Journal of Bone Research
ISSN: 2572-4916
Othman, J Bone Res 2016, 4:1
DOI: 10.4172/2572-4916.1000166
Research Article
Open Access

Mesenchymal Stem Cells as a Potential Source of Hepatocyte like Cells

Amira Othman[1,2*] **and Mai Hassan**[3]

[1]*Faculty of Medicine, Helwan University, Cairo, Egypt*

[2]*Mansoura Research Center for Cord Stem Cells, Mansoura University, Mansoura, Egypt*

[3]*Faculty of Medicine, Mansoura University, Mansoura, Egypt*

[*]**Corresponding author:** Amira Othman, Al Sikka Al Hadid Al Gharbeya, Qism Helwan, Cairo Governorate, Egypt, Tel: +20 2 25541912; E-mail: Amira.Osman@hq.helwan.edu.eg

Received date: Feb 25, 2016; **Accepted date:** Mar 11, 2016; **Published date:** Mar 15, 2016

Copyright: © 2016 Othman A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

The field of regenerative medicine is growing up rapidly in the last decades, the usage of cellular therapy such as stem cells start to grasp attention in many experimental and even clinical trial.

In this article, we mainly concentrate on the potentiality of the mesenchymal stem cell (MSC) to differentiate into hepatocyte like cells, which can overcome the problem of donor's shortage. MSC have been use lately in many *in vitro* and *in vivo* experiments, and have showed great capacity of multilinage differentiation, interestingly they have the capacity to differentiate into hepatocyte like cells. Currently many clinical trials started to involve the usage of self-MSC as a novel therapy for hepatic complication such as fibrosis or cirrhosis, which showed promising results in treatment of hepatic patients. MSC can be injected in hepatic patient to replace the cirrhotic liver, which gives a real hope for the hepatic patients as a reliable source for regeneration.

Keywords: Stem cells; Mesencymal stem cells; Hepatocyte like cells; Mesencymal stem cell culture

Introduction

Research in the field of stem cells grew out by the findings of Ernest A. McCulloch and James E. Till the two Canadian scientists. As early as 1960s McCulloch and Till initiated a series of experiments by injecting cells from bone marrow into irradiated mice, they observed newly formed nodules inside the spleens of these mice, equal in number to the injected bone marrow cells. They agreed to call these nodules 'spleen colonies', in which they concluded that each nodule arose from a single marrow cell [1]. Stem cells (SC) are further defined as the primitive cells that have the ability for extensive self-renewal and the capacity to differentiate into several cell lineages [2]. Stem cells are classified either as embryonic or adult, depending on their respective origins; embryonic stem cell (ESC) arises from blastocyst-stage while adult stem cell arises from niches of mature adult tissues and bone marrow [3].

There are three main characters of the stem cells. Firstly, they must be capable of self-renewal, i.e. SC population is maintained by undergoing symmetric or asymmetric divisions. In symmetric division, the two daughter cells preserve full SC characteristics, while only one of the two daughter cells preserves SC characteristics in asymmetric division and the other continues into a differentiation pathway. Secondly, SC should have the competency to differentiate into multiple cell lineage. Thirdly, SC should be capable of functional activity of a given tissue *in vivo* [4].

Stem cell potency

The term potency identifies the differentiation potential of the SC; i.e. the potential capability to differentiate into numerous cell types [5].

ESCs derived from the inner cell mass in the blastocyst stage, are totipotent which means that these cells can differentiate into embryonic and extraembryonic tissue. They can be propagated indefinitely *in vivo* and *in vitro*. When engrafted into immunodeficient mice. ESCs develop into teratocarcinoma tumors with mesoderm, endoderm, and ectoderm [6].

Pluripotent stem cells are the progenies of totipotent cells and have the capacity to differentiate into three germ layers: (mesoderm, endoderm, and ectoderm) [7]. While multipotent stem cells is capable of producing cells of a strictly related family of cells such as hematopoietic stem cells in which they differentiate into white blood cells, red blood cells, platelets [8]. Lastly unipotent stem cell cells can only give progeny to one cell type, besides having self-renewal capacity distinguishing them from non-stem cells e.g. muscle stem cells [9].

Stem cell and tissue regeneration

Two physiological mechanisms have been proposed for tissue regeneration in organ recovery in adults; the first is the restoration of differentiated cells by a new cell populations originated from traveling SCs in the blood. Hematopoietic cell renewal is a classic example of this kind of mechanism. While the other mechanism is the self-repair mechanism of adult differentiated functioning cells that still possess proliferative activity; hepatocytes, endothelial cells, smooth muscle cells, keratinocytes, and fibroblasts are typical example of this type [10].

Mesenchymal Stem Cells

Mesenchymal Stem Cells (MSCs) are stem cells that have the capability to differentiate into muscle, bone, cartilage, ligament, tendon, and adipose tissue, MSC can participate in the regeneration process of the these tissues. They also have the capacity to replicate

many times in culture while preserving their ability to grow in multilineage differentiation. Morphologically MSCs are described by having a small cell body with a few long cell processes [11].

Criteria for defining MSC have been approved by The International Society for Cellular Therapy (ISCT). For the cell to be classified as MSC it must have the property of adherence to the plastic in the normal culture environment besides having fibroblast like morphology [12].

MSCs can undergo osteogenic, chondrogenic, and adipogenic differentiation *in vitro*. Cultured MSCs can express CD73, CD105, CD44, CD29 and CD90 as surface markers while negatively expresse CD45, CD34, CD14, CD11b, CD19, CD79a and HLA-DR [13].

MSCs hold a unique criterion of SC which is homing, this indicates the capacity of the MSC migrate to the specific injured site [14].

Phenotypically, MSCs express multiple markers, but none of them unfortunately, is unique to MSCs. It is well known that the human adult MSCs usually do not explicit the very well-known hematopoietic stem cell markers such as CD34, CD45, CD11, nor CD14. Many co-stimulatory markers are also not expressed by MSCs such as CD86, CD80, the adhesion molecules, CD40 or CD31, CD18, PECAM 1 (platelet endothelial cell adhesion molecule 1), nor CD56 (neuronal cell adhesion molecule-1). On the other hand they can express CD73 , CD44, CD105 , CD90 (Thy-1), Stro-1 and CD71, besides the adhesion molecule vascular cell adhesion molecule (VCAM-1 or CD106) , and are known to express CD166 that is known as activated leukocyte cell adhesion molecule (ALCAM), intercellular adhesion molecule (ICAM)-1, and CD29 as well [15].

Numerous reports illustrated the segregation of human and non-human MSCs by positive antibodies selection of MSCs based on their phenotypical characterization. Other trials have used negative selection method to augment MSCs, while as hematopoietic cell lineage was removed [16,17] (Figure 1).

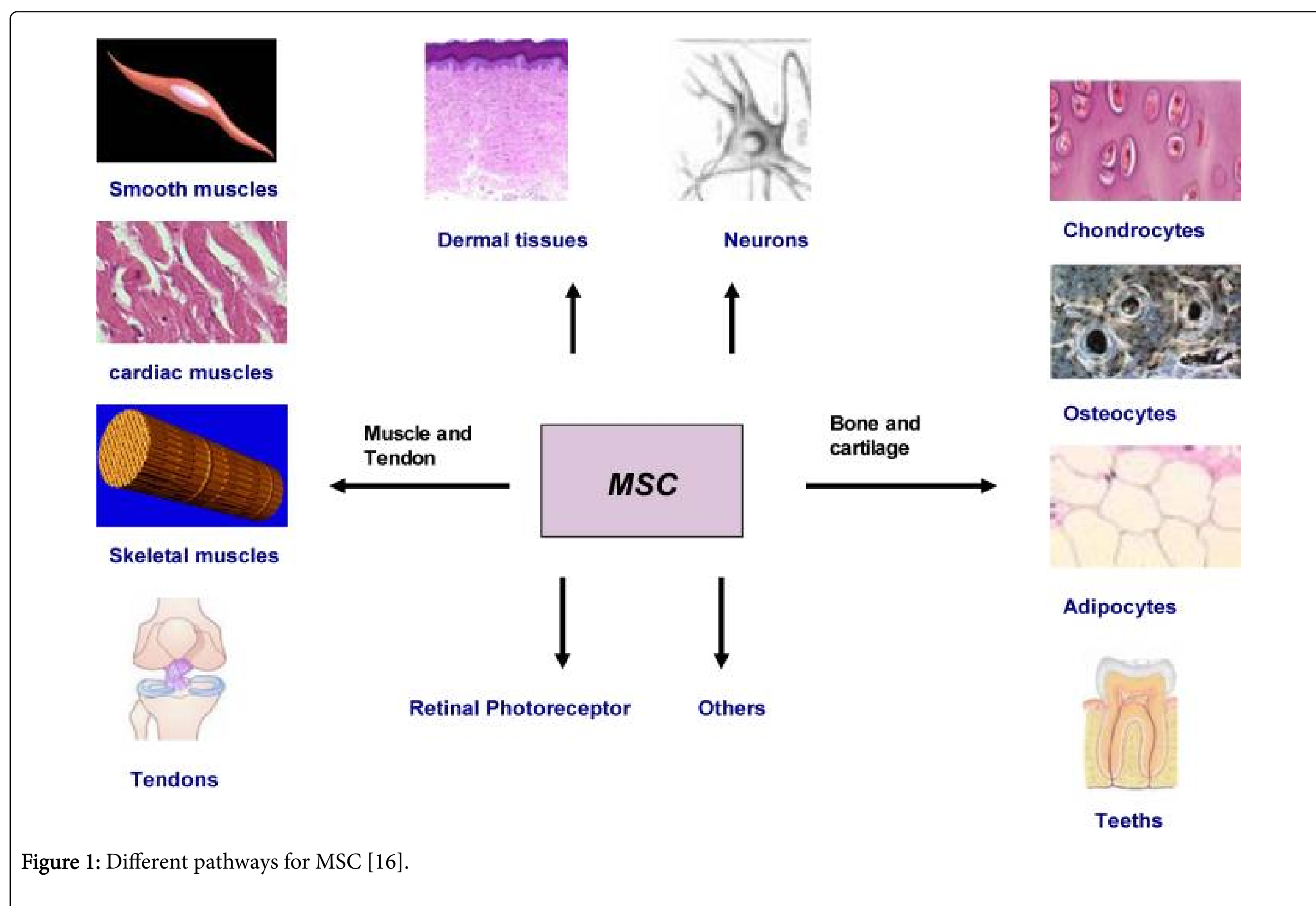

Figure 1: Different pathways for MSC [16].

Difference between human MSCs and other species

There are a dissimilarity between human MSCs and other species in expression of some molecules; for example, while human and rat MSCs do not express CD34, some reports published variable level of expression of CD34 on murine MSCs cell surface [18].

Effect of growth factors on MSC expansion

Many studies showed the effect of growth factors on MSC expansion, for the example the effect fibroblast growth factor 2 (FGF-2) on fibroblast colony formation from human MSC was studied; it showed proliferation and osteogenic differentiation of human MSC. This proved that FGF-2 had valuable effect on proliferation and bone differentiation from MSC *in vitro* [19]. Moreover, FGF-2 can maintain MSC's chondrogenic differentiation effect even after 50 doubling [20].

Serum free media augmented with and L-ascorbate and dexamethasone (Dex) were found to be crucial for colonies formation from stem cell population derived from bone marrow. These MSCs express cell markers such as STRO-1+ and STRO-1+ /VCAM-1+ whereas EGF and platelet derived growth factor (PDGF) were found to play a pivotal role in colony growth support [21].

In another study human multipotent adult progenitor, cells (MAPC) have been isolated in a serum free media containing endothelial growth factor (EGF), PDGF and insulin-like growth factor-1 (IGF-1) with dexamethasone, ascorbic acid [22] (Figure 2 and Table 1).

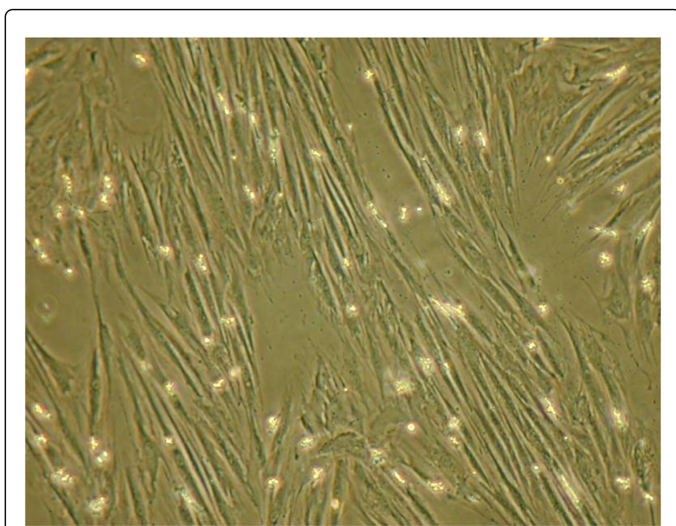

Figure 2: Mesenchymal stem cell in culture [16].

1	Adherence to plastic in standard culture conditions		
2	Phenotype	Positive	Negative
		CD105	CD45
		CD73	CD34
		CD90	CD14 or CD11b
			CD79α or CD19
			HLA-DR
3	*in vitro* differentiation: osteoblasts, adipocytes, chondroblasts (demonstrated by staining of *in vitro* cell culture)		

Table 1: Criteria for defining MSC [13].

In vitro trials for differentiation of MSCs into hepatocyte-like cells

Liver cell growth and differentiation *in vitro* are known to be affected by a specific cytokines and growth factors. The proper cytokines combination that can reach the cell microenvironment and maintain the suitable cytokines concentrations and their associated receptors over time is the crucial step of any stem cell culture system [23].

So far hepatocyte growth factor (HGF) is involved in most investigations using growth factors to stimulate the differentiation of MSCs into hepatocyte–like cells. Human umbilical cord Blood (HUCB) derived MSC shows high differentiation potentiality into hepatocytes after four weeks of culture media enriched with HGF together with either OSM (oncostatin M) or FGF-4 (fibroblast growth factor-4) [24].

MSCs isolated from HUCB are cultured on tissue culture plates or flasks containing fetal bovine serum (FBS) fortified media. FBS obtained from different source and at different time; greatly affect the MSC culture in terms of efficiency of plating and expansion [25,26].

FGF-4 is a strong mitogen for fibroblasts and endothelial cells. When FGF-4 is added to a medium contains mouse embryonic stem cells, it can induce their differentiation into cells expressing hepatocyte-related genes and surface markers [27].

Snykers et al. reported that nicotinamide (NTA) plays a role in formation of small hepatocyte colonies as it can induce the proliferation of primary hepatocytes. Upon culture with different cytokines hepatocyte-like cells, derived MSCs may show a variety of phenotypic characteristic [28].

There are various types of cytokines which have a crucial role in the hepatic differentiation of stem cells *in vitro* including [29-31]. HGF, oncostatin M, EGF, IGF, transforming growth factor (TGF), bFGF and LIF (leukemia inhibitory factor). Moreover, chemical compounds such as (Dex), retinoic acid, NTA, norepinephrine, sodium butyrate and dimethyl sulfoxide might have a role. During embryogenesis, HGF and FGF are linked to endodermal commitment. They have been identified and cloned as a strong hepatocyte mitogen; HGF is mesenchymal originated pleotropic cytokine, acting with a transmembrane protein called c-met receptor. Moreover, in the process of early hepatogenesis and liver development, HGF plays a vital role and it is involved in the process of hepatic regeneration [32].

It was found that OSM cannot induce stem cell hepatocytic differentiation by itself, but it plays a considerable role in process of hepatocyte maturation and it is produced by HSCs during the early stages of embryogenesis. While another IL-6 subfamily member is LIF, which shares OSM in signaling pathways [33].

Different Chemical compounds reported to affect the differentiation process such as insulin-transferrin-selenium (ITS) that has a chief role in promoting the survival and the proliferation of primary hepatocytes. Dex suppresses cell division that leads to encouragement of hepatocyte phenotype expression. The differentiation capacity of different liver-specific factors such as HGF, ITS, Dex and NTA were studied and compared either individually or in combination. The study showed that HGF and NTA have the major hepatogenic differentiation potential as well as in vivo functional hepatic integration [31].

Characterization of hepatocyte-like cells derived from MSCs

After *in vitro* or *in vivo* differentiation, phenotype characterization of the cells is considered a critical step, which includes not only morphological or phenotypical characterization but functional characterization as well. Under specific conditions favoring hepatocyte differentiation, it becomes easy to detect the change of cells from a fibroblast-like phenotype to the polygonal cells that has a typical shape to epithelial cells. During liver differentiation, numerous liver transcription factors as well as numerous cytoplasmic proteins are expressed selectively and can be detected using microarray analysis. Analysis of the hepatocyte-like cells derived from MSCs Show a

numerous change in the gene expression profile, which is relatively similar to those of adult and fetal hepatocytes [34].

During the early stage of MSC differentiation into hepatocyte, these cells express RNA transcripts like nestin (stem cell-specific marker), cytokeratin19 (CK19, epithelial cell marker), CX43 which is gap junction protein specific for liver stem cells and numerous early differentiation markers such as AFP, CK7, HNF4, HNF1α, HNF3β, GATA4 and C/EBPα [35].

In contrast, in the late stage of MSC differentiation, these cells express proteins as well as cell markers similar to mature hepatocytes, including connexin 32 (CX32); a hepatocyte specific gap-junction protein, (FoxM1) fork head transcription factor that plays a key step in hepatocyte differentiation [36]. CK18 an epithelial cell marker, and secretory plasma proteins like albumin, transferrin, fibrinogen, and cell adhesion molecules; also enzymes classically produced by mature hepatocytes including the cytochrome P450 subtypes 3A4 and 1A1, urea synthesis enzyme carbamoyl phosphate synthetase (CPS) gluconeogenic phosphoenol pyruvate carboxy kinase 1 and the ectopeptidase dipeptidylpeptidase type IV (CD26) [37].

Human hepatocyte-like cells can express Heppar1, a distinct marker for human hepatocytes and the differentiated hepatocytes progressively miss the expression of mesenchymal cell markers [35].

In addition to these well characterized methods, more subtle molecular biological approaches have been subjugated recently in the evaluation of hepatocyte like cell differentiation from MSCs [34].

Functions of MSCs derived hepatocyte-like cells

The key functions of liver include principally detoxification, glycogen storage as well as lipid metabolism. To assess the metabolic function of the differentiated hepatocyte like cells, some crucial tests should be carried out such as ammonia detoxification through the colorimetric di acetyl monoxime test, periodic acid-Schiff (PSA) staining can be used to detect glycogen deposition. Other tests that are used to distinguish between MSCs derived hepatocyte-like cells and the undifferentiated MSCs such as the cellular GSH and catalytic activity of GST to 1-chloro-2, 4-dinitrobenzene [36].

In vitro trials of differentiation of hepatocytes from HUC-MSCs

There are many essential factors that induce the process of *in vitro* hepatocytes differentiation of HUC-MSCs such as growth hormones , cytokines ,extracellular matrix and co-culture with other cell, Several *in vitro* models for hepatocyte differentiation have been well proven, though the preciseness of differentiation in these models is insufficient. Therefore, many efforts are still to be done to discover innovative means to facilitate HUC-MSCs differentiation into hepatocytes [37].

Different species of HUC-MSCs such as human, mouse as well as rat multipotent adult progenitor cells (MAPCs), were seeded on matrigel with both FGF-4 and HGF. They were found to have the capacity of differentiation into epitheloid cells, these cell were found to express HNF3b (hepatocyte nuclear factor-3b), GATA4, transthyretin cytokeratin 19 (CK19) and alpha-fetoprotein on the 7th day. While they expressed CK18, HNF-alpha 1 on the 4th till 28th day. Practically all human, in addition to greater number of rodent cells are positively stained for albumin on day 21 and interestingly 5% of rodent to 25% of human cells were found to be binucleated by the 21st day. They similarly showed that these cells had obtained functional features of mature hepatocytes such as secreting albumin and urea besides having high content of phenobarbital as well as cytochrome p450. Moreover, they have shown capability to take up LDL, and accumulate glycogen. MAPCs -besides its capability of expansion *in vitro* and maintaining the undifferentiated state for what is greater than hundreds of doublings has the capacity to differentiate into cells possessing the phenotype, the morphology as well as the functions of hepatocytes [38].

Another study showed that the human MSCs have a variable capacity of differentiation not only mesenchymal cell linage but can even differentiate into endoderme or neuroectodermal. In addition, the capacities to differentiate in hepatocytes like cells. They also studied the ability of HUC-MSCs to differentiate into hepatocyte-like cells. The cells were seeded under the pro-hepatogenic environment comparable to the environment used to culture the bone marrow (BM)-derived MSCs. They analyzed the expression hepatic lineage using flow cytometry, Western blot, RT-PCR, and immunofluorescence. The functional ability of the differentiated cells was evaluated by their ability to integrate DiI-acetylated low-density lipoprotein. They also showed that these cells were morphologically changed into hepatocyte-like cells, and start to express Thy-1, Flt-3, and c-Kit, on their cell surface, and showed ability to secrete α-fetoprotein, albumin, and cytokeratin-18 and 19. Furthermore, around half of these cells were found to attain the ability to transport DiI-acetylated low-density. Built on the above interpretations, they concluded that HUCB-MSCs preserve hepatogenic properties [39].

They isolated MSCs from human umblical cord blood and start culturing them in iscove modified Dulbecco medium (IMDM) enriched with FBS, FGF 4 and HGF untill reaching 70% of confluency. Alpha feto protein (AFP), urea assay, expression of CK-18 and Glycogen storage in hepatocytes was determined. Around 63.6% of the cultured cells changed into small, rounded and epitheloid on the 28th day. The cells showed increased expression of AFP starting from day 12 reaching the peak level of execration on day 28; they also expressed albumin starting from day 16 reaching the maximum level of expression of day 28. Urea was detected on day 20 showing peak on day 28. Cells expressed CK18 on day 16 while Glycogen storage was detected on day 24 [40].

Another study compared the culture of both Mononuclear cells (MNCs) obtained either from cord blood or from BM, both cells were allowed to grow in the same culture conditions, the media in both conditions was fortified by HGF, FGF 1 and FGF2. Morphological characterization were done for both cells using immunoflourscent which showed rounded, large cell and functional analysis were done using mRNA and analysis of proteins expressed from both cell lines. They found that both cells were adherent to the plate and mRNA revealed that both of them secrete albumin, AFP and CK18.

In vivo experimental trials

After being able to differentiate hepatocyte-like cells MSC research were further investigated *in vivo*. Numerous studies was done-using transplantation of MSCs in different animal models of liver injury-have displayed their differentiation into hepatocytes *in vivo*; MSCs have shown a differentiation potentiality into hepatic like cells *in vivo* besides they have been able to induce the endogenous parenchymal cells regeneration, and stimulate the fibrous matrix degradation [41].

MSCs were proved to have some anti-inflammatory properties besides being able to differentiate into hepatocytes like cells under *in*

vivo and *in vitro* conditions. Hepatocyte apoptosis was suppressed by production of secretory molecules and by adjusting the acute phase response. It was concluded that MSCs are a favorable cell population in the management of acute liver failure [42].

Another study was performed in NOD-SCID mice, the liver injury was induced by CCL4, then the animals were injected by with HUC-MSC to induce regeneration , these cells were which found in the mice regenerated liver [43].

In another model of acute liver injury using D-galactosamine, the rats were injected with MSC conditioned medium which resulted in 90% reduction of hepatocyte apoptosis, augmentation the number of hepatocyte proliferation up to three folds. Besides the decrease in the level of the injury biomarkers, together with up regulation of the hepatic genes is known to take a role in hepatic replication [44].

Clinical Trials

MSC have been used recently in many of the clinical trials to induce liver regeneration. A one-year study included 45 patients suffering from chronic hepatitis B patient with decompensated liver cirrhosis, 30 of these patients received transfusion of UC-MSC, while the other 15 received saline as a control, the treated group showed a marked decrease in the ascites volume, increased serum albumin and decreased in the serum bilirubin compared to control. While no side effect or complications were detected in either group, they concluded that UC-MSC transfusion besides being clinically safe can induce remarkable improve in patients with decompensated liver cirrhosis [45].

Another study used also transfusion of UC-MSC in patients with acute on top of chronic liver failure associated with HBV, 43 patient were included in this study, 24 received UC-MSC transfusion while the 19 were injected as a control with saline. The transfusion was given at 4 weeks interval while the follow up took place during the 48 and the 72 weeks after. There was significant increase in the survival rates in the treated group compared to the control group with increase the serum albumin and decrease in the serum bilirubin [46].

In addition, another study used BM-MSC in alcoholic cirrhotic patients. 11 patients were recruited who had been alcohol free for 6 month, the MSC were isolated from the patients' bone marrow, after being amplified for 6 month, and the patients received two injections through the hepatic artery. 12 weeks after the injection, follow up biopsies were taken which showed improve in the histological features [47].

Conclusion

Mesenchymal stem cells have the capacity to multiply and differentiate into numerous types of cell linages including hepatocytes. Many preclinical and clinical trials have been done to elicit their role in the hepatic regeneration that was proved curial. MSC provides a reliable source of hepatocytes after liver injury, beside the improvement in the liver function, this can give a hope for the hepatic patients and can decrease the gap between the availability and the demand the liver donors.

References

1. McCulloch EA, Till JE (1960) The radiation sensitivity of normal mouse bone marrow cells, determined by quantitative marrow transplantation into irradiated mice. Radiat Res 13: 115-125.
2. Orlic D, Hill JM, Arai AE (2002) Stem cells for myocardial regeneration. Circ Res 91: 1092-1102.
3. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, et al. (1998) Embryonic stem cell lines derived from human blastocysts. Science 282: 1145-1147.
4. Weissman IL (2000) Stem cells: units of development, units of regeneration, and units in evolution. Cell 100: 157-168.
5. Harvey L, Arnold B, Lawrence Z, Paul M, David B, et al. (2000) Gene Control in Development. (4thedn), WH Free man Co, New York.
6. Hwang NS, Varghese S, Elisseeff J (2008) Controlled differentiation of stem cells. Adv Drug Deliv Rev 60: 199-214.
7. Gordon MY, Blackett NM, Lewis JL, Goldman JM (1995) Evidence for a mechanism that can provide both short-term and long-term haemopoietic repopulation by a seemingly uniform population of primitive human haemopoietic precursor cells. Leukemia 9: 1252-1256.
8. Gordon GJ, Coleman WB, Hixson DC, Grisham JW (2000) Liver regeneration in rats with retrorsine-induced hepatocellular injury proceeds through a novel cellular response. Am J Pathol 156: 607-619.
9. Slack JM (2000) Stem Cells in Epithelial Tissues. Science 287: 1431-1433.
10. Asahara T, Kawamoto A (2004) Endothelial progenitor cells for postnatal vasculogenesis. Am J Physiol Cell Physiol 287: C572-C579.
11. Netter FH (1987) Musculo skeletal system: anatomy, physiology, and metabolic disorders. Summit, New Jersey.
12. Hematti P (2012) Mesenchymal stromal cells and fibroblasts: a case of mistaken identity? Cytotherapy 4: 516-521.
13. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, et al. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8: 315-317.
14. Chamberlain G, Fox J, Ashton B, Middleton J (2007) Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 25: 2739-2749.
15. Sgodda M, Aurich H, Kleist S, Aurich I, König S, et al. (2007) Hepatocyte differentiation of mesenchymal stem cells from rat peritoneal adipose tissue in vitro and in vivo. Exp Cell Res 313: 2875-2886.
16. Haynesworth SE, Barer MA, Caplan AI (1992) Cell surface antigen son human marrow derived mesenchymal cells are detected by monoclonal antibodies. Bone 13: 69-80.
17. Jang YY, Collector MI, Baylin SB, Diehl AM, Sharkis SJ (2004) Hematopoietic stem cells convert into liver cells within days without fusion. Nat Cell Biol 6: 532-539.
18. Baddoo M, Hill K, Wilkinson R, Gaupp D, Hughes C, et al. (2003) Characterization of mesenchymal stem cells isolated from murine bone marrow by negative selection. J Cell Biochem 89:1235-1249.
19. Martin P (1997) Wound healing--aiming for perfect skin regeneration. Science 276: 75-81.
20. Bianchi G, Banfi A, Mastrogiacomo M, Notaro R, Luzzatto L, et al. (2003) Ex vivo enrichment of mesenchymal cell progenitors by fibroblast growth factor 2. Exp Cell Res 287: 98-105.
21. Simmons PJ, Torok-Storb B (1991) Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. Blood 78: 55-62.
22. Reyes M, Verfaillie CM (2001) Characterization of multipotent adult progenitor cells, a subpopulation of mesenchymal stem cells. Ann N Y Acad Sci 938: 231-235.
23. Sato Y, Araki H, Kato J, Nakamura K, Kawano Y, et al. (2005) Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion. Blood 106: 756-763.
24. Hong SH, Gang EJ, Jeong JA, Ahn C, Hwang SH, et al. (2005) In vitro differentiation of human umbilical cord blood-derived mesenchymal stem cells into hepatocyte-like cells. Biochem Biophys Res Commun 330: 1153-1161.

25. Kang XQ, Zang WJ, Bao L, Li DL, Xu XL, et al. (2006) Differentiating characterization of human umbilical cord blood-derived mesenchymal stem cells in vitro. Cell Biol Int 30: 569-575.
26. Ulloa-Montoya F, Verfaillie CM, Hu WS (2005) Culture Systems for Pluripotent Stem Cells. J Biosci Bioeng 100: 12-27.
27. Xie JM, Chen JF, Gao Y, Yao KH (2006,) Hepatocyte growth factor and fibroblast growth factor-4-induced differentiation of human bone marrow mesenchymal stem cells into hepatocyte-like cells in vitro. Nan Fang Yi Ke Da Xue Xue Bao 26: 1439-1442.
28. Snykers S, Vanhaecke T, Papeleu P, Luttun A, Jiang Y, et al. (2006) Sequential exposure to cytokines reflecting embryogenesis: the key for in vitro differentiation of adult bone marrow stem cells into functional hepatocyte-like cells. Toxicol Sci 94: 330-341.
29. Lee KD, Kuo TK, Whang-Peng J, Chung YF, Lin CT, et al. (2004) In vitro hepatic differentiation of human mesenchymal stem cells. Hepatology 40: 1275-1284.
30. Chen Y, Dong XJ, Zhang GR, Shao JZ, Xiang LX (2007) In vitro differentiation of mouse bone marrow stromal stem cells into hepatocytes induced by conditioned culture medium of hepatocytes. J Cell Biochem 102: 52-63.
31. Chivu M, Dima SO, Stancu CI, Dobrea C, Uscatescu V, et al. (2009) Invitro hepatic differentiation of human bone marrow mesenchymal stem cells under differential exposure to liver-specific factors. Transl Res 154: 122-132.
32. Banas A, Yamamoto Y, Teratani T, Ochiya T (2007) Stem cell plasticity: learning from hepatogenic differentiation strategies. Dev Dyn 236: 3228-3241.
33. Forte G, Minieri M, Cossa, P, Antenucci D, Sala M, et al. (2006) Hepatocyte growth factor effects on mesenchymal stem cells: proliferation, migration, and differentiation. Stem cells 24: 23-33.
34. Stock P, Staege MS, Müller LP, Sgodda M, Völker A, et al. (2008) Hepatocytes derived from adult stem cells. Transplant Proc 40: 620-623.
35. Chen K, Wang D, Du WT, Han ZB, Ren H, et al. (2010) Human umbilical cord mesenchymal stem cells hUC-MSCs exert immunosuppressive activities through a PGE 2-dependent mechanism. Clin Immunol 135: 448-458.
36. Jones EA, Tosh D, Wilson DI, Lindsay S, Forrester LM (2002) Hepatic differentiation of murine embryonic stem cells. Exp Cell Res 272: 15-22.
37. Peister A, Mellad JA, Larson BL, Hall BM, Gibson LF, et al. (2004) Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. Blood 103: 1662-1668.
38. Allameh A, Esmaeli S, Kazemnejad S, Soleimani M (2009) Differential expression of glutathione S-transferases P1-1 and A1-1 at protein and mRNA levels in hepatocytes derived from human bone marrow mesenchymal stem cells. Toxicol In Vitro 23: 674-679.
39. Lin H, Zhang Z, Shi M, Xu RN, Fu JL, et al. (2012) Prospective controlled trial of safety of human umbilical cord derived-mesenchymal stem cell transplantation in patients with decompensated liver cirrhosis. Zhonghua Gan Zang Bing Za Zhi 20: 487-491.
40. Schwartz RE, Reyes M, Koodie L, Jiang Y, Blackstad M, et al. (2002) Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. J Clin Invest 109: 1291-1302.
41. Kang XQ, Zang WJ, Bao LJ, Li DL, Song TS, et al. (2005) Fibroblast growth factor-4 and hepatocyte growth factor induce differentiation of human umbilical cord blood-derived mesenchymal stem cells into hepatocytes. World J Gastroenterol 11: 7461-7465.
42. Jung KH, Shin HP, Lee S, Lim YJ, Hwang SH, et al. (2009) Effect of human umbilical cord blood-derived mesenchymal stem cells in a cirrhotic rat model. Liver Int 29: 898-909.
43. Dai LJ, Li HY, Guan LX, Ritchie G, Zhou JX (2009) The therapeutic potential of bone marrow-derived mesenchymal stem cells on hepatic cirrhosis. Stem Cell Res 2: 16-25.
44. Yagi H, Parekkadan B, Suganuma K, Soto-Gutierrez A, Tompkins RG, et al. (2009) Long-term superior performance of a stem cell/hepatocyte device for the treatment of acute liver failure. Tissue Eng Part A 15: 3377-3388.
45. Zhang Z, Lin H, Shi M, Xu R, Fu J, et al. (2012) Human umbilical cord mesenchymal stem cells improve liver function and ascites in decompensated liver cirrhosis patients". J Gastroenterol Hepatol 27: 112-120.
46. Shi M, Zhang Z, Xu R, Lin H, Fu J, et al. (2012) Human mesenchymal stem cell transfusion is safe and improves liver function in acute-on-chronic liver failure patients. Stem Cells Transl Med 1: 725-731.
47. Jang YO, Kim YJ, Baik SK, Kim MY, Eom YW, et al. (2014) Histological improvement following administration of autologous bone marrow-derived mesenchymal stem cells for alcoholic cirrhosis: a pilot study. Liver Int 34: 33-41.