Tumor Biol. (2015) 36:6011–6018
DOI 10.1007/s13277-015-3277-6

RESEARCH ARTICLE

Targeting delivery of lipocalin 2-engineered mesenchymal stem cells to colon cancer in order to inhibit liver metastasis in nude mice

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Received: 13 October 2014 / Accepted: 18 February 2015 / Published online: 5 March 2015

Abstract One of the major obstacles in cancer therapy is the lack of anticancer agent specificity to tumor tissues. The strategy of cell-based therapy is a promising therapeutic option for cancer treatment. The specific tumor-oriented migration of mesenchymal stem cells (MSCs) makes them a useful vehicle to deliver anticancer agents. In this study, we genetically manipulated bone marrow-derived mesenchymal stem cells with their lipocalin 2 (Lcn2) in order to inhibit liver metastasis of colon cancer in nude mice. Lcn2 was successfully overexpressed in transfected MSCs. The PCR results of SRY gene confirmed the presence of MSCs in cancer liver tissue. This study showed that Lcn2-engineered MSCs (MSC-Lcn2) not only inhibited liver metastasis of colon cancer but also downregulated the expression of vascular endothelial growth factor (VEGF) in the liver. Overall, MSCs by innate tropism toward cancer cells can deliver the therapeutic agent, Lcn2, and inhibit cancer metastasis. Hence, it could be a new modality for efficient targeted delivery of anticancer agent to liver metastasis.

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Keywords Mesenchymal stem cells · Lipocalin 2 · Liver metastasis · Nude mice · Colon cancer

Introduction

Cancer is one of the world most life-threatening and lethal diseases, and colorectal cancer is the third most common cancer and the fourth most frequent cause of cancer death worldwide [1, 2]. Scientific investigations revealed that many metastatic malignancies show strong resistance to conventional methods of cancer therapy such as surgery, chemotherapy, and radiotherapy. Therefore, many patients suffering from cancer are highly exposed to death due to tumor recurrence and therapy-related harmful complications [3–5]. On the other hand, cancer treatment methods are usually associated with many obstacles including the development of long-term side effects such as cardiac failure, renal injury, musculoskeletal problems, and pulmonary dysfunction [6, 7]. As a matter of fact, lack of tumor specificity is the major drawback of conventional cancer therapies. Therefore, numerous studies have focused on exploring new specific tumor-targeting approaches to efficiently attack cancerous cells. Recently, cell-based therapies have extensively attracted researchers' attention. One of the valuable cells in this regard is mesenchymal stem cells (MSCs) [8]. MSCs have many therapeutic potentials such as simplicity of obtaining and in vitro expansion [9], low immunogenicity [10], self-renewality and high migration potential toward the injury sites [11] These characteristics make them an appropriate candidate for cellular therapies. However, one of the interesting properties of MSCs is the specific tumor-oriented migration that rendered them to function as a new and ideal therapeutic vehicle to deliver anticancer agents directly

to the tumor sites and their metastatic environment [4]. In support of this notion, genetically modified MSCs with anticancer properties are capable of inhibiting tumor growth in experimental animal models such as those with glioma [12–14], melanoma [15], breast [16], prostate [17], and pancreatic [18, 19] cancers.

Lipocalin 2 (Lcn2) or neutrophil gelatinase-associated lipocalin (NGAL) is one of the anticancer molecules [20–22]. Although the precise role of Lcn2 remained to be controversial, several studies reported that it is implicated in pathophysiological phenomena such as inflammation, infection, injury, asthma, arthritis, and cancer [23]. Additionally, some other studies demonstrate that Lcn2 inhibits the proneoplastic factor HIF-1a, FA-kinase phosphorylation as well as vascular endothelial growth factor (VEGF) synthesis [24]. Further studies also confirmed that Lcn2 generates an antitumor effects at least on some sorts of neoplasia such as colon [20], ovary [21], and pancreas [22].

Based on these observations, in the present study, we hypothesized that genetically Lcn2-modified MSCs would specifically track the sites of metastasis formation, and target them through site-specific production of antitumor agent, i.e., Lcn2. Therefore, by exploiting the innate tendency of MSCs toward cancer cells, we exploited bone marrow-derived MSCs as efficient vehicles for the targeted delivery of expressed Lcn2 toward tumor sites. Here, we report that Lcn2-engineered MSCs are capable of homing and localizing into the metastatic liver that further inhibit the liver metastasis of colon cancer cells in nude mice.

Materials and methods

Cells isolation, expansion, and identification

Human bone marrow MSCs were isolated from the bone marrow of healthy human volunteers with informed consent. MSCs were successfully isolated as described previously [25] and cultured in 10 % fetal bovine serum Dulbecco's modified eagle's medium (DMEM; Gibco-BRL, Eggenstein, Germany). The culture dishes were incubated at 37 °C in the presence of 5 % CO_2. In order to confirm the MSCs, they were evaluated by flow cytometry for the expression of surface markers as described previously [25].

On the other hand, human colon cancer cell line SW48 was obtained from the National Cell Bank of Iran (Pasteur Institute of Iran). The cells were cultured in RPMI 1640 medium (Gibco-BRL, Germany) containing 10 % fetal bovine serum (Gibco-BRL, Germany) and 1 % penicillin streptomycin solution (10,000 U of penicillin and 10 mg of streptomycin) inside 25 cm^2 cell culture flasks. The flasks were maintained at 37 °C and 5 % CO_2.

Construction of Lcn2 recombinant plasmid and transfection into MSCs

Following isolation of Lcn2 by PCR, the product was subcloned into pEGFPN1 vector producing recombinant pEGFPN1-Lcn2 plasmid. The fidelity of the subcloned sequence was evaluated by DNA sequencing. Moreover, the amplification of a fragment of about 640 bp length via PCR using Lcn2-specific primers confirmed the translocation of the Lcn2-coding sequence into the destination vector. Next, the MSCs were transfected with 2 μg of pEGFPN1-Lcn2 plasmid using FuGENE HD (Roche, Germany) transfection reagent according to manufacturer's protocol. As a control, MSCs were transfected with pEGFPN1 (MSC-V). The expression level of Lcn2 was evaluated by reverse transcription PCR (RT-PCR), enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA), and Western blot analyses.

RT-PCR and PCR

PCR was performed in order to clone and evaluate the expression of Lcn2 and VEGF. To construct the Lcn2 expression plasmid, the 624-bp-long Lcn2 complementary DNA (cDNA) was isolated by PCR from primary vector, pcDNA3.1-Lcn2, using specific forward 5′AC*GAATTC*-ACCATGGTGCCCCTAGGTCTCCTGTGGCTG3′ and reverse 5′TA*GGATCC*GCCGTCGATACACTGGTC3′ primers. The forward primer included both the BamH1 recognition sequence (italicized) and Kozak sequence sites, while the reverse primer included EcoR1 recognition sequence site (italicized). PCR condition included a primary denaturation step of 5 min at 94 °C followed by 38 cycles of 30 s at 94 °C, 42 s at 59 °C, and 30 s at 72 °C and a final extension step of 3 min at 72 ° C.

To assess the expression of Lcn2 by RT-PCR, total RNA was extracted using TriPure reagent (Roche, Germany) according to manufacturer's protocol. RNA quality was determined by electrophoresis. Then, the extracted RNA was used at the concentration of 500 ng/μl to construct cDNA using First-Strand cDNA Synthesis SuperScript kit (Invitrogen, USA) according to manufacturer's protocol. Then, PCR was performed using internal Lcn2 primers, forward 5′TCACCT CCGTCCTGTTTA3′ and reverse 5′TGGCATACATCTTT TCGGGT3′. PCR condition included a primary denaturation step of 4 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 42 s at 57 °C, and 30 s at 72 °C and a final extension step of 2 min at 72 ° C. Finally, PCR products were analyzed by 2 % agarose gel electrophoresis. β-actin expression was evaluated for normalization.

PCR was also performed to detect SRY gene using SRY primers, forward 5′GAATATTCCCGCTCTCCGGAG3′ and reverse 5′TCTCTGAGTTTCGCATTCTGGGA3′. PCR

conditions included a primary denaturation step of 4 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 42 s at 58 °C, and 30 s at 72 °C and a final extension step of 2 min at 72 ° C. Finally, 7 μl of each PCR product was analyzed by 2 % agarose gel electrophoresis. β-actin expression was evaluated for normalization.

Additionally, to assess the expression of Lcn2 by RT-PCR, total RNA was extracted from liver tissues, and cDNA was synthesized. Then, PCR was performed using internal Lcn2 primers. PCR condition was mentioned above, and PCR products were analyzed by 2 % agarose gel electrophoresis. Also, to evaluate the VEGF expression, PCR was performed using internal VEGF primers. Primer set for VEGF included forward 5′TCCATGGATGTCTATCAGCG3′ and reverse 5′ GCTCATCTCTCCTATGTGCT3′. PCR condition included a primary denaturation step of 4 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 42 s at 59 °C, and 30 s at 72 °C and a final extension step of 2 min at 72 ° C. The PCR products were subsequently analyzed by 2 % agarose gel electrophoresis.

Quantitative real-time PCR

After cell therapy, total RNA was extracted with TriPure reagent (Roche, Germany) from liver tissue. The samples were subjected to quantitative real-time PCR analysis to indicate the increase in the level of VEGF expression (angiogenesis marker). First-strand cDNA was generated using 1 μg RNA using cDNA synthesis kit (Invitrogen, USA) according to the kit instructions. By performing PCR reactions and establishing proper thermal cycles, quantitative real-time PCR was performed in triplicate using SYBR Green PCR Master Mix (Takara, Japan) with real-time PCR 3000 system (Corbett, Sydney, Australia). PCR conditions included a primary denaturation step of 5 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 40 s at 57 °C, and 30 s at 72 °C and a final extension step of 4 min at 72 ° C. The cycle threshold (Ct) was calculated automatically, and normalization was carried out with the β-actin Ct value.

Western blot analyses

Western blot analysis was performed to evaluate the expression of Lcn2 in transfected MSCs with pcDNA3.1-Lcn2 vector at protein level. Denatured samples were run on 10–12 % SDS-polyacrylamide gel and transbloted onto PVDF membranes. The membranes were incubated with 1:200 dilution of specific human primary anti-Lcn2 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Following 2–3 h of incubation at room temperature with primary antibody, the membranes were washed with Tris-buffered saline containing 0.1 % Tween 20, then incubated with 1:2000 dilution of HRP-conjugated IgG (Dako, Glostrup, Denmark) as the secondary antibody (1 h by shaking at room temperature). Finally, after washing the membrane, a chemiluminescence solution was used to visualize the expected protein bands on PVDF membrane.

Experimental liver metastasis

Six-week-old female athymic *C57BL/6* nude mice were obtained from Cancer Models Research Center, Cancer Institute of Iran. To set up experimental liver metastasis, the mice were anesthetized with ketamine (100 mg/kg)/xylazine (10 mg/kg) mixture, and the left flank was prepared for sterile surgery. A small abdominal incision was made in the left flank, and the spleen was isolated and exteriorized. 3×10^6 SW48 cells (in 100 μl phosphate-buffered saline (PBS)) were injected into the spleen with a 27-gage needle. The spleen was turned back to the abdominal cavity, and the wound was sutured in two layers. The surgery procedure was shown in supplementary movie. Several weeks later, the mice were sacrificed. Their liver tissues were evaluated by hematoxylin and eosin (H&E) staining; afterwards, the onset of metastasis was identified on 50th day after tumor cell implantation. To examine the effect of MSC-Lcn2 on liver metastasis, after beginning of the experimental liver metastasis, four groups of mice (n=6/group, according to the similar studies [20, 26]) with preestablished liver metastasis were injected intravenously with PBS, 1×10^6 MSCs, 1×10^6 MSC-V, and 1×10^6 MSC-Lcn2 on day 55. Moreover, two groups of normal mice (with no metastasis) were injected with PBS and 1×10^6 MSCs as the control groups. Finally, on day 65 following tumor cell injection, to evaluate the presence of tumors in the liver, samples were collected, fixed with formalin, and embedded in paraffin for routine H&E staining.

Tumor targeting analysis

In order to investigate whether MSCs migrated to the metastatic liver, SRY gene was detected by PCR reaction on DNA samples from metastatic liver tissues of female mice injected intravenously with MSC-Lcn2, MSC-V, and MSC of male donor origin, at two different time points on 4th and 10th days after MSCs intravenous injection. DNA was extracted from liver tissue by genomic DNA extraction kit (Fermentas, Vilnius, Lithuania) according to manufacturer's protocol followed by PCR as described above.

Results

MSCs naturally express specific MSC-related cell surface markers. As reported previously, MSCs express typical antigenic profile of surface markers. They are positive for CD166,

CD105, CD90, and CD73 and negative for CD45 and CD34 [25].

Overexpression of Lcn2 in MSCs

To determine the overexpression of Lcn2 in MSCs, 4 days post-transfection of the MSCs, GFP was detected by fluorescent microscopy (Fig. 1a). RT-PCR was performed to evaluate the expression of Lcn2 at messenger RNA (mRNA) level. The result revealed that MSCs-Lcn2 overexpressed Lcn2 in comparison to MSCs-V, MSCs which were transfected with empty vectors (Fig. 1b). This was further confirmed by Western blot analysis to detect Lcn2 at protein level (Fig. 1c). Finally, the results were also confirmed by ELISA which were consistent with those of Western blot analysis (data not shown). To determine transient overexpression of Lcn2 in MSCs, RT-PCR was performed at different time points (1, 2, 3, 4, 7, 8, and 10 days post-transfection). The basal expression of Lcn2 increased to the highest expression level at day 4, while it decreased thereafter until day 10 (Fig. 2). These results indicate the transient overexpression of Lcn2.

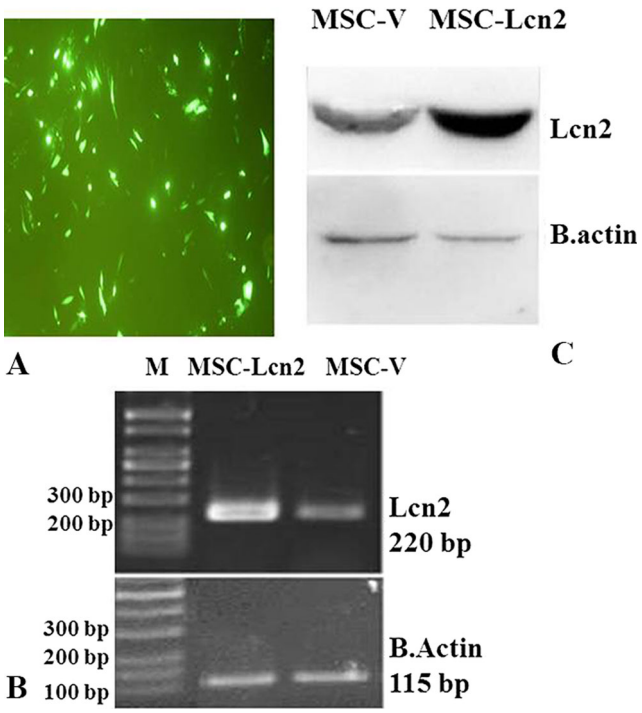

Fig. 1 Overexpression of Lcn2 in MSCs transfected with recombinant pEGFPN1-Lcn2 plasmid. **a** GFP expression in transfected MSCs was detected by fluorescent microscopy. **b** *Upper image*: RT-PCR analysis showed Lcn2 overexpression in transfected MSCs with recombinant pEGFPN1-Lcn2 plasmid (MSC-Lcn2) in comparison with the basal expression level of Lcn2 that was indicated in MSC-V which was transfected with empty vectors. *Lower image*: Expression of β-actin was used for normalization. *M*, 100 bp DNA marker. **c** Western blot analysis of Lcn2 protein expression in pcDNA3.1-Lcn2 transfected MSCs. MSC-Lcn2 revealed high levels of Lcn2 protein overexpression compared to the basic Lcn2 protein expression by MSC-V. *Lower image*: Assessment of β-actin expression as a control for both groups

MSC-Lcn2 inhibits metastasis of liver

Next, we tested the therapeutic potential of MSCs as cellular vehicles to deliver recombinant Lcn2 toward metastatic site. Initiation signs of metastasis were observed histologically at day 50 post-injection (Fig. 3a). The design of the experiment is shown in Fig. 3b. After intravenous injection of transfected MSCs, interestingly, MSC-Lcn2 showed inhibitory effects on liver metastasis growth in four of five experimental animals (Table 1. group no. 6). In contrast, there was no significant influence on liver metastasis growth in mice which were injected with MSC-V, MSC, and PBS (Table 1. group nos. 3, 4, 5). No liver metastases were observed in two control groups of normal mice injected with MSC and PBS. Next, to determine whether human Lcn2 was expressed in the liver, RT-PCR was performed on day 4 after injection. As shown in Fig. 3c, PCR band of Lcn2 was only detected in the liver of those mice transplanted with MSC-Lcn2. This suggests that MSC-Lcn2 expresses and delivers human Lcn2 to the metastatic liver.

MSCs migrate toward metastatic liver

Detection of human Y chromosome-specific SRY gene is one of the well-known methods for tracking cells. Based on PCR detection of SRY gene 4 days after injection, MSCs-Lcn2, MSCs-V, and MSCs were found in the metastatic liver and to a low amount in the liver of mice with no metastasis (Fig. 4a). Moreover, 10 days after injection, MSCs-Lcn2, MSCs-V, and MSCs were maintained in the metastatic livers of mice, and no MSCs were detected in the livers of normal mice (Fig. 4b). Overall, these results indicate that MSCs are capable of homing and localizing into metastatic tissue after systemic injection.

MSC-Lcn2 reduces VEGF expression in the metastatic liver

Angiogenesis is an essential process for tumor growth and dissemination that is mainly stimulated by vascular endothelial growth factor (VEGF), a key angiogenic activator. As shown in Fig. 5a, the basic level of VEGF expression increased in metastatic liver tissues of mice treated with MSC-V and MSC in comparison with the normal liver tissues. In contrast, VEGF expression level was reduced in metastatic liver tissues of mice treated with MSC-Lcn2. Moreover, based on quantitative real-time PCR, VEGF expression was significantly downregulated in metastatic liver tissues of mice treated with MSC-Lcn2 compared to MSCs and MSCs-V. These results suggest that Lcn2 inhibits liver metastasis through downregulation of angiogenesis (Fig. 5b).

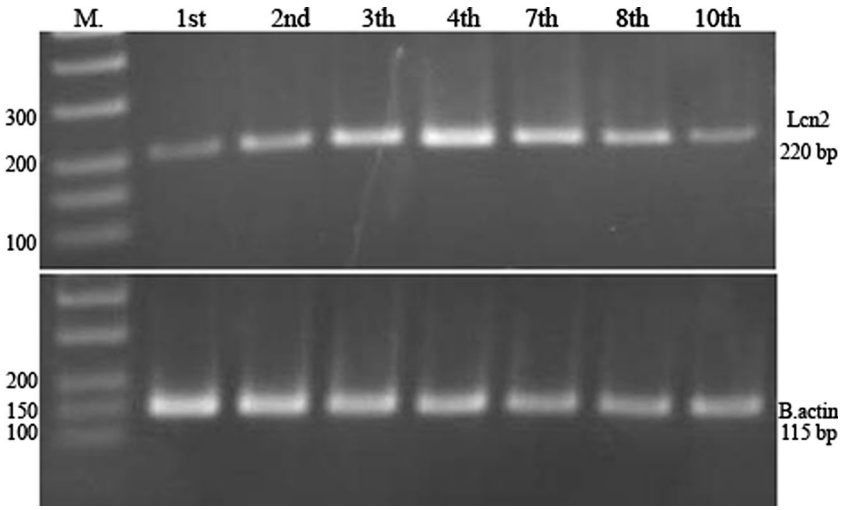

Fig. 2 Transient expression of Lcn2 in MSCs transfected with recombinant pEGFPN1-Lcn2 plasmid (MSC-Lcn2). *Upper image*: RT-PCR analysis of the Lcn2 expression levels up to 7 days after transfection of MSCs with recombinant plasmid showed the highest expression level on day 4 and a decrease in the expression thereafter. *Lower image*: Expression of β-actin was used for normalization. *M*, 100 bp DNA marker

Discussion

Although there have been significant improvements in the cancer treatment methods, cancer remains one of the top life-threatening diseases in which metastasis of cancer cells to vital organs is the major cause of death. Current cancer therapies lack sufficient specificity to tumors; therefore, they posed various side effects on normal tissues. To overcome these limitations, novel approaches have recently focused on selectively target tumors by applying various drug delivery systems such as stealth liposome [27], magnetic nanoparticles [28], and peptides [29].

In the present study, we exploited Lcn2-engineered MSCs as a cellular vector to specifically deliver the antitumor gene into the metastatic liver relying on the intrinsic tumor-oriented homing capacity of MSCs. Additionally, since Lcn2 acts as an antitumoral and antimetastatic agent in some cancers [20–22], here, we genetically manipulated MSCs with Lcn2 to target

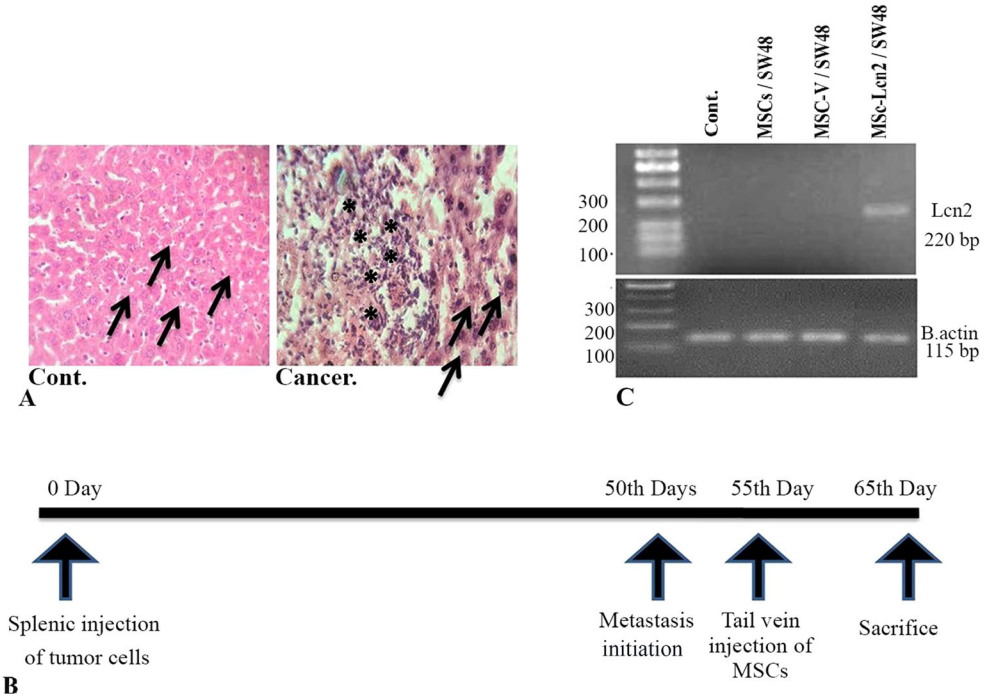

Fig. 3 Liver metastasis after intrasplenic injection of SW48. **a** 50 days after intrasplenic injection, tumor cells were visible in the liver by H&E staining in comparison with control group. Cancer cells are marked by *star* (*), and liver cells are marked by *arrow* (→). **b** Three million SW48 cells were injected intrasplenic at day 0, and initiation of liver metastases was at day 50 post-injection. Subsequently, MSCs were injected into the tail vein on day 55, and finally, the mice were sacrificed on day 65 to evaluate the presence of tumor cells in the liver. **c** Evaluation of gene delivery into liver metastases upon systemic MSC-Lcn2 administration by RT-PCR. Presence of Lcn2 within the liver samples confirmed the capability of MSC-Lcn2 to deliver Lcn2 into metastatic livers after systemic administration (*lane 4*), whereas no expression of Lcn2 was detected in the livers of mice injected with MSC-V, MSC (*lanes 2* and *3*), and control group (*lane 1*)

Table 1 Antitumor effect of MSC-Lcn2 on the growth of liver metastasis

Liver metastasis/ alive mice	Liver metastasis	Alive until 65 days	Failed mice	Total mice per group	Groups	No.
0/6	0	6	0	6	PBS	1
0/5	0	5	1	6	MSCs	2
3/4	3	4	2	6	SW48/PBS	3
4/5	4	5	1	6	SW48/MSCs	4
3/4	3	4	2	6	SW48/MSCs-pEGFPN1	5
1/5	1	5	1	6	SW48/MSCs-pEGFPN1-Lcn2	6

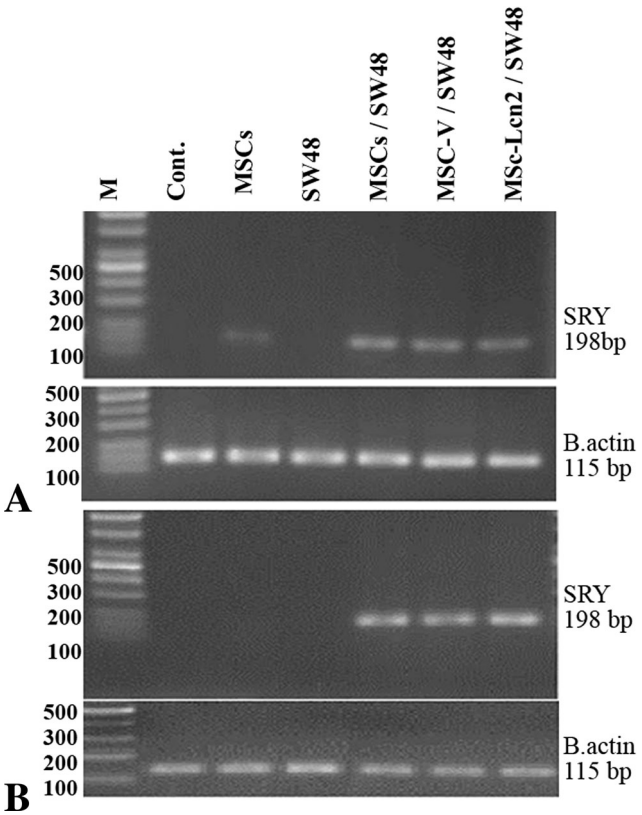

Fig. 4 Tumor homing ability of MSCs into the metastatic liver. **a** *Upper image*: PCR analysis of SRY gene on day 4 after i.v. injection of MSCs showed the presence of MSC, MSC-V, and MSC-Lcn2 in the metastatic liver (*lanes 4*, *5*, and *6*), and also the presence of MSCs in the normal liver (*lane 2*). *Lanes 1* and *3*: No MSC was injected, and MSCs were not detected in the liver. *Lower image*: Expression of β-actin was used for normalization. *M*, 100 bp DNA marker. **b** *Upper image*: PCR analysis of SRY gene on day 10 after i.v. injection of MSCs showed the maintenance of MSC, MSC-V, and MSC-Lcn2 in the metastatic liver (*lanes 4*, *5*, and *6*), and MSCs in the liver of normal mice were cleared away (*lane 2*). *Lanes 1* and *3*: No MSC was injected, and MSCs were not detected in the liver. *Lower image*: Expression of β-actin was used for normalization. *M*, 100 bp DNA marker

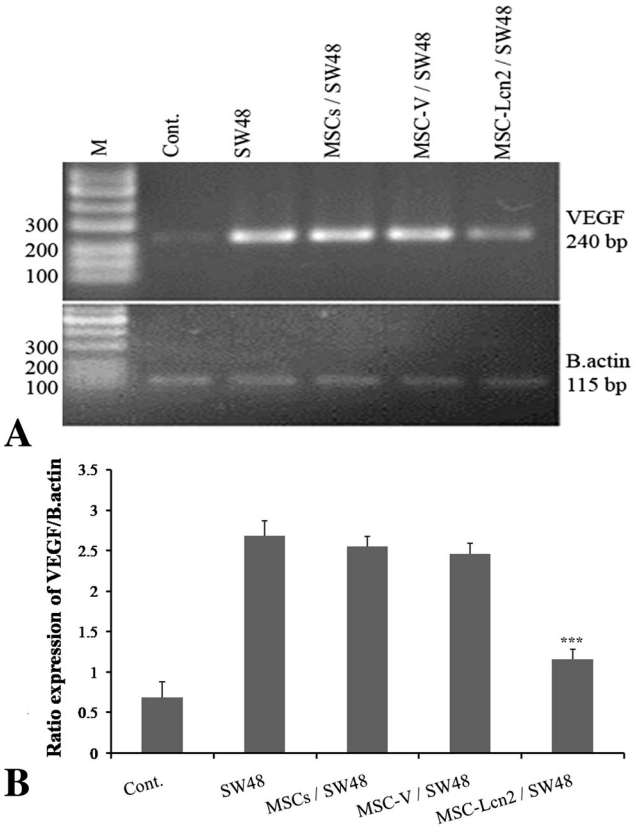

Fig. 5 Effects of MSC-Lcn2 on the angiogenesis of liver metastasis. **a** *Upper image*: RT-PCR analysis of angiogenic factor, VEGF, showed a reduction in VEGF expression in liver tissues of mice treated with MSC-Lcn2 (*lane 5*) compared to the high levels of VEGF expression in metastatic liver tissues of mice treated with MSC-V, MSC (*lanes 3* and *4*), and nontreated mice (*lane 2*). There was a basic VEGF expression in the normal liver as a control (*lane 1*). *Lower image*: Expression of β-actin was used for normalization. *M*, 100 bp DNA marker. **b** Quantitative real-time PCR analysis of VEGF showed that downregulation of VEGF expression was significantly higher in MSC-Lcn2/SW48 compared to MSCS/SW48 and MSCS-V/SW48 (Mean±SD; ***$p<0.001$)

liver metastasis through site-specific production of Lcn2 that exerts inhibitory effects on tumor cells.

We transfected MSCs with pEGFPN1 plasmid vectors. The results revealed that MSCs-Lcn2 successfully overexpress Lcn2 at both mRNA and protein levels. As a matter of fact, employing the plasmid expression system is beneficial because it offers easy transfection condition and transient expression. Considering the fact that the precise role of Lcn2 is not fully understood, we hypothesize that continuous expression of Lcn2 may be harmful. Therefore, we applied plasmid expression system in our study in order to transiently overexpress Lcn2.

Due to the importance of stem cell migration in cancer therapy, we evaluated the migratory property of MSCs. Detection of SRY gene is one of the well-known methods for tracking the cells in cellular therapy. Having applied this method at two different time points, i.e., 4 and 10 days after injection, the results revealed that MSCs-Lcn2, MSCs-V, and

MSCs migrated actively toward the metastatic liver. Moreover, systemically injected MSCs clear away from the liver tissue of normal mice after 10 days, suggesting no necessity for the existence of these cells (MSCs) in normal tissues. Recently, several research groups have proved that genetically engineered MSCs are effective in cancer treatment of animal models bearing different tumors. For instance, IL-2 and EphA2-engineered MSCs are practical to suppress glioma tumor [12, 14]. Furthermore, some studies proved that IL-12, IF-β, and TRAIL-engineered MSCs are capable of reducing lung metastasis of breast and prostate cancer [1, 12, 17]. In this study, we similarly observed significant inhibition of liver metastasis of colon cancer in nude mice injected with MSC-Lcn2. We evaluated the effect of transient overexpression of Lcn2 in genetically engineered MSCs, and we showed the overexpression of Lcn2 by MSCs in vitro throughout 10 days after transfection. In this regard, we evaluated the therapeutic effect of Lcn2 after 10 days in nude mice. Interestingly, after 10 days, we have got a positive result that confirms the therapeutic effect of Lcn2 in a short period of time (10 days). This revealed the promising effect of Lcn2 in cancer treatment. Moreover, generating fast effectiveness over a short time is also a vital factor in cancer treatment. Notably, the findings of this study supported the foregoing property. Moreover, we observed that MSCs-Lcn2 are able to migrate and reduce liver metastasis. However, the effect of Lcn2 on the survival of mice awaits elucidation. In support of our findings, Lee et al. [20] ectopically expressed Lcn2 in the KM12SM colon cancer cell line by a lentiviral vector, and a clear inhibitory effect on liver metastasis of colon cancer was observed in animal models. Moreover, Tong et al. [22] demonstrated that stable Lcn2 overexpression (MIAPaCa-2 and PANC-1) significantly inhibited cell adhesion and invasion in vitro and reduced pancreatic cancer growth in vivo. However, Li et al. [30] reported that downregulation of Lcn2 suppressed human esophageal carcinoma SHEEC cell invasion in vivo. These controversial reports regarding the role of Lcn2 depend mainly on the type of cancer.

Vascular endothelial growth factor (VEGF) is one of the key cancer-promoting molecules capable of intensifying malignancy through increasing the rate of angiogenesis. Taking this into consideration in the present study, we observed that VEGF expression was reduced in metastatic liver tissues of mice treated with MSCs-Lcn2 in comparison with those treated with MSCs-V, MSCs, and PBS. This showed the elevated levels of VEGF in their metastatic liver tissues. In support of this notion, Tong et al. [22] showed that Lcn2 suppressed VEGF expression and angiogenesis in pancreatic cancer. Moreover, Venkatesha et al. reported that Lcn2 inhibits Ras-induced VEGF production [31]. In contrast, Yang et al. [32] proved that Lcn2 promotes angiogenesis in breast cancer.

We used MSCs as means of target delivery of recombinant Lcn2 toward colon cancer cells, and study the potential capability of these engineered cells to inhibit liver metastasis. Our initial results not only indicated that MSCs-Lcn2 migrated toward cancer cells, but they also inhibited metastasis in the liver. This study highlights the importance of MSCs as a new vehicle to perform target delivery of anticancer molecules at least on colon cancer cells. Further comprehensive studies are indeed required in this regard. It is suggested that further studies mainly focus on continuous Lcn2 overexpression in stable clones for a longer period of time.

Acknowledgments This research was supported by the grant awarded from the Iranian Council of Stem Cell Technology.

Conflicts of interest None

Ethical approval All procedures performed in this study involving human participants were in accordance with the Ethical Standards of the Ethics Committee of Iranian Blood Transfusion Organization (ECIBTO) as well as the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Bone marrow samples were obtained from healthy donors with informed consent.

In addition, all procedures performed in this study involving animals were in accordance with the ethical standards of institutional guidelines and approved protocols.

References

1. Loebinger MR, Eddaoudi A, Davies D, Janes SM. Mesenchymal stem cell delivery of TRAIL can eliminate metastatic cancer. Cancer Res. 2009;69(10):4134–42. doi:10.1158/0008-5472.CAN-08-4698.
2. Ferlay JSI, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. GLOBOCAN. Cancer incidence and mortality worldwide: IARC CancerBase No. 11. 2012.
3. Bird NC, Mangnall D, Majeed AW. Biology of colorectal liver metastases: a review. J Surg Oncol. 2006;94(1):68–80. doi:10.1002/jso.20558.
4. Dai LJ, Moniri MR, Zeng ZR, Zhou JX, Rayat J, Warnock GL. Potential implications of mesenchymal stem cells in cancer therapy. Cancer Lett. 2011;305(1):8–20. doi:10.1016/j.canlet.2011.02.012.
5. Sun XY, Nong J, Qin K, Warnock GL, Dai LJ. Mesenchymal stem cell-mediated cancer therapy: a dual-targeted strategy of personalized medicine. World J Stem Cells. 2011;3(11):96–103. doi:10.4252/wjsc.v3.i11.96.
6. Schwartz CL. Long-term survivors of childhood cancer: the late effects of therapy. Oncologist. 1999;4(1):45–54.
7. Stein KD, Syrjala KL, Andrykowski MA. Physical and psychological long-term and late effects of cancer. Cancer. 2008;112(11 Suppl):2577–92. doi:10.1002/cncr.23448.
8. Hu YL, Fu YH, Tabata Y, Gao JQ. Mesenchymal stem cells: a promising targeted-delivery vehicle in cancer gene therapy. J Control Release: Off J Control Release Soc. 2010;147(2):154–62. doi:10.1016/j.jconrel.2010.05.015.
9. Compte M, Cuesta AM, Sanchez-Martin D, Alonso-Camino V, Vicario JL, Sanz L, et al. Tumor immunotherapy using gene-modified human mesenchymal stem cells loaded into synthetic extracellular matrix scaffolds. Stem Cells. 2009;27(3):753–60. doi:10.1634/stemcells. 2008-0831.
10. Sotiropoulou PA, Perez SA, Salagianni M, Baxevanis CN, Papamichail M. Characterization of the optimal culture conditions

for clinical scale production of human mesenchymal stem cells. Stem Cells. 2006;24(2):462–71. doi:10.1634/stemcells. 2004-0331.
11. Studeny M, Marini FC, Champlin RE, Zompetta C, Fidler IJ, Andreeff M. Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumors. Cancer Res. 2002;62(13):3603–8.
12. Nakamura K, Ito Y, Kawano Y, Kurozumi K, Kobune M, Tsuda H, et al. Antitumor effect of genetically engineered mesenchymal stem cells in a rat glioma model. Gene Ther. 2004;11(14):1155–64. doi:10.1038/sj.gt.3302276.
13. Choi SA, Lee JY, Wang KC, Phi JH, Song SH, Song J, et al. Human adipose tissue-derived mesenchymal stem cells: characteristics and therapeutic potential as cellular vehicles for prodrug gene therapy against brainstem gliomas. Eur J Cancer. 2012;48(1):129–37. doi:10.1016/j.ejca.2011.04.033.
14. Sun XL, Xu ZM, Ke YQ, Hu CC, Wang SY, Ling GQ, et al. Molecular targeting of malignant glioma cells with an EphA2-specific immunotoxin delivered by human bone marrow-derived mesenchymal stem cells. Cancer Lett. 2011;312(2):168–77. doi:10.1016/j.canlet.2011.07.035.
15. Maestroni GJ, Hertens E, Galli P. Factor(s) from nonmacrophage bone marrow stromal cells inhibit Lewis lung carcinoma and B16 melanoma growth in mice. Cell Mol Life Sci: CMLS. 1999;55(4):663–7.
16. Sun B, Roh KH, Park JR, Lee SR, Park SB, Jung JW, et al. Therapeutic potential of mesenchymal stromal cells in a mouse breast cancer metastasis model. Cytotherapy. 2009;11(3):289–98. doi:10.1080/14653240902807026. 1 p following 98.
17. Ren C, Kumar S, Chanda D, Kallman L, Chen J, Mountz JD, et al. Cancer gene therapy using mesenchymal stem cells expressing interferon-beta in a mouse prostate cancer lung metastasis model. Gene Ther. 2008;15(21):1446–53. doi:10.1038/gt.2008.101.
18. Kidd S, Caldwell L, Dietrich M, Samudio I, Spaeth EL, Watson K, et al. Mesenchymal stromal cells alone or expressing interferon-beta suppress pancreatic tumors in vivo, an effect countered by anti-inflammatory treatment. Cytotherapy. 2010;12(5):615–25. doi:10.3109/14653241003631815.
19. Zischek C, Niess H, Ischenko I, Conrad C, Huss R, Jauch KW, et al. Targeting tumor stroma using engineered mesenchymal stem cells reduces the growth of pancreatic carcinoma. Ann Surg. 2009;250(5):747–53. doi:10.1097/SLA.0b013e3181bd62d0.
20. Lee HJ, Lee EK, Lee KJ, Hong SW, Yoon Y, Kim JS. Ectopic expression of neutrophil gelatinase-associated lipocalin suppresses the invasion and liver metastasis of colon cancer cells. Int J Cancer J Int Du Cancer. 2006;118(10):2490–7. doi:10.1002/ijc.21657.
21. Lim R, Ahmed N, Borregaard N, Riley C, Wafai R, Thompson EW, et al. Neutrophil gelatinase-associated lipocalin (NGAL) an early-screening biomarker for ovarian cancer: NGAL is associated with epidermal growth factor-induced epithelio-mesenchymal transition. Int J Cancer J Int du cancer. 2007;120(11):2426–34. doi:10.1002/ijc.22352.
22. Tong Z, Kunnumakkara AB, Wang H, Matsuo Y, Diagaradjane P, Harikumar KB, et al. Neutrophil gelatinase-associated lipocalin: a novel suppressor of invasion and angiogenesis in pancreatic cancer. Cancer Res. 2008;68(15):6100–8. doi:10.1158/0008-5472.CAN-08-0540.
23. Halabian R, Tehrani HA, Jahanian-Najafabadi A, Habibi RM. Lipocalin-2-mediated upregulation of various antioxidants and growth factors protects bone marrow-derived mesenchymal stem cells against unfavorable microenvironments. Cell Stress Chaperones. 2013;18(6):785–800. doi:10.1007/s12192-013-0430-2.
24. Bolignano D, Donato V, Lacquaniti A, Fazio MR, Bono C, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin (NGAL) in human neoplasias: a new protein enters the scene. Cancer Lett. 2010;288(1):10–6. doi:10.1016/j.canlet.2009.05.027.
25. Mohammadzadeh M, Halabian R, Gharehbaghian A, Amirizadeh N, Jahanian-Najafabadi A, Roushandeh AM, et al. Nrf-2 overexpression in mesenchymal stem cells reduces oxidative stress-induced apoptosis and cytotoxicity. Cell Stress Chaperones. 2012;17(5):553–65. doi:10.1007/s12192-012-0331-9.
26. Kucerova L, Altanerova V, Matuskova M, Tyciakova S, Altaner C. Adipose tissue–derived human mesenchymal stem cells mediated prodrug cancer gene therapy. Cancer Res. 2007;67:6304–13. doi:10.1158/0008-5472.CAN-06-4024.
27. Adil M, Belur L, Pearce TR, Levine RM, Tisdale AW, Sorenson BS, et al. PR_b functionalized stealth liposomes for targeted delivery to metastatic colon cancer. Biomater Sci. 2013;1(4):393–401. doi:10.1039/C2BM00128D.
28. Moritake S, Taira S, Ichiyanagi Y, Morone N, Song SY, Hatanaka T, et al. Functionalized nano-magnetic particles for an in vivo delivery system. J Nanosci Nanotechnol. 2007;7(3):937–44.
29. Lu RM, Chen MS, Chang DK, Chiu CY, Lin WC, Yan SL, et al. Targeted drug delivery systems mediated by a novel Peptide in breast cancer therapy and imaging. PLoS One. 2013;8(6):e66128. doi:10.1371/journal.pone.0066128.
30. Li EM, Xu LY, Cai WJ, Xiong HQ, Shen ZY, Zeng Y. Functions of neutrophil gelatinase-associated lipocalin in the esophageal carcinoma cell line SHEEC. Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao Acta Biochim Biophys Sin. 2003;35(3):247–54.
31. Venkatesha S, Hanai J, Seth P, Karumanchi SA, Sukhatme VP. Lipocalin 2 antagonizes the proangiogenic action of ras in transformed cells. Mol Cancer Res : MCR. 2006;4(11):821–9. doi:10.1158/1541-7786.MCR-06-0110.
32. Yang J, McNeish B, Butterfield C, Moses MA. Lipocalin 2 is a novel regulator of angiogenesis in human breast cancer. FASEB J : Off Publ Fed Am Soc Exp Biol. 2013;27(1):45–50. doi:10.1096/fj.12-211730.