Surveillance for Hepatocellular Carcinoma after Autologous Stem Cell Transplantation in Cirrhosis

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ABSTRACT

BACKGROUND
During the resent years there has been interest in using bone marrow stem cells to treat liver cirrhosis. However, there is a potential concern for malignant transformation after stem cell therapy. The aim of this study was to evaluate the development of hepatocellular carcinoma (HCC) after autologous bone marrow stem cell transplantation for liver cirrhosis.

METHODS
All the patients who underwent autologous stem cell transplantation for liver cirrhosis between 2005 and 2011 at our center were enrolled. Cellular infusion was made through peripheral vein, portal vein, or hepatic artery.The patients were invited to undergo screening for hepatocellular carcinoma. The screening was made with ultrasonography and alpha-feto protein (AFP) measurement.

RESULTS
Thirty two patients (18 males) were included in the study. Mean age of patients was 45.7 years. Fifteen patients (47%) received mesenchymal stem cell (MSC), 9 (28%) received bone marrow mononuclear cells, 5 (16%) were given CD 133-positive bone marrow cells, and 3 (9%) patients received CD 34-positive bone marrow cells. Mean duration of follow up was 20.5months. Mean serum level of AFP was 2.8 ng/ml at baseline and 3.4ng/ml at the end of follow up (p= 0.3). One patient was found to have hepatocellular carcinoma three months after infusion of bone marrow mononuclear cells. The incidence rate for HCC was 1.8 cases per 100 person-years in this study.

CONCLUSION
Autologous bone marrow stem cell infusion does not appear to increase the risk of hepatocellular carcinoma. The incidence rate of HCC in this study is comparable or even less than the reported rates of HCC in cohort studies of cirrhotic patients.

KEYWORDS
Stem cells; Liver; Cirrhosis

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INTRODUCTION

During the recent yearsstem cells have been used to treat various

disorders.

Studies in early 2000s suggested that multipotent stem cells may exist among bone marrow (BM) cells; and bone marrow stem cells contribute to liver regeneration after injury.[1,2] Subsequent studies demonstrated that cell fusion rather than differentiation was the principal source of bone marrow-derived hepatocytes.[3,4]

These observations led investigators to examine the therapeutic potentials of BM-stem cells in liver disorders.

Several animal studies suggest that transplantation of BM-stem cells reduces liver fibrosis.[5,6] Subsequent uncontrolled clinical trials suggested that infusion of hematopietic CD34+ stem cells,[7-10] BM-mononuclear cells,[11,12] or BM-mesenchymal stem cells[13,14] may transiently improve liver function in cirrhosis in human.

Malignant transformation is a potential concern associated with cell based therapies. Teratoma has been reported after transplantation of pleuripotent embryonic stem cells in nude or immunocompetent mice.[15,16] Teratoma has also been observed after transplantation of induced pleuripotent stem (iPS) cells.[17] iPS cells can also lead to malignant tumors in transplanted mice. This has been attributed to induction of c-Myc gene in iPS cells.[17]

Furthermore, there is some evidence suggesting that MSCs could immortalize and transform spontaneously after long term in-vitro expansion.[18, 19]

Theoretically, malignancy is more likely to develop after cell therapy at the site of cellular engraftment. For instance, there is a report of donor-derived brain tumor development after intracerebellar injection of fetal stem cells.[20] Therefore, there is a need to assess the potential risk of HCC after cell-based therapies in patients with cirrhosis.

In this study, we aimed to investigate the incidence of HCC aftertransplantation of bone marrow stem cells in cirrhotic patients.

MATERIALS AND METHODS

Patients

We enrolled all patients who underwent BM-stem cell transplantation in ourcenter between 2005 and 2011. All patients had decompensated cirrhosis at the time of stem cell transplantation.

The patients were invited to undergo screening for hepatocellular carcinoma. The screening was made by ultrasonography and AFP measurement. For patients who were not available, data from their last follow up was used.

MSC transplantation

Bone marrow MSCs were cultured as previously described.[13] In the phase 1 study, a mean number of 31.7 million cultured MSCs were infused through a peripheral vein.[13] In the phase 2 randomized controlled study, a mean number of 240.8 million cells were infused through a peripheral vein in the MSC group (Mohamadnejad M, et al. Unpublished data 2012).

CD 34+ Bone Marrow Cell Transplantation

Bone marrow was aspirated and CD 34+ cells were isolated with miniMACS cell separator (Miltenyi Biotec, Bergisch Gladbach, Germany). Between 3 to 10 million cells were infused through hepatic artery. Details are available elsewhere.[9]

CD 133+ or Bone Marrow Mononuclear Cell Transplantation

In this ongoing randomized study, patients received bone marrow CD 133+ cells (about 3.5 million cells) or bone marrow mononuclear cells (about 500 million cells) through portal vein. The infusion was repeated after 3 months.

Statistical analysis

Data are expressed as mean ± SD and compared using the t tests. Statistical analyses were performed by SPSS version 19. P value of less than 0.05 was considered statistically significant. The incidence rate of HCC was determined by dividing the number of cases of incident HCC by the total number of person-years of follow up.

RESULTS

Thirty two patients (18 males) with decom-

pensated cirrhosis were included in the study. Mean±SD age of the patients was 45.7 ±12.8 years.

The etiology of cirrhosis included autoimmune hepatitis in 8 (25%), primary biliary cirrhosis in 4 (12.5%), hepatitis B virus in 2 (6.3%), non-alcoholic steatohepatitis(NASH) in 1 (3.1%), and cryptogenic in 17 (53.1%) patients. Mean baseline MELD ± SD score was 15.9 ± 5.2.

Fifteen patients (47%) received BM-MSCs. Four of which received MSC in the phase 1 trial, and 11 in the phase 2 trial. Also, ninepatients (28%)were given BM-mononuclear cells, 5 (16%) received CD 133-positive bone marrow cells, and 3 (9%) patients received CD 34-positive bone marrow cells (Table 1).

The patients were followed for a mean ± SD duration of 20.7± 16.6 months.

Mean ± SD serum level of AFP was 2.8±1.7 ng/ml at baseline and 3.4±3.3 ng/ml at the end of follow up (p=0.3).

One patient was found to have HCC three months after receiving BM-mononuclear cells.

This patient was a 56 year old woman with NASH-induced cirrhosis and the baseline MELD score of 15 who received 865 million autologous BM-mononuclear cells through the portal vein. Her baseline serum AFP level was 7 ng/ml which increased to 26.5 ng/ml three months after cellulcar infusion. Trans-abdominal ultrasonography revealed a 35 mm mass lesion in the right lobe of the liver. A subsequent CT scan confirmed radiologic features of HCC. The patient was referred for liver transplantation.

Total follow-up was 655 person-months and the incidence for HCC was 1.8 cases per 100 person-years.

DISCUSSION

Several uncontrolled studies have reported that autologous bone marrow stem cell transplantation transiently improves liver function in some patients with cirrhosis.[7-14] However, the efficacy of this therapeutic strategy needs to be confirmed in randomized controlled trials and the potential for malignancy needs to be sufficiently addressed.

We have previously reported the results of two uncontrolled trials of BM-MSC[13] and CD34+ hematopoietic stem cell9 transplantation and have recently completed a randomized placebo-controlled trial of BM-MSC transplantation in cirrhosis (Mohamadnejad M, et al. Unpublished data 2012). Also, a randomized placebo controlled trial of hematopietic CD133+ versus bone marrow mononuclear cell transplantation is being performed at our center.

In this study, we have evaluated the development of HCC in patients who underwent stem cell transplantation between 2005 and 2011 in any of our four trials.

Only one of our patients who had received BM-mononuclear cells developed HCC three months after transplant. We did not biopsy the lesion as histology is not required in cirrhotic patients provided that the mass is more than 2 cm in size and hasradiologic features of HCC.[21] No liver mass was reported on the baseline ultrasonography in this patient before cellular transplantation, however a mass was found on follow up ultrasonography 3 months after receiving the BM-mononuclear cells. We are not sure if the HCC developed after transplantation, or there was a small tumor before transplantation that was not detected on baseline studies.

The incidence rate of HCC in our study was 1.8 cases per 100 person-years. In natural history studies of patients with cirrhosis the incidence rates of HCC ranges from 7.1 to 2 cases per 100 person-years.[22-25] Therefore, the incidence rate of HCC in our study is comparable or even less than rates reported in patients with cirrhosis.[22-25] Another reassuring finding in our study was that the mean serum levels of AFP did not change significantly in the patients after a mean 20.5 months of follow up.

The development of donor-derived brain tumor has been reported a few years after transplantation ofallogenic neural stem cells in a patient with ataxia telangiectasia.[20]

There are some reports of spontaneous immortalization and transformation of MSCs after long term in-vitro expansion.[18,19] Theoretically, malignant

Table 1:Characteristics of patients

	Number of patients (Males)	Mean age at transplantation ± SD	Mean duration of follow up ± SD (months)	AFP±SD(ng/ml)		
				Before transplant	Follow up	*p*- value*
MSC phase 1	4 (1)	47.3±9.8	25±23.4	2.7±1.1	3.1±2.2	0.5
MSC phase 2	11 (7)	43.7±18.0	32.4±18.1	2.6±1.3	3.5±2.1	0.3
CD 34+	3 (2)	46.0±6.6	12.0±0	3.3±1.5	3.5±2.1	0.8
CD 133+	5 (2)	45.2±5.6	6.6±5.9	2.5±2.2	1.3±0.7	0.3
MNNC	9 (6)	47.7±12.2	15.1±7.5	3.2±2.4	4.6±5.6	0.4
Total	32 (18)	45.7 ±12.8	20.7± 16.6	2.8±1.7	3.4±3.3	0.3

Abbreviations: MSC: Bone marrow mesenchymal stem cells; CD 34+: Bone marrow CD 34+ stem cells; CD 133+: Bone marrow CD 133+ stem cells; MNNC: Bone marrow mononuclear cells; AFP: Serum alpha feto-protein level
**p*-value comparingserum AFP levels before transplant and at end of follow up.

transformation of the transplanted cells requires the presence of those cells in the recipient organ for several months or years.

In a previous study, we have tracked BM-mesenchymal stem cells after intrasplenic transplantation in syngenic mice.[26] Wetraced the transplanted MSCs in the recipient livers a few days to a few weeks after transplantation.However, there were almost no MSCs three months after transplantation.[26] Therefore, the transplanted MSCs are possibly cleared by the immune system in the host liver and this could decrease the possibility of malignant transformation of the transplanted cells after this period of time.

In conclusion, we found that the incidence of HCC is not increased after autologous bone marrow stem cell transplantation. While the efficacy of stem cell transplantation in cirrhosis requires to be confirmed, further safety data with longer duration of follow up is required.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

REFERENCES

1. Theise ND, Nimmakayalu M, Gardner R,Illei PB, Morgan G, Teperman L, et al. Liver from bone marrow in humans. *Hepatology* 2000;**32**:11–16.
2. Korbling M, Katz RL, Khanna A, Ruifrok AC, Rondon G, Albitar M, et al. Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N Engl J Med* 2002;**346**:738 -46.
3. Vassilopoulos G, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. *Nature* 2003;**422**:901-4.
4. 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.
5. Zhao DC, Lei JX, Chen R, Yu WH, Zhang XM, Li SN, et al. Bone marrow-derived mesenchymal stem cells protect against experimental liver fibrosis in rats. *World J Gastroenterol* 2005;**11**:3431-40.
6. Sakaida I, Terai S, Yamamoto N, Aoyama K, Ishikawa T, Nishina H, et al. Transplantation of bone marrow cells reduces CCl4- induced liver fibrosis in mice. *Hepatology* 2004;**40**:1304-11.
7. Gordon MY, Levicar N, PaiM, Bachellier P, Dimarakis I, Al-Allaf F,et al. Characterization and clinical application of human CD34+ stem/progenitor cell populations mobilized into the blood by granulocyte colony-stimulating factor. *Stem Cells* 2006;**4**:1822–30.
8. Pai M, Zacharoulis D, Milicevic MN, Helmy S, Jiao LR, Levicar N, et al. Autologous infusion of expanded mobilized adult bone marrow-derived CD34+ cells into patients with alcoholic liver cirrhosis. *Am J Gastroenterol* 2008;**103**:1952–8.
9. Mohamadnejad M, Namiri M, Bagheri M, Hashemi SM, Ghanaati H, ZareMehrjardi N, et al. Phase 1 human trial of autologous bone marrow-hematopoietic stem cell transplantation in patients with decompensated cirrhosis. *World J Gastroenterol* 2007;**13**:3359-63.
10. Levicar N, Pai M, Habib NA, Tait P, Jiao LR, Marley SB, et al. Long-term clinical results of autologous infusion of mobilized adult bone marrow derived CD34+ cells in patients with chronic liver disease. *Cell Prolif* 2008;**41** Suppl 1:115-25.

11. Terai S, Ishikawa T, Omori K, Aoyama K, Marumoto Y, Urata Y, et al. Improved liver function in patients with liver cirrhosis after autologous bone marrow cell infusion therapy. *Stem Cells* 2006;**24**:2292–8.
12. Kim JK, Park YN, Kim JS, Park MS, Paik YH, Seok JY, et al. Autologous bone marrow infusion activates the progenitor cell compartment in patients with advanced liver cirrhosis. *Cell Transplant* 2010;**19**:1237-46.
13. Mohamadnejad M, Alimoghaddam K, Mohyeddin-Bonab M, Bagheri M, Bashtar M, Ghanaati H, et al. Phase 1 trial of autologous bone marrow mesenchymal stem cell transplantation in patients with decompensated liver cirrhosis. *Arch Iran Med* 2007;**10**:459-66.
14. Kharaziha P, Hellström PM, Noorinayer B, Farzaneh F, Aghajani K, Jafari F, et al. Improvement of liver function in liver cirrhosis patients after autologous mesenchymal stem cells injection: a phase I-II clinical trial. *Eur J Gastroenterol Hepatol* 2009;**21**:1199-1205.
15. Nussbaum J, Minami E, Laflamme MA, Virag JA, Ware CB, Masino A, et al. Transplantation of undifferentiated murine embryonic stem cells in the heart: teratoma formation and immune response. *FASEB J* 2007;**21**:1345-57.
16. Fujikawa T, Oh SH, Pi L, Hatch HM, Shupe T, Petersen BE. Teratoma formation leads to failure of treatment for type I diabetes using embryonic stem cell-derived insulin-producing cells. *Am J Pathol* 2005;**166**:1781-91.
17. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature* 2007;**448**:313-7.
18. Rubio D, Garcia-Castro J, Martín MC, de la Fuente R, Cigudosa JC, Lloyd AC, et al. Spontaneous human adult stem cell transformation. *Cancer Res* 2005;**65**:3035-9.
19. Wong RS. Mesenchymal stem cells: angels or demons? *J Biomed Biotechnol* 2011;2011:459510.
20. Amariglio N, Hirshberg A, Scheithauer BW, Cohen Y, Loewenthal R, Trakhtenbrot L, et al. Donor-derived brain tumor following neural stem cell transplantation in an Ataxia Telangiectasia patient. *PLos Medicine* 2009;6:e1000029.
21. European Association For The Study Of The Liver; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012;**56**:908-43.
22. Chiba T, Matsuzaki Y, Abei M, Shoda J, Tanaka N, Osuga T, et al. The role of previous hepatitis B virus infection and heavy smoking in hepatitis C virus-related hepatocellular carcinoma. *Am J Gastroenterol* 1996;**91**:1195–1203.
23. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, et al. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. *Ann Intern Med* 1999;**131**:174-81.
24. Chiaramonte M, Stroffolini T, Vian A, Stazi MA, Floreani A, Lorenzoni U, et al. Rate of incidence of hepatocellular carcinoma in patients with compensated viral cirrhosis. *Cancer* 1999;**85**:2132-7.
25. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004;**127**:S35-50.
26. Mohamadnejad M, Sohail MA, Swenson S, Sverdlov DY, Sharma A, Hauser RG, et al. Bone marrow-derived mesenchymal stem cell transplantation to enhance the antifibrotic effect of pioglitazone in hepatic fibrosis. *Hepatology* 2009;**50**:811A.