immunomodulation. Compared with other types of MSCs, LHMSCs have several advantages, such as more secretion of protective factors. However, the following problems must be addressed: 1) how to induce differentiation of LHMSCs into functional cells that are more similar to primary hepatocytes; 2) identifying the criteria for evaluation of the degree of hepatic differentiation of LHMSC-derived cells; 3) improvement of the efficiency of cell homing to the liver after transplantation; 4) determining the number of cells required for various diseases; and 5) identifying the possible risks of LHMSC transplantation. Thus, further studies are needed to characterize LHMSCs, improve the efficacy of hepatic differentiation, and validate their therapeutic potential in liver diseases.

### Abbreviations

BMMSC: bone marrow-derived mesenchymal stem cell; EGF: epidermal growth factor; EGFR: epidermal growth factor receptor; FGF: fibroblast growth factor; HGF: hepatocyte growth factor; HLA: human leukocyte antigen; HSC: hepatic stellate cell; IFN: interferon; IL: interleukin; ITS: insulin-transferrin-selenium; LHMSC-CM: liver-derived human mesenchymal stem cell conditioned medium; LHMSC: liver-derived human mesenchymal stem cell; MHC: major histocompatibility complex; MSC: mesenchymal stem cell; MV: microvesicle; NTA: nicotinamide; OLT: orthotropic liver transplantation; OSM: oncostatin M; PAH: L-phenylalanine hydroxylase; VEGF: vascular endothelial growth factor.

### Competing interests

The authors declare that they have no competing interests.

### Authors' contributions

YW contributed to conception and writing of the manuscript; XY and EC participated in conception and acquisition of data; and LL contributed to conception of the manuscript. All authors read and approved the final manuscript.

### References

1. Hengstler JG, Brulport M, Schormann W, Bauer A, Hermes M, Nussler AK, et al. Generation of human hepatocytes by stem cell technology: definition of the hepatocyte. Expert Opin Drug Metab Toxicol. 2005;1:61–74.
2. Alison MR, Islam S, Lim SM. Cell therapy for liver disease. Curr Opin Mol Ther. 2009;11:364–74.
3. Gramignoli R, Tahan V, Dorko K, Skvorak KJ, Hansel MC, Zhao W, et al. New potential cell source for hepatocyte transplantation: discarded livers from metabolic disease liver transplants. Stem Cell Res. 2013;11:563–73.
4. Bonavita AG, Quaresma K, Cotta-de-Almeida V, Pinto MA, Saraiva RM, Alves LA. Hepatocyte xenotransplantation for treating liver disease. Xenotransplantation. 2010;17:181–7.
5. Heo JS, Choi SM, Kim HO, Kim EH, You J, Park T, et al. Neural transdifferentiation of human bone marrow mesenchymal stem cells on hydrophobic polymer-modified surface and therapeutic effects in an animal model of ischemic stroke. Neuroscience. 2013;238:305–18.
6. Castro FO, Torres A, Cabezas J, Rodriguez-Alvarez L. Combined use of platelet rich plasma and vitamin C positively affects differentiation in vitro to mesodermal lineage of adult adipose equine mesenchymal stem cells. Res Vet Sci. 2014;96:95–101.
7. Kode JA, Mukherjee S, Joglekar MV, Hardikar AA. Mesenchymal stem cells: immunobiology and role in immunomodulation and tissue regeneration. Cytotherapy. 2009;11:377–91.
8. Liu D, Xu J, Liu O, Fan Z, Liu Y, Wang F, et al. Mesenchymal stem cells derived from inflamed periodontal ligaments exhibit impaired immunomodulation. J Clin Periodontol. 2012;39:1174–82.
9. Bessout R, Semont A, Demarquay C, Charcosset A, Benderitter M, Mathieu N. Mesenchymal stem cell therapy induces glucocorticoid synthesis in colonic mucosa and suppresses radiation-activated T cells: new insights into MSC immunomodulation. Mucosal Immunol. 2014;7:656–69.
10. Kuo TK, Hung SP, Chuang CH, Chen CT, Shih YR, Fang SC, et al. Stem cell therapy for liver disease: parameters governing the success of using bone marrow mesenchymal stem cells. Gastroenterology. 2008;134:2111–21. 21 e1-3.
11. Ma XR, Tang YL, Xuan M, Chang Z, Wang XY, Liang XH. Transplantation of autologous mesenchymal stem cells for end-stage liver cirrhosis: a meta-analysis based on seven controlled trials. Gastroenterol Res Pract. 2015;2015: 908275.
12. Volarevic V, Nurkovic J, Arsenijevic N, Stojkovic M. Concise review: Therapeutic potential of mesenchymal stem cells for the treatment of acute liver failure and cirrhosis. Stem Cells. 2014;32:2818–23.
13. Friedenstein AJ, Deriglasova UF, Kulagina NN, Panasuk AF, Rudakowa SF, Luria EA, et al. Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method. Exp Hematol. 1974;2:83–92.
14. Bieback K, Kern S, Kocaomer A, Ferlik K, Bugert P. Comparing mesenchymal stromal cells from different human tissues: bone marrow, adipose tissue and umbilical cord blood. Biomed Mater Eng. 2008;18:S71–6.
15. da Silva ML, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci. 2006;119:2204–13.
16. Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood. 2001;98:2396–402.
17. in 't Anker PS, Noort WA, Scherjon SA, Kleijburg-van der Keur C, Kruisselbrink AB, van Bezooijen RL, et al. Mesenchymal stem cells in human second-trimester bone marrow, liver, lung, and spleen exhibit a similar immunophenotype but a heterogeneous multilineage differentiation potential. Haematologica. 2003;88:845–52.
18. Larijani B, Aghayan HR, Goodarzi P, Arjmand B. GMP-grade human fetal liver-derived mesenchymal stem cells for clinical transplantation. Methods Mol Biol. 2015;1283:123–36.
19. Najimi M, Khuu DN, Lysy PA, Jazouli N, Abarca J, Sempoux C, et al. Adult-derived human liver mesenchymal-like cells as a potential progenitor reservoir of hepatocytes? Cell Transplant. 2007;16:717–28.
20. Pan Q, Fouraschen SM, Kaya FS, Verstegen MM, Pescatori M, Stubbs AP, et al. Mobilization of hepatic mesenchymal stem cells from human liver grafts. Liver Transpl. 2011;17:596–609.
21. Lee JH, Park HJ, Kim YA, Lee DH, Noh JK, Kwon CH, et al. The phenotypic characteristic of liver-derived stem cells from adult human deceased donor liver. Transplant Proc. 2012;44:1110–2.
22. Herrera MB, Bruno S, Buttiglieri S, Tetta C, Gatti S, Deregibus MC, et al. Isolation and characterization of a stem cell population from adult human liver. Stem Cells. 2006;24:2840–50.
23. Brisby H, Papadimitriou N, Brantsing C, Bergh P, Lindahl A, Barreto Henriksson H. The presence of local mesenchymal progenitor cells in human degenerated intervertebral discs and possibilities to influence these in vitro: a descriptive study in humans. Stem Cells Dev. 2013;22:804–14.
24. Duret C, Gerbal-Chaloin S, Ramos J, Fabre JM, Jacquet E, Navarro F, et al. Isolation, characterization, and differentiation to hepatocyte-like cells of nonparenchymal epithelial cells from adult human liver. Stem Cells. 2007;25:1779–90.
25. Lee JH, Park HJ, Kim YA, Lee DH, Noh JK, Kwon CH, et al. Differentiation and major histocompatibility complex antigen expression in human liver-derived stem cells. Transplant Proc. 2012;44:1113–5.
26. Sana G, Lombard C, Vosters O, Jazouli N, Andre F, Stephenne X, et al. Adult human hepatocytes promote CD4(+) T-cell hyporesponsiveness via interleukin-10-producing allogeneic dendritic cells. Cell Transplant. 2014;23:1127–42.
27. Khuu DN, Scheers I, Ehnert S, Jazouli N, Nyabi O, Buc-Calderon P, et al. In vitro differentiated adult human liver progenitor cells display mature hepatic metabolic functions: a potential tool for in vitro pharmacotoxicological testing. Cell Transplant. 2011;20:287–302.

Wang *et al. Stem Cell Research & Therapy* (2016) 7:71

Page 7 of 8

28. D'Ippolito G, Schiller PC, Ricordi C, Roos BA, Howard GA. Age-related osteogenic potential of mesenchymal stromal stem cells from human vertebral bone marrow. J Bone Miner Res. 1999;14:1115–22.

29. Gotherstrom C, West A, Liden J, Uzunel M, Lahesmaa R, Le Blanc K. Difference in gene expression between human fetal liver and adult bone marrow mesenchymal stem cells. Haematologica. 2005;90:1017–26.

30. Giuliani M, Fleury M, Vernochet A, Ketroussi F, Clay D, Azzarone B, et al. Long-lasting inhibitory effects of fetal liver mesenchymal stem cells on T-lymphocyte proliferation. PLoS One. 2011;6:e19988.

31. Nasef A, Mathieu N, Chapel A, Frick J, Francois S, Mazurier C, et al. Immunosuppressive effects of mesenchymal stem cells: involvement of HLA-G. Transplantation. 2007;84:231–7.

32. Raicevic G, Najar M, Najimi M, El Taghdouini A, van Grunsven LA, Sokal E, et al. Influence of inflammation on the immunological profile of adult-derived human liver mesenchymal stromal cells and stellate cells. Cytotherapy. 2015;17: 174–85.

33. Campioni D, Rizzo R, Stignani M, Melchiorri L, Ferrari L, Moretti S, et al. A decreased positivity for CD90 on human mesenchymal stromal cells (MSCs) is associated with a loss of immunosuppressive activity by MSCs. Cytometry B Clin Cytom. 2009;76:225–30.

34. Espanol-Suner R, Carpentier R, Van Hul N, Legry V, Achouri Y, Cordi S, et al. Liver progenitor cells yield functional hepatocytes in response to chronic liver injury in mice. Gastroenterology. 2012;143:1564–75. e7.

35. Kordes C, Sawitza I, Muller-Marbach A, Ale-Agha N, Keitel V, Klonowski-Stumpe H, et al. CD133+ hepatic stellate cells are progenitor cells. Biochem Biophys Res Commun. 2007;352:410–7.

36. Vinas O, Bataller R, Sancho-Bru P, Gines P, Berenguer C, Enrich C, et al. Human hepatic stellate cells show features of antigen-presenting cells and stimulate lymphocyte proliferation. Hepatology. 2003;38:919–29.

37. Berardis S, Lombard C, Evraerts J, El Taghdouini A, Rosseels V, Sancho-Bru P, et al. Gene expression profiling and secretome analysis differentiate adult-derived human liver stem/progenitor cells and human hepatic stellate cells. PLoS One. 2014;9:e86137.

38. Tamama K, Fan VH, Griffith LG, Blair HC, Wells A. Epidermal growth factor as a candidate for ex vivo expansion of bone marrow-derived mesenchymal stem cells. Stem Cells. 2006;24:686–95.

39. Wu M, Zhou T, Liu H. Ca(2+) and EGF induce the differentiation of human embryo mesenchymal stem cells into epithelial-like cells. Cell Biol Int. 2015; 39:852–7.

40. Jung J, Zheng M, Goldfarb M, Zaret KS. Initiation of mammalian liver development from endoderm by fibroblast growth factors. Science. 1999; 284:1998–2003.

41. Farre J, Roura S, Prat-Vidal C, Soler-Botija C, Llach A, Molina CE, et al. FGF-4 increases in vitro expansion rate of human adult bone marrow-derived mesenchymal stem cells. Growth Factors. 2007;25:71–6.

42. Salehinejad P, Alitheen NB, Mandegary A, Nematollahi-Mahani SN, Janzamin E. Effect of EGF and FGF on the expansion properties of human umbilical cord mesenchymal cells. In Vitro Cell Dev Biol Anim. 2013;49:515–23.

43. Choi SC, Kim SJ, Choi JH, Park CY, Shim WJ, Lim DS. Fibroblast growth factor-2 and -4 promote the proliferation of bone marrow mesenchymal stem cells by the activation of the PI3K-Akt and ERK1/2 signaling pathways. Stem Cells Dev. 2008;17:725–36.

44. Neuss S, Becher E, Woltje M, Tietze L, Jahnen-Dechent W. Functional expression of HGF and HGF receptor/c-met in adult human mesenchymal stem cells suggests a role in cell mobilization, tissue repair, and wound healing. Stem Cells. 2004;22:405–14.

45. Forte G, Minieri M, Cossa P, Antenucci D, Sala M, Gnocchi V, et al. Hepatocyte growth factor effects on mesenchymal stem cells: proliferation, migration, and differentiation. Stem Cells. 2006;24:23–33.

46. Ghaedi M, Tuleuova N, Zern MA, Wu J, Revzin A. Bottom-up signaling from HGF-containing surfaces promotes hepatic differentiation of mesenchymal stem cells. Biochem Biophys Res Commun. 2011;407:295–300.

47. Hong SH, Gang EJ, Jeong JA, Ahn C, Hwang SH, Yang IH, et al. In vitro differentiation of human umbilical cord blood-derived mesenchymal stem cells into hepatocyte-like cells. Biochem Biophys Res Commun. 2005;330: 1153–61.

48. Sato F, Mitaka T, Mizuguchi T, Mochizuki Y, Hirata K. Effects of nicotinamide-related agents on the growth of primary rat hepatocytes and formation of small hepatocyte colonies. Liver. 1999;19:481–8.

49. Chivu M, Dima SO, Stancu CI, Dobrea C, Uscatescu V, Necula LG, et al. In vitro hepatic differentiation of human bone marrow mesenchymal stem

50. Zhou QJ, Xiang LX, Shao JZ, Hu RZ, Lu YL, Yao H, et al. In vitro differentiation of hepatic progenitor cells from mouse embryonic stem cells induced by sodium butyrate. J Cell Biochem. 2007;100:29–42.

51. Kinoshita T, Sekiguchi T, Xu MJ, Ito Y, Kamiya A, Tsuji K, et al. Hepatic differentiation induced by oncostatin M attenuates fetal liver hematopoiesis. Proc Natl Acad Sci U S A. 1999;96:7265–70.

52. Miyajima A, Kinoshita T, Tanaka M, Kamiya A, Mukouyama Y, Hara T. Role of oncostatin M in hematopoiesis and liver development. Cytokine Growth Factor Rev. 2000;11:177–83.

53. Paganelli M, Nyabi O, Sid B, Evraerts J, El Malmi I, Heremans Y, et al. Downregulation of Sox9 expression associates with hepatogenic differentiation of human liver mesenchymal stem/progenitor cells. Stem Cells Dev. 2014;23:1377–91.

54. Seymour PA, Freude KK, Tran MN, Mayes EE, Jensen J, Kist R, et al. SOX9 is required for maintenance of the pancreatic progenitor cell pool. Proc Natl Acad Sci U S A. 2007;104:1865–70.

55. Stockl S, Bauer RJ, Bosserhoff AK, Gottl C, Grifka J, Grassel S. Sox9 modulates cell survival and adipogenic differentiation of multipotent adult rat mesenchymal stem cells. J Cell Sci. 2013;126:2890–902.

56. Michalopoulos GK, Bowen WC, Mule K, Luo J. HGF-, EGF-, and dexamethasone-induced gene expression patterns during formation of tissue in hepatic organoid cultures. Gene Expr. 2003;11:55–75.

57. Yin L, Zhu Y, Yang J, Ni Y, Zhou Z, Chen Y, et al. Adipose tissue-derived mesenchymal stem cells differentiated into hepatocyte-like cells in vivo and in vitro. Mol Med Rep. 2015;11:1722–32.

58. Snykers S, Vanhaecke T, De Becker A, Papeleu P, Vinken M, Van Riet I, et al. Chromatin remodeling agent trichostatin A: a key-factor in the hepatic differentiation of human mesenchymal stem cells derived of adult bone marrow. BMC Dev Biol. 2007;7:24.

59. Zhang Q, Yang Y, Zhang J, Wang GY, Liu W, Qiu DB, et al. Efficient derivation of functional hepatocytes from mouse induced pluripotent stem cells by a combination of cytokines and sodium butyrate. Chin Med J (Engl). 2011;124:3786–93.

60. Sharma NS, Shikhanovich R, Schloss R, Yarmush ML. Sodium butyrate-treated embryonic stem cells yield hepatocyte-like cells expressing a glycolytic phenotype. Biotechnol Bioeng. 2006;94:1053–63.

61. Alizadeh E, Eslaminejad MB, Akbarzadeh A, Sadeghi Z, Abasi M, Herizchi R, et al. Upregulation of MiR-122 via trichostatin A treatments in hepatocyte-like cells derived from mesenchymal stem cells. Chem Biol Drug Des. 2016; 87:296-305.

62. Lee S, Park JR, Seo MS, Roh KH, Park SB, Hwang JW, et al. Histone deacetylase inhibitors decrease proliferation potential and multilineage differentiation capability of human mesenchymal stem cells. Cell Prolif. 2009;42:711–20.

63. Sancho-Bru P, Najimi M, Caruso M, Pauwelyn K, Cantz T, Forbes S, et al. Stem and progenitor cells for liver repopulation: can we standardise the process from bench to bedside? Gut. 2009;58:594–603.

64. Maerckx C, Tondreau T, Berardis S, van Pelt J, Najimi M, Sokal E. Human liver stem/progenitor cells decrease serum bilirubin in hyperbilirubinemic Gunn rat. World J Gastroenterol. 2014;20:10553–63.

65. Baruteau J, Nyabi O, Najimi M, Fauvart M, Sokal E. Adult human liver mesenchymal progenitor cells express phenylalanine hydroxylase. J Pediatr Endocrinol Metab. 2014;27:863–8.

66. Scheers I, Maerckx C, Khuu DN, Marcelle S, Decottignies A, Najimi M, et al. Adult-derived human liver progenitor cells in long-term culture maintain appropriate gatekeeper mechanisms against transformation. Cell Transplant. 2012;21:2241–55.

67. Defresne F, Tondreau T, Stephenne X, Smets F, Bourgois A, Najimi M, et al. Biodistribution of adult derived human liver stem cells following intraportal infusion in a 17-year-old patient with glycogenosis type 1A. Nucl Med Biol. 2014;41:371–5.

68. Sokal EM, Stephenne X, Ottolenghi C, Jazouli N, Clapuyt P, Lacaille F, et al. Liver engraftment and repopulation by in vitro expanded adult derived human liver stem cells in a child with ornithine carbamoyltransferase deficiency. JIMD Rep. 2014;13:65–72.

69. Michalopoulos GK. Liver regeneration. J Cell Physiol. 2007;213:286–300.

70. Khuu DN, Nyabi O, Maerckx C, Sokal E, Najimi M. Adult human liver mesenchymal stem/progenitor cells participate in mouse liver regeneration after hepatectomy. Cell Transplant. 2013;22:1369–80.

Wang *et al. Stem Cell Research & Therapy* (2016) 7:71

Page 8 of 8

71. Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimy M, et al. Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature. 2003;422:897–901.

72. Vassilopoulos G, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. Nature. 2003;422:901–4.

73. Fouraschen SM, Pan Q, de Ruiter PE, Farid WR, Kazemier G, Kwekkeboom J, et al. Secreted factors of human liver-derived mesenchymal stem cells promote liver regeneration early after partial hepatectomy. Stem Cells Dev. 2012;21:2410–9.

74. Ratajczak J, Wysoczynski M, Hayek F, Janowska-Wieczorek A, Ratajczak MZ. Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication. Leukemia. 2006;20:1487–95.

75. Quesenberry PJ, Aliotta JM. The paradoxical dynamism of marrow stem cells: considerations of stem cells, niches, and microvesicles. Stem Cell Rev. 2008;4:137–47.

76. Deregibus MC, Cantaluppi V, Calogero R, Lo Iacono M, Tetta C, Biancone L, et al. Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. Blood. 2007;110:2440–8.

77. Bruno S, Grange C, Deregibus MC, Calogero RA, Saviozzi S, Collino F, et al. Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. J Am Soc Nephrol. 2009;20:1053–67.

78. Herrera MB, Fonsato V, Gatti S, Deregibus MC, Sordi A, Cantarella D, et al. Human liver stem cell-derived microvesicles accelerate hepatic regeneration in hepatectomized rats. J Cell Mol Med. 2010;14:1605–18.

79. Parekkadan B, van Poll D, Megeed Z, Kobayashi N, Tilles AW, Berthiaume F, et al. Immunomodulation of activated hepatic stellate cells by mesenchymal stem cells. Biochem Biophys Res Commun. 2007;363:247–52.

80. Parekkadan B, van Poll D, Suganuma K, Carter EA, Berthiaume F, Tilles AW, et al. Mesenchymal stem cell-derived molecules reverse fulminant hepatic failure. PLoS One. 2007;2:e941.

81. Herrera MB, Fonsato V, Bruno S, Grange C, Gilbo N, Romagnoli R, et al. Human liver stem cells improve liver injury in a model of fulminant liver failure. Hepatology. 2013;57:311–9.

82. Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology. 2008; 134:1655–69.

83. Narmada BC, Chia SM, Tucker-Kellogg L, Yu H. HGF regulates the activation of TGF-beta1 in rat hepatocytes and hepatic stellate cells. J Cell Physiol. 2013;228:393–401.

84. Horiguchi K, Hirano T, Ueki T, Hirakawa K, Fujimoto J. Treating liver cirrhosis in dogs with hepatocyte growth factor gene therapy via the hepatic artery. J Hepatobiliary Pancreat Surg. 2009;16:171–7.

85. Lindroos PM, Zarnegar R, Michalopoulos GK. Hepatocyte growth factor (hepatopoietin A) rapidly increases in plasma before DNA synthesis and liver regeneration stimulated by partial hepatectomy and carbon tetrachloride administration. Hepatology. 1991;13:743–50.

86. Rockey DC, Chung JJ. Interferon gamma inhibits lipocyte activation and extracellular matrix mRNA expression during experimental liver injury: implications for treatment of hepatic fibrosis. J Investig Med. 1994;42:660–70.

87. Briquet A, Gregoire C, Comblain F, Servais L, Zeddou M, Lechanteur C, et al. Human bone marrow, umbilical cord or liver mesenchymal stromal cells fail to improve liver function in a model of CCl4-induced liver damage in NOD/SCID/IL-2Rgamma(null) mice. Cytotherapy. 2014;16:1511–8.