RESEARCH PAPER | Science | Volume : 3 | Issue : 10 | Oct 2013 | ISSN - 2249-555X

Hepatocyte derived from Rat Bone Marrow Mesenchymal Stem Cells

KEYWORDS stem cells, bone marrow mesenchymal stem cells, differentiation, hepatocyte

Faten Zahran	Ahmed Abdel.Zaher
Biochemistry Department, Faculty of Science, Zagazig University, Zagazig, Egypt.	Biochemistry Department, Faculty of Science, Zagazig University, Zagazig, Egypt.

Nermin Raafat	Mohamed Ali
Medical biochemistry departement, Faculty of Medicine, Zagazig University, Zagazig, Egypt.	Biochemistry Department, Faculty of Science, Zagazig University, Zagazig, Egypt.

ABSTRACT *Hepatic Tissue Engineering is a step toward alleviating the need for organ donors. The capacity of stem cells to differentiate into specific cell types makes them very promising in tissue regeneration and repair. However, realizing this promise requires novel methods for guiding lineage-specific differentiation of stem cells. In this study we aimed to evaluate the differentiation potential of bone marrow mesenchymal stem cells (BMSCs) into hepatocytes like cells in vitro. BMSCs isolated from rat femurs and tibias were cultured at passage 3 in the presence of hepatocyte growth factor (HGF), fibroblast growth factor-4 (FGF-4) and Oncostatin M (OSM). The medium was changed every 3 days and stored for albumin, alpha-fetoprotein (AFP) and urea assays. Expression of albumin, c-Met and alpha -fetoprotein mRNA were detected by reverse transcriptase –polymerase chain reaction (RT-PCR).*

INTRODUCTION

Stem cells are considered new promising tool for treating many disorders (zahran et al., 2012). Mesenchymal stem cells (MSCs) are multipotent cells, able to differentiate into elements of the mesodermal lineage. Bone marrow and adipose tissue represent the main sources for MSCs isolation. In the last decade, several studies have reported the plasticity of MSCs toward a hepatocyte-like phenotype. Both types of cells have been cultured under similar pro-hepatogenic conditions by sequential exposure to cytokines, growth factors and hormones reflecting their temporal expression during in vivo hepatogenesis. The different capacities of hepatic differentiation of the two subsets in vitro have been investigated. (Gómez-Lechón and Tolosa ., 2013).

Adult MSCs have generated immense research interest in cell base therapies owing to their mulipotentiality and capacity for self-renewal. Bone marrow stromal tissue has been regarded as the most likely source to obtain MSCs. Several preclinical and clinical studies have confirmed the great therapeutic potential of MSCs (Caplan, 2007). For MSCs' preclinical applications, an adequate number of cells are necessary, and considering the low number of MSCs (Wexler et al., 2003; Caplan., 2007). An extensive ex vivo expansion is required. Mesenchymal stem cells (MSC) isolated from bone marrow and differentiated into hepatocyte-like cells have increasingly gained attention for clinical cell therapy of liver diseases because of their high regenerative capacity. (Brückner et al., 2013).

Hepatocytes derived from mesenchymal stem cells (MSCs) hold great potential for cell-based therapies for liver diseases. The cell-based therapies are critically dependent on the hepatic differentiation of the MSCs with a high efficiency and on a considerable scale (Ji Ru et al., 2012).

MATERIALS AND METHODS:

Isolation and culture of mesenchymal stem cells from rat bone marrow

Isolation of MSCs from bone marrow as previously described (Samad and Masoud., 2007) with some modifications. Eight-week-old male Sprague-Dawley rat was sacrificed by cervical dislocation and their femurs and tibias were carefully cleaned from skin by pulling towards the foot, which is cut at the anklebone. The muscle and connective tissue were removed from both the tibias and the femurs by scraping the diaphysis of the bones cleaning then pulling the tissue towards the ends of the bone. The bones were put in ethyl alcohol 10% for sterilization and leave it for some seconds. The ends of the tibia and femur were cut by sharp scissors. A 27-gauge needle were inserted and flushed with Dulbecco's Modified Eagle's Medium DMEM (lonza, Belgium), and collected in a 15-ml tube. Nucleated cells were isolated with a density gradient Ficoll/Paque (Lymphocyte Separation Medium 1.077, (sigma Aldrich, USA). Four milliliters of diluted BM was slowly folded into the same amount of Ficoll lymphocyte separation medium and was then centrifuged at 400 rpm speed for 20 min in a cooling centrifuge at -20oC so that, BM mononuclear cells were separated.

Culture of separated mononuclear cells

The mononuclear cell suspension obtained was re-suspended in DMEM medium with 10% FBS and 1% penicillin-streptomycin mixture (10 IU/10 IU/25mg, Lonza Belgium) Cells were cultured at a concentration of 5x10 6 per 25-cm 2 culture flask; they were then incubated at 37 oC in 5% humidified CO2 incubator (Heraeus, Germany).

After 1 day, non-adherent cells were removed by two to three washes with PBS and adherent cells were further cultured in DMEM. The medium was changed every 3 days until the monolayer of adherent cells reaches 70-80% confluence, then trypsinization made for cell splitting by trypsin-EDTA solution for passage 1. The number of cells were evaluated by haemocytometer and cellular viability by the Try- pan Blue Exclusion test , each 250-300 × 10^3 cells inoculated in 25 cm^2 culture flask that were incubated at 37 ^{0}C and 5 % CO_2. Cell cultivation was maintained up to the 3rd passage.

Flow cytometer analysis

Cells were characterized using cell surface markers by fluorescence-activated cell sorting (FACS) analysis (Daniel et al., 2010).The cells were stained with different fluorescently labeled monoclonal antibodies (mAb) Using the anti-rat CD105- PE mAb, CD34- FITC mAb (BD PharMingen, Franklin Lakes, NJ). The fluorescence intensity of the cells was

Volume : 3 | Issue : 10 | Oct 2013 | ISSN - 2249-555X

evaluated by flow cytometer. In brief, 5×10^5 cells (in100 µl PBS/0.5% BSA/2mmol/L EDTA) were mixed with 5 µl of the fluorescently labeled mAb and incubated in the dark at 2-8 ^{0}C for 30-45 min. washing with PBS containing 2% BSA was done twice and the pellet was re-suspended in PBS and analyzed immediately on flow cytometer to evaluate the fluorescence intensity of the cells.

Hepatocyte differentiation

Hepatocyte differentiation as previously described (Kang et al., 2005) with some modifications. The cultured cells at passage 3 were seeded in the plastic culture flasks at 5×10^5/bottle. When the cells grow at 70% confluence, the control group was continuously cultured in DMEM supplemented with 10 %FBS, 1% penicillin- streptomycin mixture. The hepatocyte differentiation group was cultured in DMEM supplemented with 10 % FBS, 20 ng/mL HGF, 10 ng/mL FGF-4, 10 ng/mL OSM,1% penicillin- streptomycin mixture. Each flask was supplemented with 5 mL medium, which was changed every 3 days. The medium was collected for albumin, alpha fetoprotein (AFP) and urea assays.

Hepatocyte functional activity

Albumin and Alpha fetoprotein assay:

ALB and Alpha fetoprotein secretion were measured at various times throughout the cell differentiation. Undifferentiated bone marrow cells and cultural bone marrow cells did not secrete any ALB. The albumin secretion rate was quantified using the rat albumin ELISA quantitation kit (Kamiya Biomedical Company cat. number KT-354) and Alpha fetoprotein assay using (DRG Cat. Number EIA-1468) according to instructions provided in the kit.

Urea production:

Urea production and secretion by hepatocytes were detected at various time points throughout differentiation by colorimetric assay according to manufacturer protocol (Diamond,Egypt) (Buga et al., 1996).

Reverse transcription-polymerase chain reaction (RT-PCR)

RNA extraction

Total RNA was isolated from cell lysates using RNA extraction kit (RNeasy Mini Kit Qiagene catalog number 74104) as described by the manufacturer and digested with DNase I (Fermentas) to remove genomic DNA before cDNA was synthesized. cDNA synthesis was performed using the reverse transcription-PCR protocol of the first strand synthesis kit as described by the manufacturer protocol. (QuantiTect Rev. Transcription Kit Qiagene catalog number 205310) (Alireza Khoshde et al., 2012).

Polymerase Chain Reaction (PCR)

For PCR, 5 µl cDNA was incubated with 29.5 µl water, 4 µl 25 mM MgCl2, 1 µl dNTPs (10 mM), 5 µl 10× PCR buffer, 0.5 µl (2.5 U) Taq polymerase and 2.5 µl of each primer containing 10 pmol. Specific PCR products were generated.All PCR experiments were performed using a Gene Amp PCR (Perkin-Elmer, USA).The following specific oligonucleotide primers were used:

Albumin
5'-AAGGCACCCCGATTACTCCG-3' (sense)
5' TGCGAAGTCACCCATCACCG-3' (antisense)
AFP
5' -AGGCTGTACTCATCATTAAACT-3' (sense)
5'-ATATTGTCCTGGCATTTCG-3' (antisense)
c-Met
5'-CAGTGATGATCTCAATGGGCAAT-3' (sense)
5'-AATGCCCTCTTCCTATGACTTC-3' (antisense)
β-actin
5'-AGAGGGAAATCGTGCGTGAC-3' (sense)
5'-AGGAGCCAGGGCAGTAATC-3' (anti-sense)

Amplification reactions were carried out for 35 cycles at 94 ^{0}C for 1 min, 58 ^{0}C for 1 min, and at 72^{0}C for 1 min for albumin; at 94^{0}C for 45 s, at 51^{0}C for 45 s and at 72 ^{0}C for 45 s for AFP; at 94^{0}C for 1 min, at 60^{0}C for 1 min and at 72^{0}C for 1 min for C-met and at 94^{0}C for 30 s, at 55^{0}C for 45 s, and at 72 ^{0}C for 45 s for β-actin (Shu et al., 2004).

Gel electrophoresis

The reaction products were subjected to 2% agarose gel electrophoresis and visualized by ethidium bromide staining. The reaction products were 649 bp (albumin), 484 bp (AFP), 725-bp (c-Met) and 353 bp (β-actin), respectively. Housekeeping gene β-actin was used as an internal control, adult liver tissue was used as a positive control.

RESULTS

Cell culture

The cultures were observed by using an invert light microscope. Attachment of spindle-shaped cells to tissue culture plastic flask was observed after 1 day of culture of bone marrow mesenchymal stem cells (BMSCs). Primary cultures reached 70-80% confluence in approximately 7-9 days for BMSCs, during the passages, the cell growth tended to accelerate and morphology of cells changed gradually. Cells become more flat-shape with increasing in passage number (figure 1).

Immune phenotypic characterization:

Cultures of third passage BMSCs were analyzed for expression of cell-surface markers. BMSCs were negative for the hematopoietic lineage marker CD34 and positive for CD105 (figure 2).

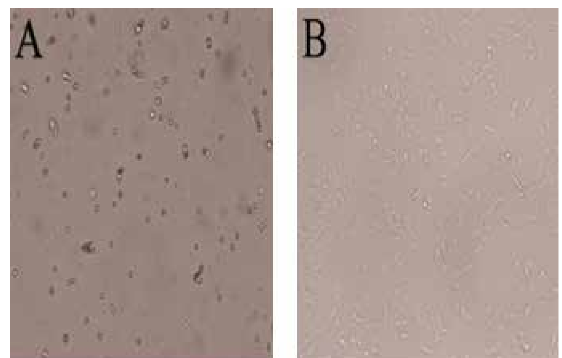
Hepatocyte functional activity

Table (1): Concentration of AFP, albumin and urea in the medium at different time points (mean ±SE). (n=5)

	7 day of differentiation	14 day of differentiation	21 day of differentiation
Alpha feto protein (IU/L)	16 ±.5	29 ±1.1	49 ±0.9
Albumin (mg/L)	-	0.8±0.6	1.9 ±0.14
Urea (mmole/L)	-	-	4.8 ±0.17

Gene expression of liver specific markers

To assess the direct differentiation of bone marrow cells into hepatocyte lineages we examined mRNA expression of endodermal and liver specific genes including albumin, alpha fetoprotein which could not be detected in fresh bone marrow cells (figure 4).In bone marrow cells' differentiated culture group, albumin, alpha feto protein mRNA expressions first appeared within 7 days of differentiation and lasted throughout differentiation time.



Volume : 3 | Issue : 10 | Oct 2013 | ISSN - 2249-555X

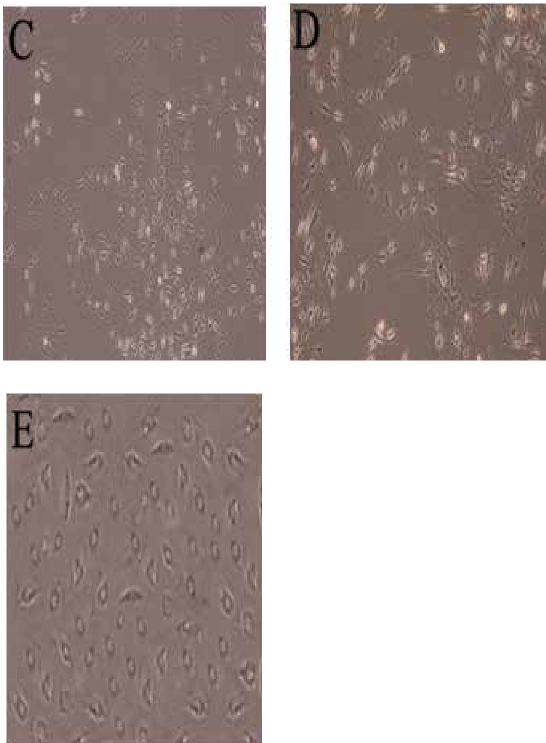


Figure 1(A-E): cell morphology before and after differentiation. (A) Image of MSC after isolation Scale bar=100 μm. (B) MSC culture at passage 1 Scale bar=100 μm. (C) MSC culture at passage 2 Scale bar=100 μm. (D) MSC culture at passage 3 (E) Differentiated bone marrow cells on day 21 Scale bar=200 μm.

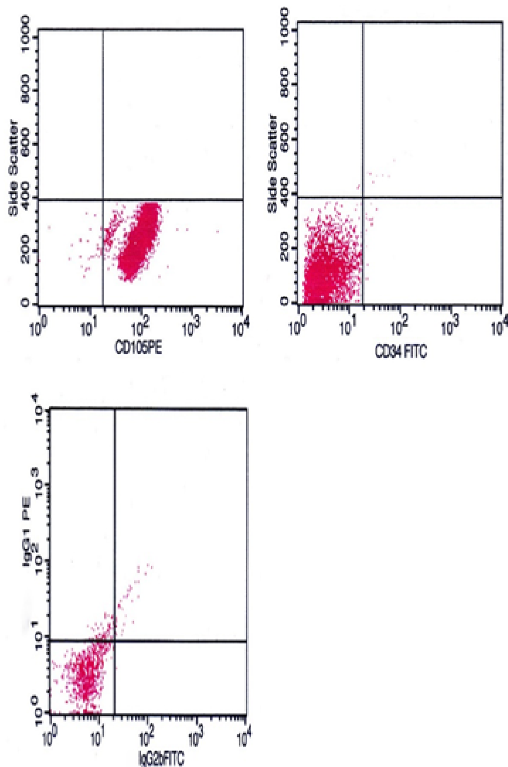


Figure (2): Flow cytometry analysis of cell surface markers in MSCs expressed CD105 but did not express CD34. The surface marker expression pattern corresponds to BM-MSC.

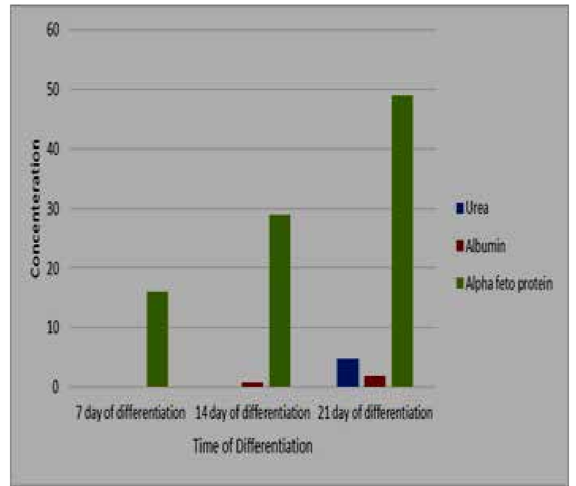
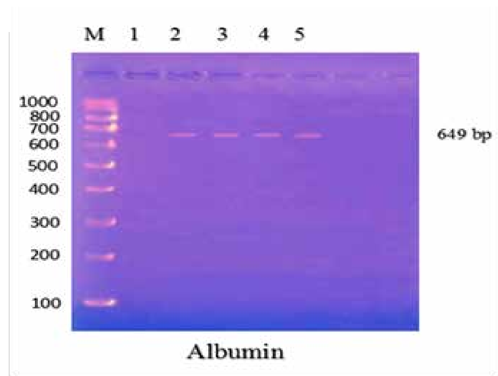
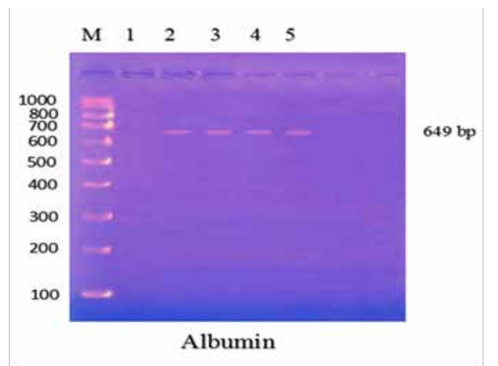
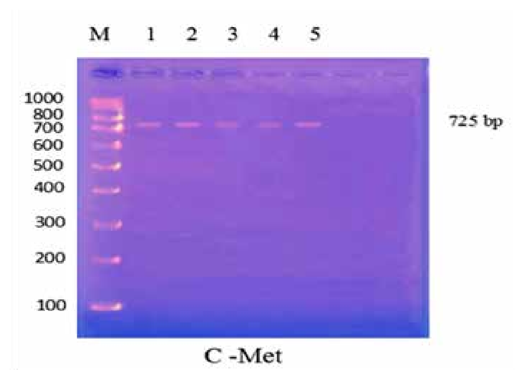


Figure (3): Concentration of AFP, albumin and urea in the medium at different time points of differentiation.







Volume : 3 | Issue : 10 | Oct 2013 | ISSN - 2249-555X

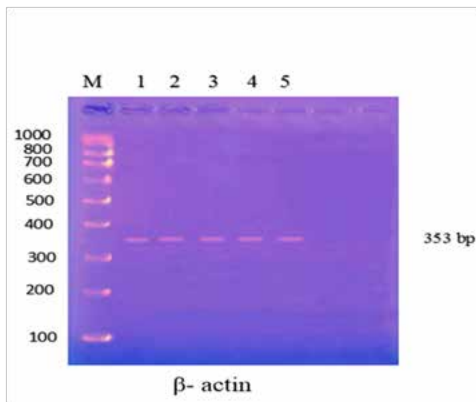


Figure (4). mRNA expression of Alpha fetoprotein, albumin, c-Met and β- actin gene.as detected by RT-PCR, Lane M: PCR marker with 100 bp ladder Lane 1, negative control (rat MSC) .Lanes 2-4, (7, 14, and 21 days after differentiation respectively, lane 5 positive control .

DISCUSSION:

A study like the current investigation provides some insights on hepatic differentiation of rat bone marrow mesenchymal stem cells in vitro. Using such data, investigators will be able to use hepatocyte like cells differentiated from bone marrow MSCs in drug biotransformation studies and other studies.

Mesenchymal stem cells (MSCs) could differentiate into cells of all mesodermal origin, including adipocytes, osteocytes, chondrocytes and myocytes (zuk et al., 2002). Besides, these MSCs are also capable of "trans differentiation" into ectodermal cells, such as neural cells (Woodbury et al., 2000) and hepatocyte like cells (Shu et al., 2004; Sgodda et al., 2007; Liang et al 2011; Ye et al., 2012).

In this study Bone marrow was harvested from Eight-week-old male Sprague-Dawley rat, young male rats were chosen because it was observed experimentally that mesenchymal stem cells found in young more than adult and also found in male more than female, that are consistent with others (Asumda and Chase, 2011; Katsara et al., 2011).Femurs and tibias were carefully cleaned from muscles and then put it in ethyl alcohol 10% to ensure that is no any contamination , then the collected bone marrow were cultured in plastic flask. Plastic flask was used because mesenchymal stem cells unlike other cells in bone marrow can attached on plastic flask in short time. Bone marrow cells were cultured in DMEM which contain the nutrient needed to the cells and PH=7.2-7.4 that suitable for cells, FBS was added to the media which contain growth factors that help cells for growth and proliferation and the flask was incubated at 37°C like temperature of body, Then bone marrow cells were cultured in DMEM with FBS and 1% antibiotic (penicillin-streptomycin mixture) in plastic flask. Media of bone marrow cells were changed after 24 hrs to get rid of non-adherent cells and leave adherent cells which mostly are MSCs.

The morphological changes of cells have been noted every day, cells showed spindle shape after about one day, it was noted that cells become more flat-shape with increasing in passage number (figure 1) and that are in line with (Samad and Masoud., 2007), but their study were on C57BL/6 mouse bone marrow.

Immunophenotyping characterization were done by flowcytometery to know that surface markers were present or not (Asumda and Chase., 2011; Cheuk et al., 2011). Flowcytometry measurements showed that bone marrow MSCs were negative for the haematopoietic lineage marker CD34 and positive for CD105 and these results were in agreement with others (somia et al., 2013; Julian Braun et al., 2013).

The hepatocyte differentiation protocol used in this study have been demonstrated as an efficient and convenient way to induce MSCs to hepatic lineage, the results showed that the differentiated cells displayed the hepatocyte like morphology, expression of hepatic specific gene and protein, functioned as adult liver cells, these results are in accordance with previous studies (Wang et al., 2004; Seo et al., 2005; Chien et al., 2006; Sgodda et al., 2007).Some researchers believe that microenvironment played an important role in differentiation of stem cells (Tosh and Slack 2002). The differentiation of stem cells is controlled under microenvironment. Stem cells have reciprocity with adjacent cells, ECM and cytokines. They also have effects on differentiation of stem cells. (Schwartz et al., 2002; Fiegel et al., 2003), plated marrow stem cells on matrigel, fibronectin or collagen matrix and differentiated them into hepatocyte-like cells. Matrigel consists of a mixture of ECM components. Fibronectin and collagen matrix are two of ECM components. But in our study, MSCs cultured in plastic culture flasks not treated with matrigel or fibronectin. However, MSCs still differentiated into hepatocytes, suggesting that it is not important to culture MSCs on certain media in vitro. Maybe, cytokines play an important role in differentiating rat MSCs into hepatocytes. It seemed that the ECM could potentially modulate the local concentration of cytokines and cytokines could regulate stem cell proliferation and differentiation. After being cultured with HGF, adult human MSCs could also differentiate into hepatocytes in vitro (Fiegel et al., 2003). HGF was first identified as a blood-derived mitogen for hepatocytes. HGF and its receptor c-Met are the key factors for liver growth and function. FGF-4 is mitogenic for fibroblasts and endothelial cells. Mouse embryonic stem cells grown in medium supplemented with FGF-4 could differentiate into cells expressing hepatocyte-specific genes and antigens (Ruhnke et al., 2003). It has been reported that treatment of the cultures with OSM increased the cell size of hepatocytes and enhanced cell differentiation (Lazaro et al., 2003), by co-operation of HGF and FGF, OSM the differentiation of MSCs was triggered and MSCs developed into hepatocytes.

Hepatocyte differentiation of BMSCs was characterized by Morphologic change of the cells, Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and hepatocyte functional activity.

Morphologic change of MSCs was apparent, cell shape changed from spindle and fibroblast-like to round and epithelia-like, this results agree with (Shi et al., 2005) as shown in (figure 1).

The differentiation of MSCs into hepatocytes, revealed that hepatic specific gene C-Met was already expressed by MSCs without differentiation protocol. After induction, MSCs also expressed albumin and alpha fetoprotein besides C-Met. Albumin and alpha fetoprotein mRNA appeared on day 7 after differentiation (figure 4).It was found that C-Met (a receptor for hepatocyte growth factor) mRNA already expressed by MSCs without differentiation protocol. C-Met mRNA was detected by RT-PCR in freshly isolated BM cells as well as in normal rat liver as a positive control (Figure 4). Our results indicate that BMSCs can respond to HGF, this result in agreement with (Oh et al., 2000).

Albumin (ALB) and AFP mRNA appeared on 7th day of differentiation and these results are in accordance with (Alireza Khoshd et al., 2012), with prolonged period of induction, the expression of ALB was gradually increased and that of AFP was decreased. These findings were consistent with (Zhou et al., 2004). These results suggested that, MSCs could be induced into hepatocyte-like cells in vitro.

In our study, the cells that were differentiated into hepatocyte-like cells could produce urea, secrete albumin and AFP. Urea production was characterized by hepatocyte activity, although kidney tubular epithelium also produced urea. In

Volume : 3 | Issue : 10 | Oct 2013 | ISSN - 2249-555X

contrast, albumin and AFP production were specific tests for the presence and metabolic activity of hepatocytes. Also it was found that AFP could be detected throughout the differentiation process, from day 7, the level of AFP increased significantly, suggesting that MSCs began to secrete AFP, before day 14, the concentration of albumin could not be measured by ELISA assay because there was small amount of albumin in the medium, albumin was secreted by MSCs from day 14, and urea was significantly secreted from day 21 as shown in figure (3). The data suggested that rat MSCs can be differentiated into hepatocytes by induction of FGF-4 and HGF, OSM our data are consistent with (kang et al., 2005)

CONCOLUSION

Hepatic stem cells, especially bone marrow-derived hepatic stem cells may be therapeutically useful for treating a variety of diseases that affect the liver. This has been proved in some animal models (Lagasse et al., 2000, Zhan et al., 2003).

MSCs could be isolated, expanded, and maintained in vitro in an undifferentiated state for more than 100 population doublings(Jiang et al.,2002), and differentiate into hepatocytes. Our finding suggesting that MSCs may be used as an ideal cells for in vivo therapy of genetic or acquired disorders of the liver and also can be used in bio-artificial liver devices. Moreover, MSCs might be used as a new potential therapeutic modality for severe acute pancreatitis. We hope that bone marrow MSCs will play the most important role in therapy of liver diseases in the future.

REFERENCE

Alireza Khoshde, Abbas S. Lotfi, Masoud Soleimani, Morteza Daliri, Ali Mota and Behzad Adibi (2012) .Evaluation of Biochemical Markers in Hepatocyte like Cells Differentiated from Adipose Derivation Stem Cells .World Applied Sciences Journal 16 (5): 693-698. | | Asumda FZ, Chase PB, (2011).Age-related changes in rat bone-marrow mesenchymal stem cell plasticity. BMC Cell Biol. 12; 12:44. | Buga GM, Singh R, Pervin S, Rogers NE, Schmitz DA, Jenkinson CP, Cederbaum SD, Ignarro LJ. (1996). Arginase activity in endothelial cells: inhibition by NG-hydroxy-L-arginine during high-output NO production. Am J Physiol; 271: H1988-H1998. | Caplan, A. I. (2007). Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J. Cell Physiol. 213: 341-347. | | Chien, C. C., Yen, B. L., Lee, F. K., Lai, T.H., Chen, Y. C., Chan, S. H. and Huang, H. I. (2006). In vitro differentiation of human placenta derived multipotent cells into hepatocyte-like cells. Stem Cells24: 1759-1768. | | Cheuk-Kwan Sun, Chia-Hung Yen3, Yu-Chun Lin, Tzu-Hsien Tsai, Li-Teh Chang, Ying-Hsien Kao, Sarah Chua, Morgan Fu, Sheung-Fat Ko, Steve Leu and Hon-Kan Yip. (2011). Autologous Transplantation of Adipose- Derived Mesenchymal Stem Cells Markedly Reduced Acute Ischemia Reperfusion Lung Injury in a Rodent Model. Journal of Translational Medicine, 9:118. | | Daniel LA, Dan Z, Lyndsey L, Adams, Brandon K, Matthew D, Erik J. (2010). Donor-matched comparison of dental pulp stem cells and bone marrow-derived mesenchymal stem cells in a rat model. Tissue Eng Regen Med.; 4(1): 73–81. | | Fiegel HC, Lioznov MV, Cortes-Dericks L, Lange C, Kluth D, Fehse B, Zander AR. (2003). Liver-specific gene expression in cultured human hematopoietic stem cells. Stem Cells 2003; 1: 98-104. | | Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM. (2002). Pluripotency of mesenchymal stem cells derived from adult marrow. Nature; 418: 41-49. | | Julian Braun, Andreas Kurtz, Neslihan Barutcu, Juliane Bodo, Andreas Thiel, and Jun Dong: (2013). Concerted Regulation of CD34 and CD105 Accompanies Mesenchymal Stromal Cell Derivation from Human Adventitial Stromal Cell. Stem cells and development. 22(5): 815-827. | | Katsara O, Mahaira LG, Iliopoulou EG, Moustaki A, Antsaklis A, Loutradis D, Stefanidis K, Baxevanis CN, Papamichail M, Perez SA. (2011). Effects of donor age, gender, and in vitro cellular aging on the phenotypic, functional, and molecular characteristics of mouse bone marrow derived mesenchymal stem cells. Stem Cells Dev.; 20(9):1549 | | Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman IL, Grompe M. (2000). Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med; 6: 1229-1234. | | Lazaro, C.A., E.J. Croager, C. Mitchell, J.S. Campbell, C. Yu, J. Foraker, J.A. Rhim, G.C. Yeoh and N. Fausto,(2003). Establishment, characterization and long-term maintenance of cultures of human fetal hepatocytes. Hepatology, 38: 1095-1106. | | Liang Peng, Hai-peng Zhu, Yu-bao Zheng, Chan Xie, Yu-tian Chong and Zhi-liang Gao (2011). Mesenchymal stem cells differentiate into hepatocyte-like cells under different induction systems in hepatitis B patients with liver failure. African Journal of Biotechnology Vol. 10(5), pp. 840-847. | | María José Gómez-Lechón and Laia Tolosa (2013) .Hepatogenic Differentiation: Comparison between Adipose Tissue-Derived Stem Cells and Bone Marrow Mesenchymal Stem Cells Stem Cells and Cancer Stem Cells .10: 45-57. | Ru Ji, Ning Zhang, Nan You, Qiang Li, Weihui Liu, Nan Jiang, Jie Liu, Hongtao Zhang, Desheng Wang, Kaishan Tao and Kefeng Dou (2012). The differentiation of MSCs into functional hepatocyte-like cells in a liver biomatrix scaffold and their transplantation into liver-fibrotic mice. Biomaterials 33: 8995–9008. | | Ruhnke M, Ungefroren H, Zehle G, Bader M, Kremer B, Fandrich F. (2003).Long-term culture and differentiation of rat embryonic stem cell-like cells into neuronal, glial, endothelial, and hepatic lineages. Stem Cells; 4: 428-436. | | S. Brücknera, H.-M. Tautenhahna, S. Winklera, P. Stocka, S. Jonasa, M. Dollingerb, B. Christa (2013) .Isolation and Hepatocyte Differentiation of Mesenchymal Stem Cells from Porcine Bone Marrow—"Surgical Waste" as a Novel MSC Source Transplantation Proceedings 45: 2056–2058. | | Sai-Nan Shu, Lai Wei, Jiang-Hua Wang, Yu-Tao Zhan, Hong-Song Chen, Yu Wang.(2004). Hepatic differentiation capability of rat bone marrow-derived mesenchymal stem cells and hematopoietic stem cells World Journal of Gastroenterology.10 (19):2818-2822. | | Samad Nadri & Masoud Soleimani (2007). Isolation murine mesenchymal stem cells by positive selection. In Vitro Cell.Dev.Biol.—Animal 43:276–282. | | Schwartz RE, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, Lenvik T, Johnson S, and Hu WS, Verfaillie CM. (2002). Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. J Clin Invest; 109: 1291-1302. | | Seh-Hoon Oh, Masahiro Miyazaki , Hirosuke Kouchi, Yusuke Inoue, Masakiyo Sakaguchi,Toshiya Tsuji, Nobuyuki Shima, Kanji Higashioz and Masayoshi Namba.(2000). Hepatocyte Growth Factor Induces Differentiation of Adult Rat Bone Marrow Cells into a Hepatocyte Lineage in Vitro. Biochemical and Biophysical Research Communications. 279,500 –504. | | Seo, M. J., Suh, S. Y., Bae, Y. C. and Jung, J. S. (2005). Differentiation of human adipose stromal cells into hepatic lineage in vitro and in vivo. Biochem. Biophys. Res. Commun. 328: 258-264. | | Sgodda, M., Aurich, H., Kleist, S., Aurich, I., Konig, S, Dollinger, M. M., Fleig, W. E. and Christ, B. (2007). Hepatocyte differentiation of mesenchymal stem cells from rat peritoneal adipose tissue in vitro and in vivo. Exp. Cell Res.313: 2875-2886. | | Somia H. Abd-Allah, Sally M. Shalaby, Heba F. Pasha, Amal S. El-Shal, Nermin Raafat, Sheren M. Shabrawy, Hanan A. Awad, Mona G. Amer, Mahmoud A. Gharib, Eman A. El Gendy, Amal A. Raslan, Hassan M. El-Kelawy (2013). Mechanistic action of mesenchymal stem cell injection in the treatment of chemically induced ovarian failure in rabbits. cytotherapy . 15: 64-75. | | Tosh D, Slack JM. (2002). How cells change their phenotype. Nature Rev Mi Cell Biol ; 3: 187-194. | Wang, P. P., Wang, J. H., Yan, Z. P., Hu, M. Y., Lau, G. K., Fan, S. T. and Luk, J. M. (2004). Expression of hepatocyte-like phenotypes in bone marrow stromal cells after HGF induction. Biochem. Biophys. Res. Commun.320: 712-716. | | Wexler, S. A., Donaldson, C., Denning-Kendall, P., Rice, C., Bradley, B. and Hows, J. M. (2003). Adult bone marrow is a rich source of human mesenchymal stem cells but umbilical cord and mobilized adult blood are not. Br. J. Haematol. 121: 368-374. | | Woodbury D, Schwarz EJ, Prockop DJ, Black IB. (2000). Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res; 61: 364-370. | | Xin-Qin Kang, Wei-Jin Zang, Tu-Sheng Song, Xiao-Li Xu, Xiao-Jiang Yu, Dong-Ling Li, Ke-Wei Meng, Sheng-Li Wu, Zhi-Ying Zhao. (2005). Rat bone marrow mesenchymal stem cells differentiate into hepatocytes in vitro. World Journal of Gastroenterology; 11(22):3479-3484. | | Xiao-lei SHI, Yu-dong QIU, Qiang LI, Ting XIE, Zhang-hua ZHU, Lei-lei CHEN, Lei LI, Yi-tao DING (2005). Hepatocyte-like cells from directed differentiation of mouse bone marrow cells in vitro. Acta Pharmacologica Sinica ; 26 (4): 469–476. | | Ye Danna, Tanthanuch W, Thumanu K, Sangmalee A, Parnpai R, Heraud P.(2012). Discrimination of functional hepatocytes derived from mesenchymal stem cells using FTIR microspectroscopy. Analys.137, 4774-4784. | Zahran F, Salama M, Lotfy A, El-Deen IM (2012). Isolation and characterization of adipose tissue-derived stem cells: An in vitro study. Basic Research Journal of Medicine and Clinical Sciences 1 (5) Pp 88-94. | Zhan YT, Wei L, Chen HS, Cong X, Fei R, Wang Y. (2003). Differentiation of bone marrow stem cells in rat hepatic fibrogenesis environment. Zhonghua Ganzangbing Zazhi. 11: 673-675. | | Zhou YM, Hu DR, Yao P, Fan GR (2004). Study of mouse marrow cells differentiation into a hepatocyte lineage in vitro. Chin. J. Hepatol. 12: 722-725. | | Zuk, P.A., M. Zhu, P. Ashjian, D.A. Ugarte, J.I. Huang, H. Mizuno, Z.C. Alfonso, J.K. Fraser, P. Benhaim and M.H. Hedrick, 2002. Human adipose tissue is a source of multipotent stem cells. Mol. Biol. Cell., 13: 4279-4295. |