Suppression of orthotopically implanted hepatocarcinoma in mice by umbilical cord-derived mesenchymal stem cells with sTRAIL gene expression driven by AFP promoter

Cihui Yan a,b,c,d,1, Ming Yang a,1, Zhenzhen Li a, Shuangjing Li a, Xiao Hu a, Dongmei Fan a, Yanjun Zhang a,**, Jianxiang Wang a,**, Dongsheng Xiong a,*

a State Key Laboratory of Experimental Hematology, Department of Pharmacy, Institute of Hematology & Hospital of Blood Diseases, Chinese Academy of Medical Sciences & Peking Union Medical College, Number 188, Nanjing Road, Heping District, Tianjin, China
b Department of Immunology, Tianjin Medical University Cancer Institute and Hospital, China
c National Clinical Research Center of Cancer, China
d Key Laboratory of Cancer Immunology and Biotherapy, Tianjin, China

1 These authors contributed equally to this work.

Article info
Article history:
Received 16 November 2013
Accepted 13 December 2013
Available online 6 January 2014

Keywords:
Mesenchymal stem cell
TRAIL
Alpha-fetoprotein
Targeted therapy
Hepatocarcinoma

Abstract
Mesenchymal stem cells (MSCs) are promising vehicles for delivering therapeutic agents in tumor therapy. Human umbilical cord-derived mesenchymal stem cells (HUMSCs) resemble bone marrow-derived MSCs with respect to hepatic differentiation potential in injured livers in animals, while their hepatic differentiation under the hepatocarcinoma microenvironment is unclear. In this study, HUMSCs were isolated and transduced by lentiviral vectors coding the soluble human tumor necrosis factor-related apoptosis-inducing ligand (sTRAIL) gene driven by alpha-fetoprotein (AFP) promoter to investigate the therapeutic effects of these HUMSC against orthotopically implanted hepatocarcinoma in mice. We showed that HUMSCs can be transduced by lentivirus efficiently. HUMSCs developed cuboidal morphology, and expressed AFP and albumin in a two-step protocol. HUMSCs were capable of migrating to hepatocarcinoma in vitro as well as in vivo. In the orthotopical hepatocarcinoma microenvironment, the AFP promoter was activated during the early hepatic differentiation of HUMSCs. After intravenous injected, MSC.AFPILZ-sTRAIL expressed sTRAIL exclusively at the tumor site, and exhibited significant antitumor activity. This effect was stronger when in combination with 5-FU. The treatment was tolerated well in mice. Collectively, our results provide a potential strategy for targeted tumor therapy relying on the use of the tumor tropism and specific differentiation of HUMSCs as vehicles.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Mesenchymal stem cells (MSCs), first described by Friedenstein et al. [1], are characterized as adherence to plastic, expression of specific surface antigens CD105, CD90 and CD73 but lack of CD34 and CD45, and multi-differentiation potential into osteoblasts, adipocytes and chondroblasts [2]. MSCs hold a great promise as vehicles for targeted delivery and local production of agents in tumor therapy because they can be easily isolated and expanded to a large number of cells required for clinical use, have tumor tropism, and can be genetically engineered with viral vectors [3,4]. Human umbilical cord tissue represents a convenient, abundant and economic source of adult MSCs. Human umbilical cord-derived MSCs (HUMSCs) share characters of conventional bone marrow-derived MSCs, which provides an idea chose for intensive studies and application of MSCs.

Gene therapy using therapeutic agents driven by the tumor- or tissue-specific promoter is a promising approach for the treatment of cancer. The alpha-fetoprotein (AFP) promoter, which is reactivated in hepatocellular carcinoma, has been widely applied to regulate the cytotoxic gene expression to kill the tumor cells selectively [5–7]. And the expression vectors and adenovirus were usually used as vehicles for this targeted therapy. While some problems must be concerned, such as low infective efficiency of targeted cells, non-specific infection to normal tissues and potential immunogenicity resulted from viral proteins. Thus, potential therapeutic strategies are urgently needed to overcome these limitations. Accumulated evidences indicate that human bone marrow, umbilical blood and adipose tissue-derived MSCs are...
capable of differentiating into hepatocytes in vitro as well as in vivo, expressing the specific markers for hepatocytes such as AFP, albumin (ALB), CK18 and CK19, and exhibiting hepatic function such as uptake of low density lipoprotein, synthesis of glycogen and the activity of liver drug enzyme cytochrome P450 [8–11]. During the hepatic differentiation of MSCs, AFP expression increases at the early stage and disappears at the later stage. As a result, the AFP promoter, which is reactivated specifically at the early hepatic differentiation of MSCs, could be used to selectively control the expression of a therapeutic agent in MSC-base gene therapy although there is no report so far.

TNF related apoptosis-inducing ligand (TRAIL), a member of tumor necrosis factor (TNF) superfamily, is a promising candidate for cancer therapy as it induces apoptosis in a wide variety of human cancer cell lines, while largely sparing normal cells [12,13]. As a type 2 transmembrane protein, TRAIL can be cleaved by specific proteases and the extracellular region forms a soluble molecule. Soluble TRAIL (sTRAIL) (amino acids 114–281) forms a homotrimer which is a pivotal structure for receptor recognition and apoptotic function [12,14]. The isoleucine zipper (ILZ), a derivation of leucine zipper, is known to be a strong trimerization domain and required for the apoptotic TRAIL protein secreted into the culture supernatant [14]. Nevertheless, recombinant human sTRAIL displays a short half-life (30–60 min) in vivo, which would limit the use of sTRAIL in clinical.

In the present study, we designed a specifically targeted therapeutic system, in which HUMSCs were engineered to specifically secrete cytotoxic ILZ-sTRAIL protein which was regulated by AFP promoter, and investigated its antitumor effect in combination with 5-fluorouracil (5-FU) on the orthotopically implanted hepatocarcinoma in mice.

2. Materials and methods

2.1. HUMSCs preparation and cell culture

HUMSCs were isolated from the gelatinous Wharton’s jelly (WJ) of the human umbilical cord by methods previously described [15]. HUMSCs were subcultured at a density of 4000 cells/cm² in DF-12 medium (Invitrogen, USA) supplemented with 2 mmol/L L-glutamine and 10% FCS (Gibco, USA). Passages 3–5 were used for the following experiments. The human hepatocellular carcinoma cell line HepG2, breast cancer cell line MCF-7, mouse embryonic fibroblast cell line 3T3 (Institute of Hematology & Blood Diseases Hospital Chinese Academy of Medical Sciences & Peking Union Medical College, PUMC, Tianjin, China), and human embryonic kidney cell-derived 293 T cell line (kindly provided by Professor Cheng Tao, PUMC) were maintained in DMEM (Invitrogen, USA) supplemented with 2 mmol/L l-glutamine, 100 units/ml penicillin (HyClone, USA), 100 μg/ml streptomycin (HyClone, USA) and 10% FCS. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO2.

2.2. Luciferase assay for the specificity of AFP promoter

The sequence of alpha-fetoprotein (AFP) specific promoter was amplified from pCL3 plasmid (Cyagen Bioscience, USA) and inserted into pGL3 basic vector (Kpn I and Nhe I) to construct pGL3 AFP Firefly luciferase reporter vector (pGL3 AFP vector). HepG2, MCF-7 and 3T3 cells incubated in the six-well culture plates were transfected with Firefly luciferase-expressing vectors (pGL3 AFP, pGL3 basic, pGL3 control and pGL3 CMV) respectively, and Renilla luciferase-expressing vector (pRL-TK) was co-transfected into these three cell lines as an internal control. These cells were cultured for additional 2 days. Cultures were prepared and analyzed using the dual luciferase reporter assay system (Promega, USA). The relative luciferase activity was calculated as the ratio of Firefly luciferase activity to Renilla luciferase activity. Each data point was averaged from two replicates of three separate experiments.

2.3. Hepatogenic differentiation in vitro

Hepatogenic differentiation was conducted in two-ways as described previously [8]. The hepatogenesis was assessed by detecting AFP and ALB expression, the specific maker genes for liver cells, by RT-quantitative PCR and Western blot.

2.4. Transient transfection of 293 T cells

293 T cells were transfected with pLentIRLZ-STRAIL or pLentIRCopGFP (control) using lipofectamin<sup>®</sup>2000 (Invitrogen, USA). After 48 h of transfection, cell supernatant was collected by centrifugation at 500 × g for 10 min at 4 °C to clear 293 T cells and applied in vitro studies.

2.5. Production of lentiviral vectors

The lentiviral particles produced by 293 T cells were conducted according to the System Biosciences (SBI, Canada) protocol. The lentiviral-containing supernatant was collected at 48 h post-transfection and spun at 500 × g for 5 min, filtered through a 0.44 μm pore size filter (Millipore, USA) and used to transduce HUMSCs immediately or stored at −80 °C.

2.6. Transduction of MSCs

HUMSCs were plated at a density of 2 × 10<sup>5</sup> cells per well in T-25 cm plastic culture flasks and incubated overnight at 37 °C. On the next day, medium was removed and 3 ml of appropriate fresh medium containing lentiviral supernatants at MOI 8 and 8 μg/ml of polybrene (Sigma, USA) was added. The medium was removed after 8 h and 10% FCS DF-12 medium was added. HUMSCs were incubated for the indicated time and DiRed fluorescence was observed under fluorescence microscope (Nikon, Japan).

2.7. In vitro migration study

The migratory ability of HUMSCs was determined using Transwell plates in vitro as described previously [3]. The number of cells that had migrated to the lower side of the filter was counted under a light microscope with five high-power fields (>400). Experiments were done in triplicate.

2.8. In vivo migration of HUMSCs

The human hepatocarcinoma HepG2 cell line was used to build orthotopic liver tumor model in Balb/c athymic nude mice as described previously [16]. All animals (male, age 6 weeks, Peking Union Medical College, PUMC, China) received humane care during the study and had free access to water and laboratory chow. Animal studies were approved by the IACUC of the Institute of Hematology & Hospital of Blood Diseases, PUMC. When the orthotopic tumor model had been successfully developed after 7 days of orthotopic implantation, 5 × 10<sup>6</sup> MSC.CMV luciferase-expressing MSCs were injected into mice for migration detection. Bioluminescence imaging (BLI) was performed using IVIS-Xenogen 100 system (Caliper Lifesciences, USA) at the indicated time. In brief, mice were anesthetized by intraperitoneal administration of 100 μl of 20 mg/ml pentobarbital sodium. Each mouse received 15 mg/ml α-luciferin (Promega, USA) at a dosage of 150 mg/kg, i.p. 10 min prior to imaging. All images represent a 5 min exposure time.

2.9. In vivo differentiation of HUMSCs

Firstly, MSC.AFP Luci cells were orthotopically injected into the tumor-burned liver for differentiation detection. And BLI was detected as described above. Then, MSC.AFP.I.LZ-sTRAIL cells, in which LZ-sTRAIL was fused with CopGFP at the N-terminus, were i.v. injected into mice. After 1-week of injection, mice were sacrificed and the livers were removed for the specific expression of the targeted gene by fluorescence microscopy and Western blot.

2.10. Treatment of orthotopic hepatic carcinoma model

After 7 days of orthotopic tumor inoculation, mice were randomized into eight groups and five mice were included in each group as following: (1) PBS control; (2) 5-FU; (3) HUMSCs; (4) HUMSCs and 5-FU; (5) MSC.AFP.CopGFP; (6) MSC.AFP.CopGFP and 5-FU; (7) MSC.AFP.I.LZ-sTRAIL; (8) MSC.AFP.I.LZ-sTRAIL and 5-FU. The responsive HUMSCs engineered or not were i.v. injected at a dose of 5 × 10<sup>5</sup> cells in each mouse. 5-FU was i.p. injected at a dosage of 10 mg/kg for successive 5 days from the next day of HUMSCs injection. At day 60 after treatment started, mice were sacrificed. The tumor was dissected from each mouse, measured and weighed. The volume is expressed in mm<sup>3</sup> using the formula: V = 0.5 a × b<sup>2</sup> where a and b are the long and short diameters of the tumor, respectively. The serum level of liver enzymes alanine aminotransferase (ALT) and aspartate transaminase (AST) was assessed by spectrophotometer (Nanjing JiaCheng Bioengineering Institute, China).

2.11. Statistical analysis

Data are represented as mean ± SD. Differences between groups were examined for significant differences by ANOVA LSD or Dunnett post hoc procedure. Values of P < 0.05 were considered to be statistically significant and that of P < 0.01 were considered to be highly statistically significant.

3. Results

3.1. Hepatic differentiation of HUMSCs in vitro

In the absence of serum, cell proliferation arrested. In the presence of HGF and bFGF, the fibroblastic morphology of HUMSCs was lost and cells developed a broadened morphology by the end of the induction step. In the presence of oncostatin M, dexamethasone and ITS<sup>®</sup>, elongated ends disappeared and a cuboidal morphology...
of hepatocytes developed with increasing time of differentiation. After prolonged culture, abundant granules appeared in the cytoplasm of differentiated cells (Fig. 1A).

The mRNA expression of AFP, an early maker gene of hepatocytes, increased and rank the top at day 12, then decreased sharply. The mRNA expression of ALB increased along the time of differentiation (Fig. 1B). The protein expression of AFP rose dramatically at day 6 and decreased afterward. The protein expression of ALB increased and was detected at all time points post-induction. While undifferentiated cells did not express AFP or ALB (Fig. 1C).

3.2. Construction of lentiviral expression vectors

We successfully cloned AFP specific promoter (2810 bp) from pUp plasmid. The specific transcriptional activity of AFP promoter, which was defined as the ratio of Firefly luciferase (fLuc) activity to Renilla luciferase activity, was tested firstly before vector construction followed. The mean pGL3 AFP ratios were 49.05 ± 5.47, 1.69 ± 0.24 and 6.88 ± 0.61 in HepG2, MCF-7 and 3T3 cells, respectively (Fig. 2A), indicating the specific transcriptional activity of AFP promoter in AFP-positive cells. Then, lentiviral expression vectors which included the targeted genes driven by CMV or AFP promoter were successfully constructed (Fig. 2B). HUMSCs could be transduced efficiently by lentivirus containing the ILZ-sTRAIL gene controlled by AFP promoter without affecting their growth. The DsRed fluorescence could be observed even after 26 days of transduction (Fig. 2C) without the CopGFP fluorescence (data not shown).

3.3. Inhibitory effect of ILZ-sTRAIL on the growth of human HepG2 cells

To obtain ILZ-sTRAIL, 293 T cells were transfected with plasmid pLentiR.ILZ-sTRAIL, in which ILZ-sTRAIL expression was controlled by CMV promoter, and the supernatant containing ILZ-sTRAIL (9.475 ± 0.786 ng/ml) was collected from 293 T cell culture (Fig. 3A) and used in the following studies in vitro. ILZ-sTRAIL inhibited the growth of HepG2 cells in a concentration-dependent manner. And this effect was significantly enhanced when combined with 5-FU (Fig. 3B). The therapeutic index (CI) of

![Fig. 1. Hepatic differentiation of HUMSCs in vitro. (A) Morphological changes at the indicated time during differentiation. (B) Differentiated HUMSCs expressed hepatocyte-specific marker genes by quantitative real-time PCR. (C) Differentiated HUMSCs expressed hepatocyte-specific proteins by Western blot.](image-url)
Combination treatment showed that ILZ-sTRAIL plus 5-FU exhibited an additive inhibitory effect on the proliferation of HepG2 cells (Table 1). The level of Bcl-2 protein expression decreased, and Bax expression increased, caspases involved in apoptosis such as caspase-8, 9, 3 and PARP were cleaved and activated. And all these changes were much more significant when ILZ-sTRAIL and 5-FU were combined in the treatment (Fig. 3C).

3.4. Migration capacity of HUMSCs to hepatocarcinoma in vitro and vivo

The migration potential in HUMSCs was tested firstly using Transwell plates in vitro. Only a few cells migrated toward serum-free medium, while the migration of HUMSCs was stimulated significantly by the conditioned medium from HepG2 cells (Fig. 4A).

---

**Fig. 2.** Plasmid construction. (A) The specific activity of AFP promoter in different cell lines. Cells were co-transfected with firefly luciferase reporter plasmid (containing different promoters needed tested) and Renilla luciferase reporter plasmid (used as an internal control) at a ratio of 50:1. The activity of luciferase was quantified 48 h later. (B) Schematic representation of lentiviral expression vectors constructed. CMV promoter; AFP promoter; EF1 promoter; luciferase; signal peptide; CopGFP; ILZ-sTRAIL; DsRed. (C) High transduction efficiency in HUMSCs by lentivirus.

**Fig. 3.** Inhibitory effect of ILZ-sTRAIL on the growth of hepatocarcinoma HepG2 cells. (A) ELISA detection measuring ILZ-sTRAIL released in the supernatant of 293 T cell culture after transient transfection. (B) Cells were cultured (1 x 10^4 cells/well) overnight and exposed to different concentration of ILZ-sTRAIL in the presence or absence of 5-FU for 72 h and tested by CCK8 assay. **P < 0.01 compared with corresponding concentration of ILZ-sTRAIL treatment alone. (C) Western blot showed apoptosis induced by 4 ng/ml ILZ-sTRAIL.
Moreover, the migratory activity of HUMSCs appeared in a concentration-dependent manner (Fig. 4B). The migration of modified HUMSCs was in a similar pattern as that of unmodified HUMSCs. Next, to monitor tumor tropism of HUMSCs in vivo, orthotopic hepatic carcinoma model was successfully built in Balb/c mice (Fig. 4C), and HUMSCs constitutively expressing fluc reporter gene (MSC.CMVLu) were injected intravenously. Representative BLI analysis in live animals revealed that after 1 day of injection, intensive fluc imaging signals were only detected in lung, suggesting that a mount of HUMSCs were entrapped by capillaries in lung. After 2 days of injection, the signals in lung decreased substantially; in contrast, the signal intensity in the liver slightly increased and strengthened on day 5 (Fig. 4D). No detectable signals were observed in the other organs.

3.5. Hepatic differentiation of HUMSCs in vivo

To verify hepatic differentiation under the microenvironment of orthotopically implanted hepatocarcinoma, we inoculated HUMSCs labeled with fluc driven specifically by AFP promoter (MSC.AFPILuc) in situ in tumor-bearing livers and found that the fluc signal was weak after 1 day of injection, increased and reached the peak on day 7 after injection. Then the activity decreased rapidly and was not detectable after 9 days of injection (Fig. 5A). We also used sham operation as negative control and did not detect any fluc signal in all organs. Next, we injected MSC.AFPILZ-sTRAIL intravenously. 7 days later, we found that HUMSCs migrated to the tumor site and produced the targeted gene identified both by CopGFP fluorescence (Fig. 5B) and by ILZ-sTRAIL protein expression (Fig. 5C) owing to the activation of AFP promoter.

3.6. Antitumor potential of MSC.AFPILZ-sTRAIL against orthotopically implanted hepatic carcinoma

In the in vivo study, mice were sacrificed after 60 days of the start treatment, and the tumors in livers were dissected and weighed. As shown in Fig. 6A, obvious tumor regression was observed in the MSC.AFPILZ-sTRAIL group (p < 0.05) and the MSC.AFPILZ-sTRAIL plus 5-FU group (p < 0.01). And the combination treatment exhibited stronger antitumor effect when compared with MSC.AFPILZ-sTRAIL treatment alone. The pathological result showed that tumor cells in control groups grew vigorously and had bigger trachychromatic nucleuses. However, tumor cells in the groups treated with MSC.AFPILZ-sTRAIL, either with 5-FU or not, appeared pyknotic and necrotic, and had extensive lymphocytic infiltration. This phenomenon was even obvious in the combination treatment group (Fig. 6B). The serum level of ALT and AST decreased significantly in these two groups (ALT, P < 0.01; AST, P < 0.05) (Fig. 5C). During the whole treatment period, there were no significant differences in the body weight in all groups (P > 0.05) (Fig. 5D).

4. Discussion

In this study, we efficiently engineered HUMSCs to specifically secret ILZ-sTRAIL driven by AFP promoter via lentiviral transduction. Our results showed that these cells can migrate toward hepatocarcinoma and undergo hepatic differentiation in vitro as well as in vivo. MSC.AFPILZ-sTRAIL exhibited significant antitumor effect on the orthotopically implanted hepatocarcinoma, which was mediated by ILZ-sTRAIL induced apoptosis and strengthened.

---

Table 1
The therapeutic index (CI) of ILZ-sTRAIL and 5-FU combination treatment. Cells were cultured (1 x 10⁴ cells/well) overnight and exposed to different concentration of ILZ-sTRAIL in the presence or absence of 5-FU for 72 h and tested by CCK8 assay. CI < 1, synergistic; CI = 1, additive; CI > 1, antagonistic.

<table>
<thead>
<tr>
<th>5-FU (µg/ml)</th>
<th>ILZ-sTRAIL (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>0.5</td>
<td>1.1</td>
</tr>
<tr>
<td>2</td>
<td>0.93</td>
</tr>
</tbody>
</table>

---

Fig. 4. Tumor tropism of HUMSCs in vitro and vivo. (A) The migratory capacity of HUMSCs in response to conditioned medium of HepG2 cells was determined using Transwell plates. SFM (serum-free medium) used as a negative control. (B) The number of cells migrated to the lower side of the filter was counted under a light microscope with five high-power fields (x400). "**P < 0.01 compared with SFM. Experiments were done in triplicate. (C) Orthotopically implanted hepatic carcinoma model was well established. Livers were removed and examined histopathologically at day 7 after transplantation. (D) HUMSCs migrated to orthotopical hepatic carcinoma in vivo. HUMSCs labeled with constitutively expressed firefly luciferase were i.v. injected into tumor-bearing mice and monitored by bioluminescence imaging using Xenogen imaging system at the indicated time.
when combined with 5-FU. This is an original report that the homing capacity and differentiation potential are combined together in the strategy of human derived MSCs as vehicles delivering targeted agents for tumor therapy.

The effect of MSCs on tumor growth is always in debate, which might be due to the factors of the different source of MSCs, the ratio of each cell population performed in animal models, the location of the lesion, and alternative administration route et al. It is reported that hepatocellular carcinoma (HCC) derived MSCs promote HCC cell proliferation and invasion [17]. And tumor cells mixed with bone marrow-derived MSCs transplanted subcutaneously exhibited elevated capability of proliferation, rich angiogenesis in tumor tissues and highly metastatic ability [18,19]. However, HUMSCs, used in our study, did not promote the growth of orthotopically hepatic carcinoma. Several recent studies demonstrate that HUMSCs express high level of pro-apoptotic and tumor suppressor genes [20] having the properties of non-tumorigenicity [21] or anti-tumorigenicity [22], and unable transform into tumor-associated fibroblasts (TAFs) [23]. As a result, HUMSCs represent an ideal source of MSCs in targeted therapy.

We used an orthotopical human hepatocarcinoma xenograft model to imitate the microenvironment of liver cancer in vivo and evaluate the migratory ability, differentiation potential and therapeutic efficacy of HUMSCs. The tumor tropism of HUMSCs was observed obviously in our study. However, in healthy or sham mice, HUMSCs did not home to liver and disappeared quickly in vivo (data not shown). Tumors can be regarded as wounds that do not heal; the shared tropism of MSCs in site of an injury tissue and tumor is thought to result from the similarities of the microenvironment [24]. The exact process underlining the migration of MSCs to tumor site is not clear. Two possible mechanisms have been proposed, one is that the released chemokines/cytokines increases the migration of MSCs [25]; another is that the interaction of cytokines or chemokines with their corresponding receptors would induce the migration of MSCs towards tumor microenvironment [26]. As tumor-bearing mice had a tumor microenvironment resembling of an unresolved wound, HUMSCs could migrate specifically and localize at the tumor site. Our result showed that the accumulation of HUMSCs in liver presented in delayed phase. We presume that although some amount of HUMSCs were trapped by pulmonary capillaries and a part of HUMSCs that had arrived in liver died from the unsuitable environment, the HUMSCs remaining in liver could survive and grow after a short adaptive phase.

It has been reported that in the hepatic injured and cirrhotic rat, human MSCs from bone marrow as well as umbilical blood are capable of differentiating into hepatocytes and improve hepatic function [9,27]. On the contrary, the differentiation of bone marrow cells into mature hepatocytes is much low efficiency under physiologic conditions as a selection strategy is required for the differentiation [28]. However, the hepatic differentiation of HUMSCs is little reported. In our experiment, hepatic differentiation of HUMSCs was detected in the livers bearing orthotopically implanted hepatocarcinoma, but not in the site of orthotopically implanted breast cancer (data not shown), indicating that the specific microenvironment of hepatocarcinoma would act as a selective pressure to stimulate hepatic differentiation of HUMSCs. This result is also confirmed by a recent study which demonstrates that HUMSCs could be a promising stem cell source to generate hepatocyte-like cells as transcription factors involved in liver development and liver progenitor markers are highly expressed in HUMSCs [29]. The
relative shorter expression phase of AFP here could result from the different sources of MSCs, test methods and animal models.

As we found that AFP gene expressed specifically at the early stage of hepatic differentiation of HUMSCs under the specific environment of hepatocarcinoma, we genetically engineered HUMSCs to express ILZ-sTRAIL controlled by AFP promoter to inhibit the growth of orthotopical hepatocarcinoma. This therapeutic strategy takes advantage of the tumor tropism and hepatic differentiation of HUMSCs together. Firstly, HUMSCs migrated to tumor site which provided the prerequisite of hepatic differentiation. Then, hepatic differentiated HUMSCs supported re-activation of AFP promoter. Consequently, ILZ-sTRAIL was expressed, secreted, localized and concentrated in the tumor tissue exclusively but not in the peritumoral tissue or other organs. As a result, significant antitumor effect was achieved and no obvious side effects were detected. The improvement of liver function may be associated with the reduced tumor burden and repair and generation by MSC.AFPILZ. The tumor tropism of HUMSCs both decreases the required quantity of HUMSCs injected and escapes the probability of unrelated action resulting from the cytotoxic agents secreted. Even though a number of HUMSCs migrated to other organs and tissues, for example, local inflammation tissues in non-tumor-burned organs or lung capillaries in which HUMSCs were trapped, the cytotoxic agent cannot be expressed as the specific microenvironment devoting to hepatic differentiation was not provided and the AFP promoter would not be active. Furthermore, we found that hepatic differentiated HUMSCs tended to localize in the area of adjacent tumor or even the inner of solid tumor, and the expression of ILZ-sTRAIL exclusively in tumor tissue but not in the peritumoral liver tissue. It seems that cytokines involved in migration and differentiation also distribute gradiently in liver just as in the overall level. As a result, HUMSCs accumulating in tumor site are influenced more deeply by the tumor microenvironment, more susceptible to hepatic differentiation and express targeted agents specifically. The precise mechanism should be investigated further in future.

It is reported recently that bone marrow-derived MSCs from mice differentiate into the stromal compartment of hepatocarcinoma xenografts and express stromal protein Tie2 and
CCL5. These engineering modified MSCs can deliver tissue-specific suicide gene which expression is controlled by Tie2 or CCL5 promoter and suppress the growth of hepatocarcinoma in vivo [30]. As HUMSCs are more inclined to develop hepatic differentiation [29], in our studies, we use the AFP promoter which is active in the early phase of hepatic differentiation to regulate specifically the expression of ILZ-sTRAIL. Only HUMSCs developing hepatic differentiation in the specific microenvironment of hepatocarcinoma but not those developing stromal differentiation could secret the therapeutic agent, which greatly enhance the selectivity and specification of targeted tumor therapy. As an isoleucine zipper was added to sTRAIL to facilitate trimer formation which was the active form of sTRAIL, and the secreted ILZ-sTRAIL collected from the culture supernatant of 293 T cells did not undergo purification process but was used directly, the concentration of ILZ-sTRAIL used in our experiments was much lower than that used in the previous reports. In agree with the previous studies [31,32], we found that 5-FU sensitized tumor cells to TRAIL-induced apoptosis by regulating the expression of some members of Bcl-2 family and activation of caspase signal pathway. Though it is reported that 5-FU can increase the expression of death receptor 4 and 5 (DR4 and DR5) of TRAIL [33], we did not find significant changes in our study (data not shown). Interesting, ILZ-sTRAIL delivered and selectively expressed by HUMSCs in vivo exhibited much greater capacity compared with that in vitro. This may be partially resulted from activation of systemic antitumor immunity via TRAIL-induced apoptosis with the evidence that much lymphocyte infiltration was found in the groups treated with MSCAPFIL2-sTRAIL.

5. Conclusion

We reported here a promising therapeutic strategy of MSC-based gene therapy against hepatocarcinoma. The expression of therapeutic agents was regulated by the activation of AFP promoter during the early stage of hepatic differentiation of HUMSCs. This therapeutic strategy takes good advantages of tumor tropism and hepatic differentiation potential of HUMSCs, which provides a potential way for targeted therapy. However, the interaction between MSCs and microenvironment is complicated and the exact mechanism should be further explored before this therapeutic strategy is translated into clinical.

Acknowledgments

This work is supported by grants from the Chinese National Natural Sciences Foundation (Grant Nos. 30873091, 3071291), National High-tech R&D Program of China (863 program Grant 2011AA020118) and the Natural Sciences Foundation of Tianjin, People’s Republic of China (No. 05YFGZCX02800). We thank Dr. Linlin Jiang for the animal study.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2013.12.037.

References


