Inhibitory effect of therapeutic genes, cytosine deaminase and interferon-β, delivered by genetically engineered stem cells against renal cell carcinoma

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Received March 6, 2018; Accepted October 18, 2019

DOI: 10.3892/or.2020.7570

Abstract. Although the effects of stem cells expressing anticancer genes on tumor growth have been demonstrated by many researchers in various types of cancer, relatively few studies have investigated their inhibitory effects on cancer metastasis. In the present study, we examined the inhibitory effects of cytosine deaminase (CD)/5-fluorocytosine (5-FC) and interferon-β (IFN-β) using genetically engineered neural stem cells (hNSCs) in a cellular and metastasis model of renal cell carcinoma (RCC). The CD/5-FC method has the advantage of minimizing damage to normal tissues since it selectively targets cancer cells by the CD gene, which converts prodrug 5-FC to the drug 5-fluorouracil. Moreover, we used hNSCs as a tool to effectively deliver the anticancer genes to the tumor site. These stem cells are known to possess tumor-tropism because of chemoattractant factors expressed in cancer cells. Therefore, we ascertained the expression of these factors in A498 cells, a cell line of RCC, and identified the A498-specific migration ability of hNSCs. We also confirmed that the proliferation of A498 cells was significantly reduced by therapeutic hNSCs in the presence of 5-FC. Furthermore, we established an A498 metastasis model. In the animal experiment, the weight of the lungs increased in response to cancer metastasis, but was normalized by hNSCs expressing CD and/or IFN-β genes, while the incidence of liver metastasis was suppressed by the hNSCs. Overall, the results of this study demonstrate that stem cells expressing anticancer genes have the potential for use as an alternative to conventional therapy for metastatic cancer.

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Key words: engineered stem cells, renal cell carcinoma, metastasis, cytosine deaminase, interferon-β

Introduction

Renal cell carcinoma (RCC) derived from the lining of the proximal convoluted tubule is the most common tumor of the kidney and is diagnosed with high incidence (1,2). Indeed, RCC accounts for more than 90% of kidney neoplasms and about 2% of malignant tumors (3,4). Approximately 50% of patients with RCC have been reported to have low survival rates because of metastasis (5). The most common metastatic sites are the lung parenchyma (50-60%) and liver (30-40%) (6). Traditional cytotoxic chemotherapeutic agents and immunotherapy generally have poor effects on RCC (7). Surgery has therapeutic effects for patients whose tumors are limited to the kidney, but 20 to 40% of patients develop metastasis after surgery (8,9). Moreover, recurrence and metastasis have been reported in RCC patients undergoing radical surgery (10). Therefore, research efforts are needed to establish effective treatment strategies for the treatment of malignant RCC.

Gene-directed enzyme prodrug therapy (GDEPT) is an effective strategy for cancer therapy that can minimize adverse drug reactions as it selectively inhibits cancer cells using the bystander effect of suicide genes that convert pro-drugs to drugs near the tumor sites (11,12). If there are vehicles by which these genes can be effectively transferred toward cancer cells, these genes are expressed near the neoplasm (13). For example, *Escherichia coli* cytosine deaminase (CD), a suicide gene, alters 5-fluorocytosine (5-FC) to its effective form, 5-fluorouracil (5-FU) (11,14). 5-FU specifically inhibits DNA synthesis and induces cell death in human tumors (15,16). In addition, interferon-β (IFN-β), a type I IFN known to block the synthesis phase of the cell cycle, inhibits cancer proliferation at high concentrations, but is problematic due to excessive toxicity (17,18). Therefore, it is necessary to investigate promising treatments using tools that can efficiently convey anticancer genes (e.g., CD and IFN-β) while minimizing biological cytotoxicity in normal tissues.

In the present study, we used HB1.F3 cells (human neural stem cells) derived from a 15-week-old fetus as delivery tools (19). hNSCs are suitable for the treatment of metastatic tumors because they were effectively recruited to a distant lesion in a previous study (20). We transduced HB1.F3 cells to express CD and/or IFN-β genes. One of these was HB1. F3.CD cells expressing only the CD gene (19), while the other was HB1.F3.CD.IFN-β cells expressing CD and IFN-β genes. Many researchers have demonstrated that these cells migrate to the tumor and interfere with cancer cell proliferation (21-23). Furthermore, we confirmed the anticancer effect of HB1.F3.CD and HB1.F3.CD.IFN-β cells using RCC A498 cells, in a cellular and metastasis model. Overall, the results of this study suggest that hNSC therapy is a powerful approach for the treatment of patients with metastasized nodules.

Materials and methods

Cell culture and media. All hNSCs (HB1.F3, HB1. F3.CD and HB1.F3.CD.IFN-β) were kindly provided by Dr Seung U. Kim of the University of British Columbia, Vancouver, BC, Canada. A498 cells were purchased from the Korean Cell Line Bank. Hyclone™ Dulbecco's modified Eagle's medium (DMEM, Hyclone Laboratories) supplemented with 10% fetal bovine serum (GE Healthcare), 10 mM HEPES (Invitrogen Life Technologies; Thermo Fisher Scientific, Inc.), 10 U/ml penicillin and streptomycin of 100 μg/ml (Cellgro Mediatech) was used as the culture medium for all cell lines.

Reverse transcriptase polymerase chain reaction (PCR) analysis. The RNAs of HB1.F3.CD, HB1.F3.CD.IFN-β and A498 cells were isolated using Trizol (Invitrogen Life Technologies; Thermo Fisher Scientific, Inc.), while M-MLV RT (iNtRON Biotechnology) was used to obtain cDNAs. The expression of the anticancer genes was confirmed using primers for human glyceraldehydes-3-phosphate dehydrogenase (GAPDH, sense primer 5'-ATGTTCGTCATGGGTGTG AACCA-3' and antisense 5'-T GGCAGGTTTTTCTAGACG GCAG-3'), CD (sense primer 5'-GCGCGAGTCACCGCC AGCCACACCACGGC-3' and antisense, 5'-GTTTGTAAT CGATGGCTTCTGGCTGC-3') and IFN-β (sense primer, 5'-AAAGAAGCAGCAATTTTCAG-3' and antisense, 5'-TTT CTCCAGTTTTTCTTCCA-3') genes in HB1.F3.CD and HB1.F3.CD.IFN-β cells. In addition, stem cell factor (SCF, sense primer, 5'-CCAGCTCCCTTAGGAATGACA-3' and antisense, 5'-TAAATGAGACCCAAGTCCCGC-3'), vascular endothelial growth factor (VEGF, sense primer, 5'-AAA ACACAGACTCGCGTTGC-3' and antisense, 5'-GGCCGC GGTGTGTCTA-3') and C-X-C chemokine receptor type 4 (CXCR4, sense primer, 5'-ATCCCTGCCCTCCTGCTGACT ATTC-3' and antisense, 5'-GAGGGCCTTGCGCTTCTG GTG-3'), which are chemoattractant factors, were amplified from A498 cells by PCR. PCR amplification of cDNAs was performed at 30 cycles in denaturation for 30 sec at 95˚C, annealing for 30 sec at 58˚C, and extension for 30 sec at 72˚C. All PCR products were separated from 1.5% agarose gel containing NEO Green (NEO Science) by electrophoresis. The results of electrophoresis were confirmed using LuminoGraph II (ATTO Corp.).

Cell viability assay. A498 cells (2,000 cells/well) were seeded in 96-well plates on day 1. After 24 h, 5-FC and 5-FU of each concentration diluted in phosphate buffered saline (PBS) were applied once a day for three days. In another 96-well plate, A498 (1,000 cells/well) and hNSCs (2,000 cells/well) were co-cultured to confirm the effects of HB1.F3.CD and HB1.F3.CD.IFN-β cells expressing anticancer genes *in vitro*. 5-FC of each concentration was applied under the same conditions in wells containing A498 and hNSCs, after which EZ-Cytox reagent (iTSBiO) was added to wells according to the manufacturer's protocols. After 2 h, the optical densities (ODs) (450 nm) were measured using a microplate reader (BioTek Instruments, Inc.) to confirm the viability of the A498 cells.

Transwell assay. Transwells (BD Biosciences) with an 8.0 μm pore size were used to demonstrate the tumor-tropic ability of hNSCs toward A498 cells. Briefly, A498 cells were separately seeded in a 24-well plate (lower chambers) on day 1. Negative control (NC) groups included only media without A498 cells. After incubation for 24 h, three types of hNSCs were seeded into the Transwells (upper chambers) pre-coated with fibronectin (Sigma-Aldrich; Merck KGaA). There were 6 groups: HB1.F3 with or without A498 cells, HB1.F3.CD with or without A498 cells and HB1.F3.CD.IFN-β with or without A498 cells. Within 24 h, the membranes of the Transwells were treated with 3.7% formaldehyde (Sigma-Aldrich; Merck KGaA), and then permeabilized with 100% methanol (Sigma-Aldrich; Merck KGaA). All membranes of the Transwells were then stained with crystal violet and the number of migrated stem cells was counted using an IX-73 inverted microscope (Olympus) and Cell Sense Dimension (Olympus Corp.).

Metastasis model of RCC. Experiments were conducted with approval from the Chungbuk National University Institutional Animal Care and Use Committee (CBNUA-1089-17-01). We purchased 4 week-old female athymic nude mice (KOATECH) and allowed them to acclimatize to the experimental environment for one week. Thirty-fine mice were randomly grouped by weight (initial weight 22-26 g). A498 cells ($2x10^6$ cells) pre-stained with CMFDA Green Cell Tracker (CMFDA) were mixed in PBS and then injected into the tail vein.

Treatment of genetically engineered hNSCs in a mouse model. We divided the mice into five experimental groups (seven mice per group). Group 1 (NC group) was a negative control group without injections. Group 2 (A498 group) was injected with A498 cells alone. One week after injection of the A498 cells, hNSCs pre-stained with CM-DiI Red Cell Tracker (CM-DiI) were injected into the tail vein (group 3 (F3 group) injected with HB1.F3; groups 4 (CD group) injected with HB1.F3.CD and groups 5 (CD-β group) injected with HB1.F3.CD.IFN-β). Next, 5-FC (200 mg/kg/day) was intravenously injected into mice in the F3, CD and CD-β groups three times a week (24). The injection cycle of hNSCs and 5-FC was repeated three times. At 30 days after the A498 injection, mice of all groups were euthanized by inhalation with dimethyl ether, after which their organs were extracted, weighed and stored in 10% formalin solution.

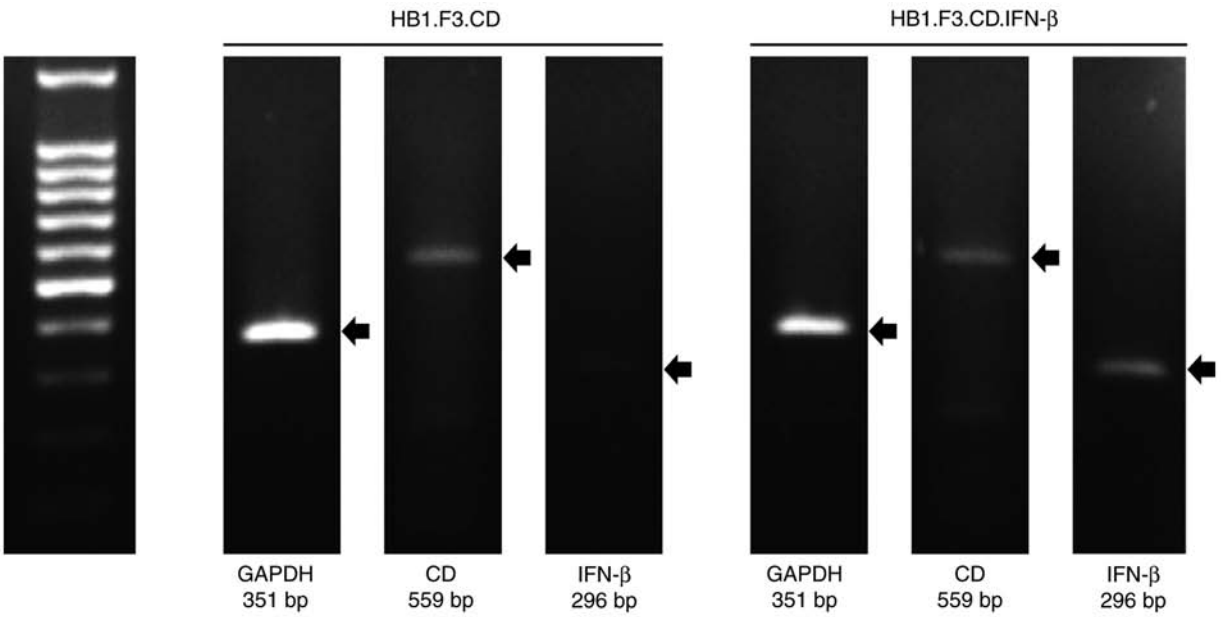

Figure 1. Expression of CD and IFN-β genes in HB1.F3.CD and HB1.F3.CD.IFN-β cells, which are genetically engineered hNSCs. Extraction/synthesis of total RNA/cDNA was performed to confirm the expression of anticancer genes (CD and IFN-β) in both types of hNSCs. The synthesized cDNA was amplified by PCR and separated by electrophoresis. The results of electrophoresis were confirmed using LuminoGraph II, and GAPDH was used as an internal control for comparison with CD and IFN-β in both types of hNSCs. GAPDH, 351 bp; CD, 559 bp; IFN-β, 296 bp. CD, cytosine deaminase; IFN-β, interferon-β; hNSCs, genetically engineered human neural stem cells.

Fluorescence analysis. Tissue sections on slides were prepared as previously described (22). Briefly, slides were treated with DAPI solution (600 nM) for fluorescence analysis, mounted with cover glasses and then analyzed using an IX-73 inverted microscope (Olympus). Blue images indicate the nuclei of all cells including hNSCs and A498 cells. Green indicates CMFDA pre-stained A498 cells; red, CM-DiI pre-stained hNSCs.

Data analysis. All experiments were repeated at least three times. Statistical analyses were conducted using GraphPad Prism (v5.0; GraphPad Software). Groups were compared by two-way analysis of variance (ANOVA) followed by post hoc Dunnett's or Tukey's multiple comparison tests or Student's t-tests. Data are presented as the means ± standard error of the means. P-values <0.05 were considered statistically significant.

Results

Expression of CD and/or IFN-β genes in HB1.F3.CD and HB1.F3.CD.IFN-β cells. We conducted PCR to confirm the expression of anticancer genes in the genetically engineered hNSCs using GAPDH, CD and IFN-β primers. The GAPDH gene was used as an internal control of each cell and the PCR products were electrophoresed on the same gel. As a result, the CD gene was expressed in both HB1.F3.CD and HB1.F3.CD.IFN-β cells and the IFN-β gene was expressed only in HB1.F3.CD.IFN-β cells (Fig. 1).

Inhibition effect of CD/5-FC and IFN-β gene in vitro. To confirm the inhibitory effect of 5-FU against A498 cells, wells seeded with only A498 cells were treated with 5-FC and 5-FU, and the cell viability was evaluated. As shown in Fig. 2B, A498 cells were significantly influenced by treatment with 5-FU (500 μg/ml) compared to the 5-FC (500 μg/ml) group, and a significant reduction in cell viability was observed in response to all concentrations of 5-FU (Fig. 2A). Moreover, 5-FC was applied to wells that contained co-cultured A498 cells and hNSCs to evaluate the anti-proliferative effects of CD gene-expressing hNSCs that convert 5-FC into 5-FU. The cell viability of A498 cells decreased significantly in the HB1.F3.CD group compared to the HB1.F3 group at all concentrations, and the synergism of the CD and IFN-β genes was confirmed in the HB1.F3.CD.IFN-β group (Fig. 2C and D).

A498-specific migration ability of hNSCs. hNSCs are known to have cancer-specific migration capabilities induced by chemoattractant factors expressed in cancer cells. Therefore, PCR was conducted to confirm the expression of these factors in A498 cells. The chemoattractant factors SCF, CXCR4 and VEGF were expressed in A498 cells (Fig. 3A). The GAPDH gene was used as an internal control of A498 cells.

Transwells were used to identify the migration capability of hNSCs toward A498 cells *in vitro*. The membranes of the Transwells were stained with crystal violet solution and the number of hNSCs that migrated in the direction of the lower chambers, where the A498 cells were located, was determined by counting using an IX-73 inverted microscope (Fig. 3B). As shown in Fig. 3C, a higher number of hNSCs migrated in the A498 groups than that noted in the NC groups.

Changes in lungs in the metastasis model. To confirm the metastasis-suppressive effects of hNSCs *in vivo*, a metastasis model was established as described in Materials and methods. After

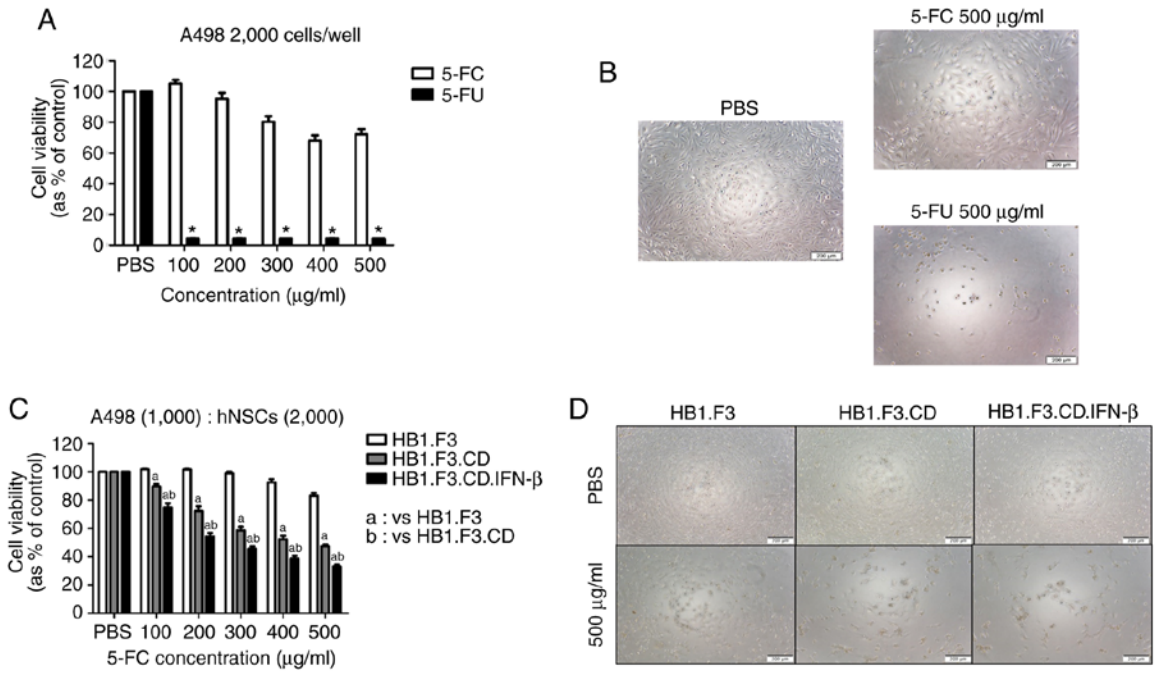

Figure 2. A498-inhibitory effects of hNSCs expressing CD and/or IFN-β genes against A498 cells in a cell viability assay. (A and B) A498 cells ($2x10^3$ cells/well) seeded on 96-well plates were treated with pro-drug (5-FC) or drug (5-FU) of each concentration (0-500 μg/ml) once a day for 3 days. After 3 days, cell viability assay and microscopic analysis were performed. (C and D) A498 cells ($1x10^3$ cells/well) were co-cultured with three types of hNSCs in 96-well plates. These 3 groups were treated with the six concentrations of 5-FC once a day for 3 days. After microscopic analysis, cell viability was analyzed using an EZ-Cytox kit. A PBS group was used as a negative control, meaning that the concentration of treated 5-FC or 5-FU was 0 μg/ml. Scale bars, 200 μm. *P<0.05 vs. 5-FC, ^{a}P<0.05 vs. HB1.F3, ^{b}P<0.05 vs. HB1.F3.CD. CD, cytosine deaminase; IFN-β, interferon-β; hNSCs, genetically engineered human neural stem cells; 5-FC. 5-fluorocytosine; 5-FU, 5-fluorouracil.

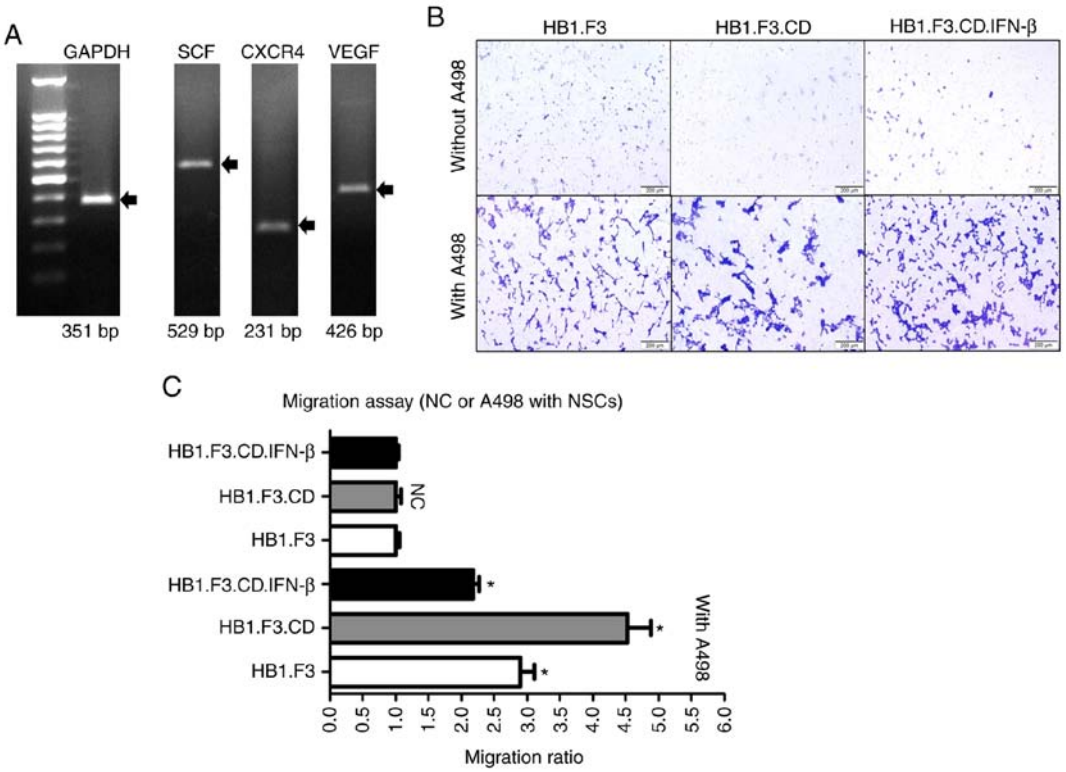

Figure 3. Identification of A498-specific migration ability of hNSCs by PCR and Transwell assay. (A) The chemoattractant factors SCF (529 bp), CXCR4 (231 bp), and VEGF (426 bp) are expressed in A498 cells as determined by PCR. The results of PCR were identified by LuminoGraph II. (B and C) A498 cells ($1x10^5$ cells/well) were seeded into the lower chambers of 24-well plates, while three types of hNSCs ($1x10^5$ cells/well) were seeded into the upper chambers of the Transwells that had been pre-coated using fibronectin. After incubation for 24 h, membranes of the Transwells were stained with crystal violet. hNSCs that migrated were enumerated using the cellSense Dimension software (Olympus Life Science Solutions). NC groups did not contain the A498 cells in the lower chambers. Scale bars, 200 μm. hNSCs, genetically engineered human neural stem cells; NC, negative control; SCF, stem cell factor; VEGF, vascular endothelial growth factor; CXCR4, C-X-C chemokine receptor type 4.

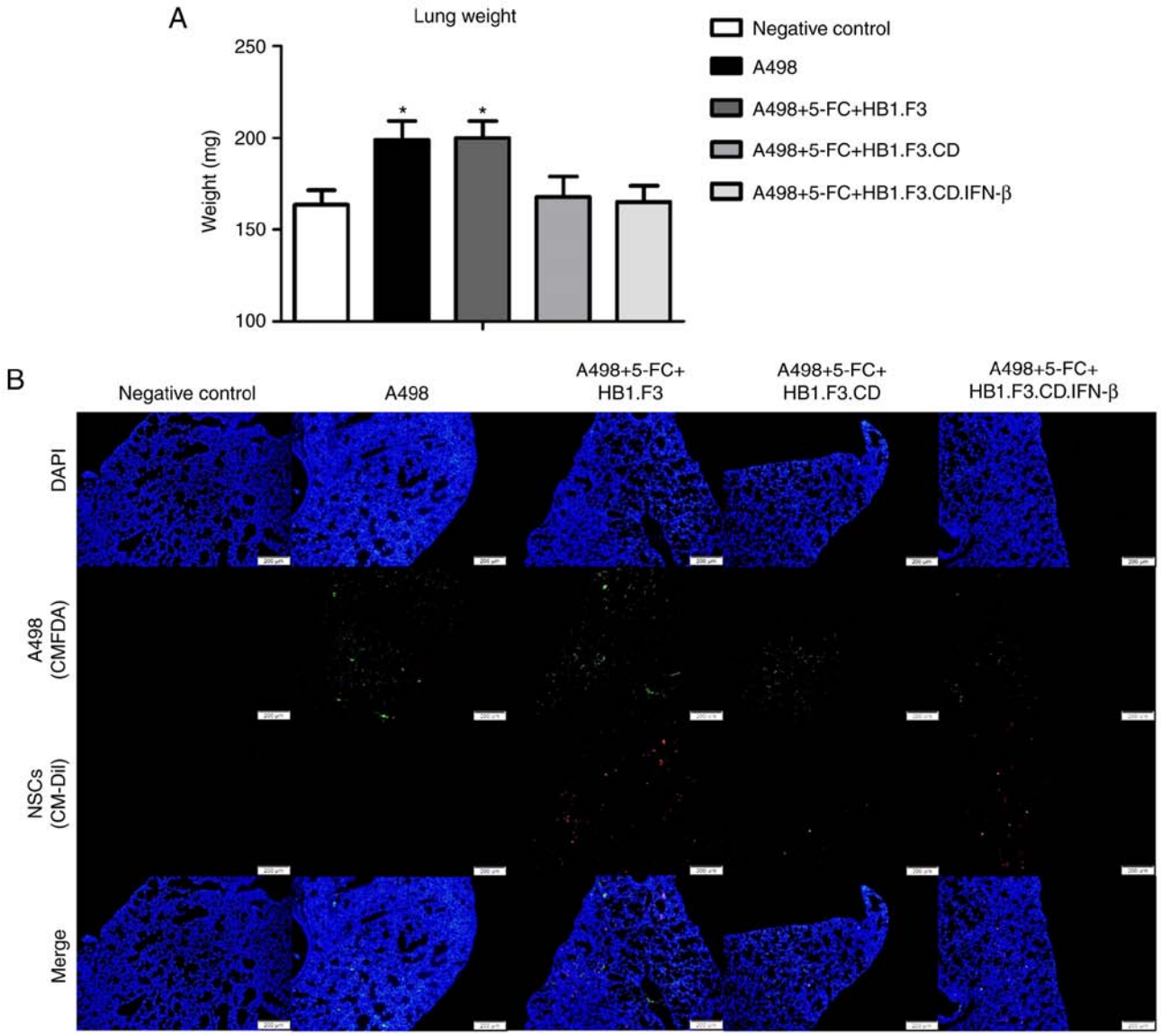

Figure 4. Changes in the lungs by hNSCs expressing anticancer genes in the metastasis model. Lungs of mice injected with CMFDA pre-stained A498 cells and CM-DiI pre-stained hNSCs were extracted. (A) The weight of the lungs before fixation in formalin solution was measured. The graph was drawn using GraphPad Prism (v5.0; GraphPad Software). (B) Lungs fixed in 10% formalin solution were sectioned with a microtome. Tissue sections were analyzed by fluorescence microscopy after DAPI counterstaining. Scale bars, 200 μm. DAPI (blue) indicates nuclei of all cells, CMFDA (green) indicates A498 cells and CM-DiI (red) indicates hNSCs. *P<0.05 vs. negative control. hNSCs, genetically engineered human neural stem cells.

the experiment was terminated, the weight of the lungs from all mice was determined (Fig. 4A). Lung weight was significantly increased in the A498 and F3 (A498+5-FC+HB1.F3) groups compared to the NC (negative control) group. However, the lung weights of the CD (A498+5-FC+HB1.F3.CD) and CD-β (A498+5-FC+HB1.F3.CD.IFN-β) groups were unchanged compared to the NC group. Fluorescence analysis revealed green fluorescence (A498 cells) in all groups except the NC group, while red fluorescence (hNSCs) was confirmed in the F3, CD and CD-β groups (Fig. 4B).

Metastasis inhibition effect of HB1.F3.CD and HB1.F3.CD. IFN-β cells in the liver. The weights of the extracted livers were measured using the same method as for the lungs. Unlike the results for the lungs, there were no significant differences in the weights of the livers between any of the groups and the NC (negative control) group (Fig. 5A). However, as shown in

Table I. Reduction in the incidence of metastasis by hNSCs expressing CD and/or IFN-β genes.

	Incidence of metastasis	
Groups	Lung	Liver
Negative control	0/7	0/7
A498	7/7	5/7
A498+5-FC+HB1.F3	7/7	5/7
A498+5-FC+HB1.F3.CD	7/7	4/7
A498+5-FC+HB1.F3.CD.IFN-β	7/7	1/7

All mice (7 per group) were sacrificed and evaluated for metastasis by fluorescence analysis. CD, cytosine deaminase; IFN-β, interferon-β; hNSCs, genetically engineered human neural stem cells.

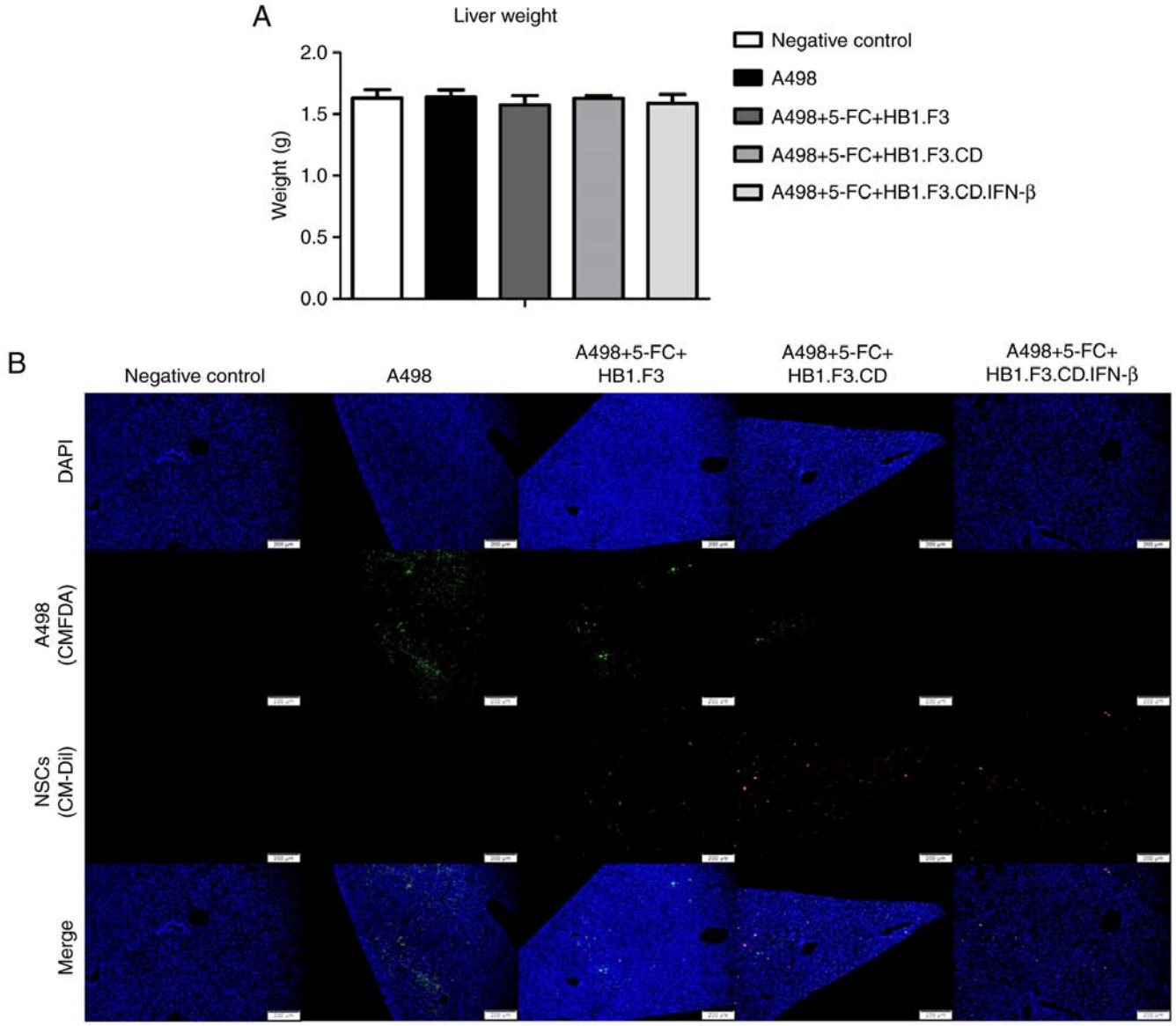

Figure 5. Inhibition of liver metastasis by intravenously injected therapeutic stem cells. A498 cells pre-stained with CMFDA and hNSCs pre-stained with CM-DiI were injected intravenously. (A) After termination of the experiment, livers were harvested and weighed. (B) Tissue slides with sectioned liver tissue were stained with DAPI solution for fluorescence analysis. Scale bars, 200 μm. DAPI (blue) indicates nuclei of all cells, CMFDA (green) indicates A498 cells and CM-DiI (red) indicates hNSCs. hNSCs, genetically engineered human neural stem cells.

Table I, there was a significant difference in the incidence of metastases. In particular, the CD-β (A498+5-FC+HB1.F3.CD.IFN-β) group showed significantly fewer incidences of metastases than the other groups in the liver. Fluorescence analysis of livers from the F3 (A498+5-FC+HB1.F3), CD (A498+5-FC+HB1.F3.CD) and CD-β (A498+5-FC+HB1.F3.CD.IFN-β) groups revealed a difference in the number of mice in which green fluorescence was confirmed, but red fluorescence was present in all mice (Fig. 5B). Taken together, these results indicate that the incidence of metastasis differs between CD/5-FC and IFN-β treated and non-treated groups.

Discussion

Renal cell carcinoma (RCC), which is the most common renal cancer, spreads to the lymph nodes, lungs and liver when metastasized (25). If it spreads to organs outside the kidney, multiple therapies such as chemotherapy and surgery are attempted, but most cases of RCC are resistant to traditional treatment modalities (26). A previous study revealed that 40% of patients diagnosed with RCC die of cancer progression, 25-30% of whom are identified with metastatic RCC (27). Therefore, it is necessary to discover promising treatments that can serve as an alternative to conventional therapies.

In the present study, we used genetically engineered human neural stem cells (hNSCs) as a vehicle that can specifically deliver anticancer genes to tumor sites. It is well known that hNSCs have the ability to target multiple tumor types by the interaction with chemoattractant factors (27-30). According to a previous study, hNSCs preferentially migrated to hypoxic areas in glioma xenografts. Hypoxia is a critical microenvironment of cancer and hypoxia-induced migration of hNSCs to glioma region was found to be mediated by the interactions between chemoattractant factors such as SDF-1/CXCR4, uPA/uPAR,

VEGF/VEGFR2 (31). We confirmed the tumor-specific tropism of hNSCs targeting A498 RCC cells in a PCR and Transwell assay. As a result, three chemoattractant factors such SCF, CXCR4 and VEGF were found to be expressed in the A498 cells, and the number of migrated hNSCs was 2-5 times higher in the A498 cell-seeded groups than that in the negative control (NC) groups. Accordingly, HB1.F3.CD and HB1.F3.CD.IFN-β cells have the potential to minimize adverse effects due to A498-specific targeting ability.

CD/5-FC therapy, a type of gene-directed enzyme prodrug therapy (GDEPT), has considerable advantages compared to conventional chemotherapy as the CD gene can convert the inactive drug 5-FC to an effective drug near cancer cells. Moreover, we confirmed the synergism of CD and IFN-β genes in a cell viability assay. The cell viability of A498 cells was reduced by approximately 10-50% by the CD gene alone according to the concentration of 5-FC, and decreased by about 70% in HB1.F3.CD.IFN-β cells expressing both genes.

Many previous studies have demonstrated the ability of these stem cells to inhibit tumor growth in xenograft models of various types of cancer (16,22). However, few studies have investigated the inhibitory effects of therapeutic hNSCs in metastasis models. Therefore, to verify the metastasis-inhibitory effect of HB1.F3.CD and HB1.F3.CD.IFN-β cells against RCC, A498 cells were injected into mice intravenously to establish a metastasis model. After all injections were completed, the lungs and livers, the most common metastatic sites of RCC, were extracted. The weight of the extracted organs did not differ in regards to the livers, but the weight of the lungs was altered by hNSCs/5-FC. Lung weights were 120% higher in the A498 and F3 groups than that noted in the NC group, but the CD (A498+5-FC+HB1.F3.CD) and CD-β (A498+5-FC+HB1.F3.CD.IFN-β) groups injected with hNSCs expressing anticancer genes showed similar results to that of the NC group. Furthermore, as shown in Table I, the incidence of liver metastases was significantly reduced in the CD-β group. Fluorescence analysis revealed that there were no A498 cells in some mice, while stem cells were present in the livers of all mice in the CD-β group. These findings indicate that metastasis of A498 cells is inhibited by hNSCs expressing anticancer genes in the presence of 5-FC. In the present study, the metastasis of RCC in lung and liver by A498 RCC cells and anti-metastatic effect of hNSCs were confirmed by the presence of A498 cells and hNSCs in lung and liver and their weight changes. However, the identification of altered expression of metastasis-specific markers such as cytokeratins and vimentin in lung and liver by A498 cells and hNSCs via immunohistochemistry are still required to be conducted in subsequent studies. In addition, the issues concerned associated with stem cell delivery need to be identified and solved in further studies. In the present study, although hNSCs were found in both lung and liver where A498 cancer cells resided in advance, the pulmonary first-pass effect occurring during intravenous stem cell delivery (which means that most of stem cells are trapped in the lungs before they can play a role) is considered as a major obstacle (32). Achieving a superior stem cell effect has been considered to involve placing the cells into the lesion to date. Therefore, various attempts should be made to solve the pulmonary first-pass effect and maximize the tumor-specific tropism of hNSCs, its inherent property.

The results of this study demonstrated that HB1.F3.CD and HB1.F3.CD.IFN-β cells with 5-FC diminished the cell viability of RCC and retarded metastasis to other organs in a cellular and metastasis model. In conclusion, hNSC therapy in combination with current therapies is expected to exhibit superior efficacy for patients suffering from metastatic RCC.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Basic Science Research Program (2020R1A2C2006060) and the Global Research and Development Center (GRDC) Program (2017K1A4A3014959) through the National Research Foundation (NRF) of Korea funded by the Ministry of Science and ICT.

Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

KCC and SUK conceived and designed the study. GSK and SMK performed the experiments and analyzed the results. GSK completed the draft of the stufdy and KCC revised the manuscript. SUK and GL provided some materials and essential techniques for this study. GL and KCC reviewed and edited the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Experiments were conducted with approval from the Chungbuk National University Institutional Animal Care and Use Committee (CBNUA-1089-17-01).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests to report.

References

1. Williams RT, Yu AL, Diccianni MB, Theodorakis EA and Batova A: Renal cancer-selective englerin A induces multiple mechanisms of cell death and autophagy. J Exp Clin Cancer Res 32: 57, 2013.
2. Curti BD: Renal cell carcinoma. JAMA 292: 97-100, 2004.
3. Gupta K, Miller JD, Li JZ, Russell MW and Charbonneau C: Epidemiologic and socioeconomic burden of metastatic renal cell carcinoma (mRCC): A literature review. Cancer Treat Rev 34: 193-205, 2008.

4. Basu B and Eisen T: Perspectives in drug development for metastatic renal cell cancer. Target Oncol 5: 139-156, 2010.
5. Saini S, Liu J, Yamamura S, Majid S, Kawakami K, Hirata H and Dahiya R: Functional significance of secreted frizzled-related protein 1 in metastatic renal cell carcinomas. Cancer Res 69: 6815-6822, 2009.
6. Matias M, Casa-Nova M, Borges-Costa J and Ribeiro L: Unusual head metastasis of kidney cancer. BMJ Case Rep 2013: bcr2013200004, 2013.
7. Zhang F, Shang D, Zhang Y and Tian Y: Interleukin-22 suppresses the growth of A498 renal cell carcinoma cells via regulation of STAT1 pathway. PLoS One 6: e20382, 2011.
8. Amato RJ: Renal cell carcinoma: Review of novel single-agent therapeutics and combination regimens. Ann Oncol 16: 7-15, 2005.
9. Lane BR, Rini BI, Novick AC and Campbell SC: Targeted molecular therapy for renal cell carcinoma. Urology 69: 3-10, 2007.
10. Antonelli A, Cozzoli A, Zani D, Zanotelli T, Nicolai M, Cunico SC and Simeone C: The follow-up management of non-metastatic renal cell carcinoma: Definition of a surveillance protocol. BJU Int 99: 296-300, 2007.
11. Huber BE, Austin EA, Richards CA, Davis ST and Good SS: Metabolism of 5-fluorocytosine to 5-fluorouracil in human colorectal tumor cells transduced with the cytosine deaminase gene: Significant antitumor effects when only a small percentage of tumor cells express cytosine deaminase. Proc Natl Acad Sci USA 91: 8302-8306, 1994.
12. Nawa A, Tanino T, Luo C, Iwaki M, Kajiyama H, Shibata K, Yamamoto E, Ino K, Nishiyama Y and Kikkawa F: Gene directed enzyme prodrug therapy for ovarian cancer: Could GDEPT become a promising treatment against ovarian cancer? Anticancer Agents Med Chem 8: 232-239, 2008.
13. Qiao J, Doubrovin M, Sauter BV, Huang Y, Guo ZS, Balatoni J, Akhurst T, Blasberg RG, Tjuvajev JG, Chen SH and Woo SL: Tumor-specific transcriptional targeting of suicide gene therapy. Gene Ther 9: 168-175, 2002.
14. Ye L, Tao K, Yu Y and Wang G: Reduction of G0 phase cells of colon cancer caco-2 cells may enhance 5-fluorouracil efficacy. J Biomed Res 24: 64-68, 2010.
15. Kim KY, Kim SU, Leung PC, Jeung EB and Choi KC: Influence of the prodrugs 5-fluorocytosine and CPT-11 on ovarian cancer cells using genetically engineered stem cells: Tumor-tropic potential and inhibition of ovarian cancer cell growth. Cancer Sci 101: 955-962, 2010.
16. Yi BR, Kang NH, Hwang KA, Kim SU, Jeung EB and Choi KC: Antitumor therapeutic effects of cytosine deaminase and interferon-β against endometrial cancer cells using genetically engineered stem cells in vitro. Anticancer Res 31: 2853-2861, 2011.
17. Garrison JI, Berens ME, Shapiro JR, Treasurywala S and Floyd-Smith G: Interferon-beta inhibits proliferation and progression through S phase of the cell cycle in five glioma cell lines. J Neurooncol 30: 213-223, 1996.
18. Ren C, Kumar S, Chanda D, Kallman L, Chen J, Mountz JD and Ponnazhagan S: Cancer gene therapy using mesenchymal stem cells expressing interferon-beta in a mouse prostate cancer lung metastasis model. Gene Ther 15: 1446-1453, 2008.
19. Kim SU, Nakagawa E, Hatori K, Nagai A, Lee MA and Bang JH: Production of immortalized human neural crest stem cells. Methods Mol Biol 198: 55-65, 2002.
20. Jeong SW, Chu K, Jung KH, Kim SU, Kim M and Roh JK: Human neural stem cell transplantation promotes functional recovery in rats with experimental intracerebral hemorrhage. Stroke 34: 2258-2263, 2003.
21. Kim GS, Heo JR, Kim SU and Choi KC: Cancer-Specific inhibitory effects of genetically engineered stem cells expressing cytosine deaminase and interferon-β against choriocarcinoma in xenografted metastatic mouse models. Transl Oncol 11: 74-85, 2017.
22. Park GT, Kim SU and Choi KC: Anti-proliferative effect of engineered neural stem cells expressing cytosine deaminase and interferon-β against lymph node-derived metastatic colorectal adenocarcinoma in cellular and xenograft mouse models. Cancer Res Treat 9: 79-91, 2017.
23. Kim SU, Jeung EB, Kim YB, Cho MH and Choi KC: Potential tumor-tropic effect of genetically engineered stem cells expressing suicide enzymes to selectively target invasive cancer in animal models. Anticancer Res 31: 1249-1258, 2011.
24. Li C, Penet MF, Winnard P Jr, Artemov D and Bhujwalla ZM: Image-guided enzyme/prodrug cancer therapy. Clin Cancer Res 14: 515-522, 2008.
25. Singer EA, Gupta GN, Marchalik D and Srinivasan R: Evolving therapeutic targets in renal cell carcinoma. Curr Opin Oncol 25: 273-280, 2013.
26. Schwartz MJ, Liu H, Hwang DH, Kawamoto H and Scherr DS: Antitumor effects of an imidazoquinoline in renal cell carcinoma. Urology 73: 1156-1162, 2009.
27. Lam JS, Leppert JT, Belldegrun AS and Figlin RA: Novel approaches in the therapy of metastatic renal cell carcinoma. World J Urol 23: 202-212, 2005.
28. Lash GE, Warren AY, Underwood S and Baker PN: Vascular endothelial growth factor is a chemoattractant for trophoblast cells. Placenta 24: 549-556, 2003.
29. Koshiba T, Hosotani R, Miyamoto Y, Ida J, Tsuji S, Nakajima S, Kawaguchi M, Kobayashi H, Doi R, Hori T, *et al*: Expression of stromal cell-derived factor 1 and CXCR4 ligand receptor system in pancreatic cancer: A possible role for tumor progression. Clin Cancer Res 6: 3530-3535, 2000.
30. Yi BR, O SN, Kang NH, Hwang KA, Kim SU, Jeung EB, Kim YB, Heo GJ and Choi KC: Genetically engineered stem cells expressing cytosine deaminase and interferon-β migrate to human lung cancer cells and have potentially therapeutic anti-tumor effects. Int J Oncol 39: 833-839, 2011.
31. Zhao D, Najbauer J, Garcia E, Metz MZ, Gutova M, Glackin CA, Kim SU and Aboody KS: Neural stem cell tropism to glioma: Critical role of tumor hypoxia. Mol Cancer Res 6: 1819-1829, 2008.
32. Fischer UM, Harting MT, Jimenez F, Monzon-Posadas WO, Xue H, Savitz SI, Laine GA and Cox CS Jr: Pulmonary passage is a major obstacle for intravenous stem cell delivery: The pulmonary first-pass effect. Stem Cells Dev 18: 683-692, 2009.