Pancreatic tumor mass in a xenograft mouse model is decreased by treatment with therapeutic stem cells following introduction of therapeutic genes

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Abstract. Pancreatic cancer is the fourth most common cause of cancer-related mortality. In the present study, we employed 2 types of therapeutic stem cells expressing cytosine deaminase (CD) with or without human interferon-β (IFN-β), HB1.F3.CD and HB1.F3.CD.IFN-β cells, respectively, to selectively treat pancreatic cancer. The CD gene converts the non-toxic prodrug, 5-flurorocytosine (5-FC), into the toxic agent, 5-fluorouracil (5-FU). In addition, human IFN-β is a potent cytokine that has antitumor effects. To generate a xenograft mouse model, PANC-1 cells ($2x10^6$/mouse) cultured in DMEM containing 10% FBS were mixed with Matrigel and were subcutaneously injected into BALB/c nu/nu mice. In the migration assay, the stem cells expressing the CD or IFN-β gene effectively migrated toward the pancreatic cancer cells, suggesting the presence of chemoattractant factors secreted by the pancreatic tumors. In the co-culture and MTT assay, antitumor activity of the therapeutic stem cells was observed in the presence of 5-FC was shown that the growth of PANC-1 cells was inhibited. Furthermore, these effects were confirmed in the xenograft mouse model bearing tumors originating from PANC-1 cells. Analyses by histological and fluorescence microscopy showed that treatment with the stem cells resulted in the inhibition of pancreatic cancer growth in the presence of 5-FC. Taken together, these results indicate that stem cells expressing the CD and/or IFN-β gene can be used to effectively treat pancreatic cancer and reduce the side-effects associated with conventional therapies.

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Introduction

Pancreatic cancer is one of the most lethal human cancers and continues to be a major health problem. Despite considerable efforts made in the past 50 years, conventional therapeutic approaches, such as surgery, radiation, chemotherapy, or combinations of these modalities, have little impact on the course of this aggressive neoplasm (1). Additionally, current treatments or therapies used to treat human cancer have low selectivity or specificity against tumor cells and often result in systemic toxicity that damages normal healthy tissues. Thus, novel strategies with enhanced therapeutic efficiency and selectivity for treating pancreatic cancer are needed.

Stem cells have recently gained considerable attention from researchers given their possible clinical use for developing new cancer treatments. While traditional chemotherapy involves the administration of drugs, genetically engineered stem cells (GESTECs) can be used to induce the *in vivo* production of therapeutic agents (2-7). This technique enables the replacement of damaged genes or insertion of additional genes with new functions. For example, human neural stem cells (hNSCs) were found to have therapeutic potential and tumor tropism for treating malignant tumors in the human brain including medulloblastomas and gliomas (8-10). This supports the possibility of using hNSCs as a carrier to deliver genes to cells in tumor sites for tumor-specific enzyme/prodrug systems with concomitant prodrug administration (11).

HB1.F3 cells are immortalized hNSCs derived from the human fetal brain at 15 weeks of gestation using the amphotropic replication-incompetent retroviral vector, v-myc (12,13). Clonal HB1.F3.CD cells are derived from parental HB1.F3 cells transfected with the *Escherichia coli* (*E. coli*) cytosine deaminase (CD) gene (12). Additionally, clonal HB1.F3.CD. IFN-β cells are derived from parental HB1.F3.CD cells, and express both the *E. coli* CD and human interferon-β (IFN-β) genes (5). This type of clonally isolated multipotent HB1.F3 cells has the ability to self-renew and differentiate into cells of neuronal and glial lineages both *in vivo* and *in vitro* (12). The CD/ 5-fluorocytosine (5-FC) system is a gene-directed enzyme/ prodrug therapy (GEPT) (14-18) in which the non-toxic prodrug 5-FC is converted into a cytotoxic metabolite, 5-fluorouracil (5-FU) (19,20). 5-FU inhibits DNA synthesis in cells and results

in cytotoxicity (21,22). This GEPT system has been experimentally tested against several types of tumors including colorectal and prostate cancers, demonstrating that GEPT appears to be an effective therapy for these tumors (23-26). In our previous studies, diverse sources of stem cells, neural, amniotic-fluid and amniotic membrane, were employed to express therapeutic genes for selectively targeting different types of human cancers including primary and metastatic cancers (2-4,7,26-28).

In the present study, we aimed to ascertain whether 2 types of therapeutic stem cells, HB1.F3.CD and HB1.F3.CD.IFN-β, can selectively migrate toward human pancreatic cancer cells. As a cancer cell model, human pancreatic carcinoma cells, PANC-1, were employed to investigate whether therapeutic stem cells migrate and target this type of cancer in *in vitro* and *in vivo* models. We also evaluated the therapeutic value of these cells in an enzyme/prodrug system that could serve as a novel pancreatic cancer therapy. The antitumor and tumor-tropic properties of GESTECs provide the potential for treating invasive tumors without any side-effects (8-10,29). By selectively delivering therapeutic genes to tumor cells, GESTECs expressing CD and IFN-β may have synergistic antitumor effects against pancreatic cancer cells.

Materials and methods

Cell culture. The PANC-1 epithelioid cells line derived from human pancreatic carcinoma of ductal cell origin was obtained from the Korean Cell Line Bank (Seoul, Korea). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) (both from HyClone Laboratories, Inc., Logan, UT, USA), 1% penicillin/streptomycin (Cellgro Mediatech, Inc., Manassas, VA, USA), 1% HEPES (Invitrogen Life Technologies, Carlsbad, CA, USA), and 0.1% anti-mycoplasma plasmocin (InvivoGen, San Diego, CA, USA) at 37˚C in a humidified atmosphere of 5% CO_2-95% air. HB1.F3, HB1.F3.CD and HB1.F3.CD.IFN-β cells, along with human dermal fibroblasts (HDFs), were also cultured in the same media as indicated above. All cells were trypsinized with 0.05% trypsin/0.02% EDTA (PAA Laboratories, Austria) in Mg^{2+}/Ca^{2+}-free HBSS.

Reverse transcription (RT)-PCR. To confirm the expression of CD and/or the IFN-β gene in the HB1.F3.CD and HB1.F3.CD.IFN-β cells, RT-PCR was performed. The presence of these chemoattractant molecules and their interaction with specific receptors, such as stem cell factor (SCF)/c-KIT, CXC chemokine receptor 4 (CXCR4), and vascular endothelial growth factor (VEGF)/VEGFR1 and VEGFR2 receptors, in the GESTECs and related ligands in PANC-1 cells were detected by RT-PCR.

RNA extraction was performed using TRIzol reagent (Invitrogen Life Technologies). One microgram of total RNA was reversely transcribed into complementary DNA (cDNA) using murine leukemia virus reverse transcriptase (MMLV-RT; iNtRON Biotechnology, Sungnam, Korea), 10 pM dNTPs (Bioneer Co., Daejeon, Korea), 200 pM nonamer random primers (Takara Bio, Inc., Shiga, Japan), 0.5 µl RNase inhibitor (iNtRON Biotechnology), and 5X RT buffer. The cDNA prepared from this procedure was amplified by PCR performed with 0.2 µmol/l of each reverse and forward primer,

2.5 units of Taq polymerase, 0.2 mmol/l dNTP and 10X PCR buffer (all from iNtRON Biotechnology). PCR reaction for these chemoattractant factors (ligands and receptors) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a positive control was carried out in a PTC-100 thermal cycler (MJ Research Inc., Waltham, MA, USA) with 30 cycles of denaturation at 95˚C for 30 sec, annealing at 58˚C for 30 sec, and extension at 72˚C for 30 sec. The PCR products were separated on a 1.5% agarose gel containing ethidium bromide (EtBr), and the results of electrophoresis were analyzed using Gel Doc 2000 (Bio-Rad Laboratories, Hercules, CA, USA). The sequences of the reverse and forward primers along with the predicted product sizes are shown in Table I.

Cell viability assay. To investigate the effects of 5-FC and 5-FU on PANC-1 cells, a cell viability assay was conducted. PANC-1 cells were seeded in 96-well plates (5,000 cells/well) and cultured in 100 µl culture medium supplemented with 5% FBS. Next, 5-FC and 5-FU (Sigma-Aldrich, St. Louis, MO, USA) were serially diluted with phosphate-buffered saline (PBS) (final concentration 0.1, 0.3, 0.5, 1 and 5 mmol/l) and were used to treat the cells for 3 days. A 3-(29)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to measure cell viability on day 5. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (10 µl of a 10 mg/ml stock) was added to each well, and the plates were incubated for 4 h at 37˚C. Supernatants were then removed, and 100 µl of dimethyl sulfoxide (DMSO, 99.0%; Junsei Chemical Co. Ltd., Tokyo, Japan) was added to each well to dissolve the resultant formazan crystals. Cell viability of the PANC-1 cells was measured at 540 nm using an ELISA plate reader (Molecular Devices, Sunnyvale, CA, USA) in duplicates.

To measure cell growth inhibition resulting from treatment with 5-FC, PANC-1 cells (1,250 cells/well) were seeded in 96-well plates and cultured in 100 µl cell culture medium with 5% FBS. After seeding, HB1.F3, HB1.F3.CD or HB1.F3.CD.IFN-β cells (3,750 cells/well) in 100 µl cell culture medium containing 5% FBS were added to the cultures and incubated for 24 h. Next, the cells were treated with different concentrations (0.1, 0.3, 0.5, 1 and 5 mmol/l) of 5-FC for 3 days. A MTT assay was performed in duplicate to measure cell viability on day 5.

In vitro migration assay. To ascertain whether GESTECs are capable of migrating toward pancreatic cancer cells, PANC-1 cells and HDFs (1x10⁵ cells/500 µl/well) were seeded in 24-well plates in DMEM containing 10% FBS and incubated for 6 h at 37˚C. The cells were then incubated in fresh 2% FBS containing DMEM for 24 h in 37˚C. Transwell plates (8 µm; BD Biosciences, Franklin Lakes, NJ, USA) coated with fibronectin (250 µg/ml; Sigma-Aldrich) were placed in empty 24-well plates and incubated overnight in 37˚C. Using a general protocol, 2 µM of chloromethylbenzamido-1,1'-di-octadecyl-3,3,3'-tetramethyl-indocarbocyanine perchlorate (CM-DiI; Invitrogen Life Technologies) was used to label the HB1.F3, HB1.F3.CD or HB1.F3.CD.IFN-β cells (1x10⁵ cells/well) which were plated in the upper chambers of the Transwell plates (11,26). The cells were then cultured in DMEM supplemented with 2% FBS for 24 h at 37˚C. The PANC-1 cells and HDFs were subsequently stained with 200 ng/ml 4',6-diamidino-2-phenylindole solution (DAPI; Invitrogen

Table I. Sequences of the oligonucleotide primers used in this study and the predicted PCR product sizes.

mRNA	Oligo-sequences (5'-3')	Expected size (bp)
CD	Forward: GCGCGAGTCACCGCCAGCCACACCACGGC Reverse: GTTTGTATTCGATGGCTTCTGGCTGC	559
SCF	Forward: ACTTGGATTCTCACTTGCATTT Reverse: CTTTCTCAGGACTTAATGTTGAAG	505
c-KIT	Forward: GCCCACAATAGATTGGTATTT Reverse: AGCATCTTTACAGCGACAGTC	570
CXCR4	Forward: CTCTCCAAAGGAAAGCGCAGGTGGACAT Reverse: AGACTGTACACTGTAGGTGCTGAAATCA	558
IFN-β	Forward: AAAGAAGCAGCAATTTTCAG Reverse: TTTCTCCAGTTTTTCTTCCA	296
VEGF	Forward: AAGCCATCCTGTGTGCCCCTGATG Reverse: GCTCCTTCCTCCTGCCCGGCTCAC	377
VEGFR2	Forward: ACGCTGACATGTACGGTCTAT Reverse: GCCAAGCTTGTACCATGTGAG	438
GAPDH	Forward: ATGTTCGTCATGGGTGTGAACCA Reverse: TGGCAGGTTTTTCTAGACGGCAG	351

CD, cytosine deaminase; SCF, stem cell factor; CXCR4, CXC chemokine receptor 4; IFN-β, interferon-β; VEGF, vascular endothelial growth factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Life Technologies) in the dark for 10 min at 37˚C. Each well was then washed with PBS, and the upper side of the Transwell membrane was scraped to remove cells that had not migrated into the membrane. Cells stained with CM-DiI and DAPI were examined by fluorescence microscopy (IX71 inverted microscope; Olympus, Tokyo, Japan).

Pancreatic cancer xenograft mouse models. All animal experimental procedures were approved by the Animal Care Committee of Chungbuk National University. Twenty-one 6-week-old male BALB/c nude mice were purchased from the Central Animal Laboratory (SLC, Shizuoka, Japan). The mice were acclimated to a controlled environment (22-24˚C with 40-60% relative humidity, a 12-h light/dark cycle, and frequent ventilation) for 1 week prior to the experiments. After this period, PANC-1 cells ($2x10^6$ cells/mouse) were injected into the subcutaneous dorsal thoracic region of each mouse. The resulting tumor volume was measured using a caliper every week and was calculated using the formula: (0.5236 x length x width x height).

In vivo antitumor effect of GESTECs. To evaluate the therapeutic effects of the GESTECs, the xenograft mice were divided into 3 groups that were i) treated with 0.85% saline as a negative control, ii) inoculated with HB1.F3.CD cells ($8x10^6$ cells/mouse/2 weeks) in the presence of 5-FC (500 mg/kg/day), or iii) inoculated with HB1.F3.CD.IFN-β cells ($8x10^6$ cells/mouse/2 weeks) in the presence of 5-FC (500 mg/kg/day). The experiment was conducted for 4 weeks after GESTECs injection. After tumor volumes reached 200 mm³, GESTECs stained with CM-DiI were injected in areas surrounding the tumor mass. Injections were performed twice a week during the experimental period. Two days after injection of the GESTECs, the control group received intraperitoneal injections of normal saline (100 μl), while the other mice with GESTECs were injected with 5-FC (500 mg/kg/day in 100 μl saline) every day during the experimental period. One day after the last 5-FC administration, the mice were sacrificed and tumors were excised.

Histopathology. Tumors excised from the mice during necropsy were fixed in a 10% normal formalin solution (Sigma-Aldrich). The fixed samples were cut into 4- to 6-mm sections, embedded in paraffin, and cut into 3-μm sections using a microtome (Leica, Wetzlar, Germany). The sections were mounted on slides and stained with hematoxylin and eosin (H&E) (Sigma-Aldrich). The stained slides were then examined by light microscopy using a BX51 microscope (Olympus).

Fluorescence analysis. To observe localization of the GESTECs in the xenograft tumor mass, DAPI staining as a counterstaining was performed on prepared tumor section slides. After rehydration, the slides were fixed in a 10% normal formalin (Sigma-Aldrich) solution for 10 min, washed twice in PBS and incubated with DAPI for 10 min at 37˚C. The stained slides were examined with an IX71 inverted microscope.

Statistical analysis. Data from each experiment are presented as the means ± standard deviation (SD) or standard error of the mean (SEM) in *in vitro* and *in vivo*. Differences between each group were evaluated with one-way ANOVA and Tukey's test using Prism GraphPad (v5.0; GraphPad software, San Diego, CA, USA). P-values <0.05 were considered to indicate statistically significant results.
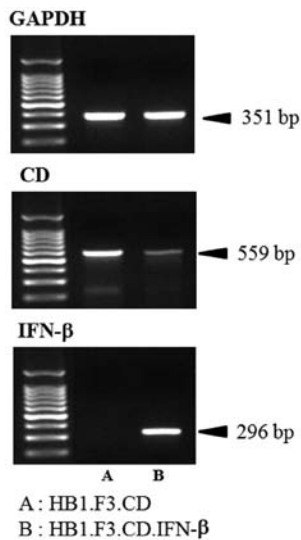
Figure 1. Expression of *E. coli* CD and human IFN-β genes in the engineered stem cells. After extracting RNA from the HB1.F3.CD and HB1.F3.CD.IFN-β cells, cDNA was synthesized by RT reaction. The PCR products were separated by 1.5% agarose gel electrophoresis. GAPDH (351 bp) was used as an internal control. Expression of *E. coli* CD (559 bp) and human IFN-β (296 bp) was detected. Lane A, HB1.F3.CD cells; lane B, HB1.F3.CD.IFN-β cells.

Results

Identification of CD and IFN-β gene expression in the GESTECs. Expression of CD and IFN-β genes in the HB1.F3.CD and HB1.F3.CD.IFN-β cells was confirmed by RT-PCR. cDNA synthesized from GESTECs RNA was amplified. The CD gene (559 bp) was identified in both HB1.F3.CD and HB1.F3.CD.IFN-β cells as shown in Fig. 1. In addition, the IFN-β gene (296 bp) was observed in the HB1.F3.CD.IFN-β cells but not in the HB1.F3.CD cells (Fig. 1). GAPDH (351 bp) was used as an internal control.

In vitro migration of GESTECs toward pancreatic cancer cells. To measure the ability of GESTECs to migrate toward PANC-1 pancreatic cancer cells, a modified Transwell migration assay was performed. HB1.F3.CD and HB1.F3.CD.IFN-β cells stained with CM-DiI and DAPI were examined by fluorescence microscopy (Fig. 2). Compared to DAPI-stained HDFs as a control, the migratory capacity of GESTECs toward PANC-1 cells was significantly greater (Fig. 2). This result indicates that therapeutic stem cells are capable of migration towards human PANC-1 pancreatic cancer cells.

Identification of chemoattractant factors in PANC-1 cells and GESTECs. To determine whether the PANC-1 cells and GESTECs express chemoattractant molecules, RT-PCR reaction for several chemoattractant ligands and their respective receptors was performed. In the GESTECs, expression of the chemoattractant receptors VEGFR2 and c-KIT was observed while CXCR4 gene expression was not found (Fig. 3). In the PANC-1 cells, VEGF (a chemoattractant ligand) was expressed but SCF was not (Fig. 3).

Cell viability assay. Cell viability was assessed using two methods. First, the cytotoxic effects of 5-FC and 5-FU on PANC-1 cells were assessed. Although treatment with

5-FC did not affect cancer cell growth, 5-FU noticeably decreased cell proliferation by 85% at a concentration of 0.5 mmol/l (Fig. 4A). Secondly, the cytotoxic effect of 5-FC at different concentrations (0.1-5 mmol/l) was examined on PANC-1 cells co-cultured with GESTECs. PANC-1 cell growth was markedly inhibited by co-culturing with HB1.F3.CD or HB1.F3.CD.IFN-β cells in the presence of 5-FC. When the number of GESTECs was fixed, cell proliferation of PANC-1 was decreased by increasing concentrations of 5-FC (0.1-5 mmol/l) (Fig. 4B). When the cytotoxic effects of HB1.F3.CD and HB1.F3.CD.IFN-β were compared, no statistically significant difference was detected (Fig. 4B).

Tumor mass formation in a mouse xenograft model. PANC-1 cells were injected into the subcutaneous dorsal thoracic region of 21 BALB/c nude male mice, and the stem cells were administered into these mice following tumor formation in the presence of a prodrug, 5-FC (Fig. 5A). Tumor volume (V) was measured using a caliper every week on the subcutaneous dorsal thoracic area of each mouse using the formula: V = (0.5236 x length x width x height). After tumor formation, pancreatic tumor growth in the mice was significantly inhibited up to 50% by HB1.F3.CD or HB1.F3.CD.IFN-β cells in the presence of 5-FC (500 mg/kg/day) (Fig. 5B). This result indicated that HB1.F3.CD and HB1.F3.CD.IFN-β cells had effective *in vivo* therapeutic activity in the presence of the prodrug 5-FC. However, a significant synergistic effect of simultaneous CD and IFN-β expression was not observed when the effect of the HB1.F3.CD.IFN-β cells were compared with that of the HB1.F3.CD cells in the *in vitro* cell viability assay (Fig. 5B).

Histological analysis of the pancreatic tumor mass. To identify changes in histological features of the pancreatic tumor masses from the mice, a histopathological analysis was performed on tumor tissues stained with H&E. Typical tumor morphology, such as active mitosis and a high nucleus/cytoplasm ratio, was observed in tissues from the control mice that were not treated with GESTECs (Fig. 6A). In contrast, the pancreatic tumor masses from mice treated with HB1.F3.CD or HB1.F3.CD.IFN-β cells in the presence of 5-FC showed extensive necrosis characterized by pyknosis and destruction of cell morphology (Fig. 6A).

Fluorescent analysis to detect GESTEC migration. To explore the mechanisms underlying tumor growth inhibition by GESTECs, a fluorescence analysis was performed using GESTECs stained with CM-DiI and DAPI-stained PANC-1. As observed with fluorescence microscopy (Fig. 6B), CM-DiI-stained GESTECs (red) migrated toward the PANC-1 cells (blue). This result indicates that the GESTECs effectively migrated toward the pancreatic tumor mass. This migration of therapeutic stem cells expressing a suicide gene and IFN-β to the sites of pancreatic tumor formation may induce the antitumor activity in the presence of a prodrug, 5-FC.

Discussion

Novel strategies that overcome the limits of conventional cancer therapies such as radiotherapy, chemotherapy and
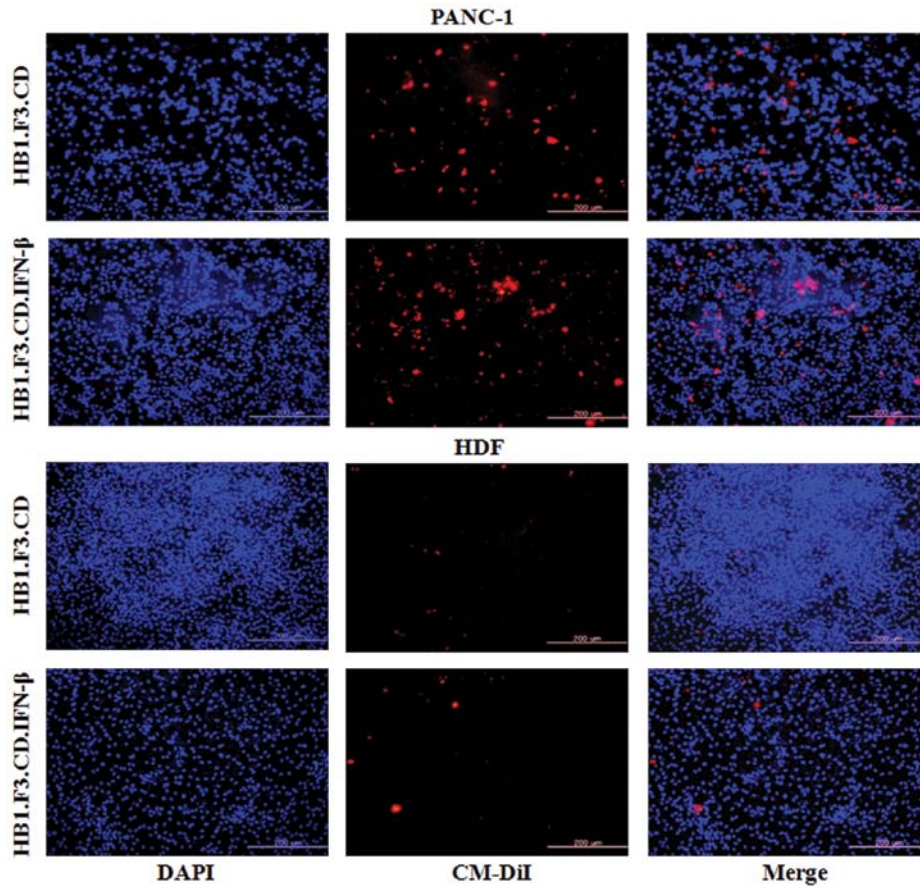
Figure 2. *In vitro* migration of the GESTECs toward PANC-1 pancreatic cancer cells. PANC-1 cells and human dermal fibroblasts (HDFs) as a control were seeded in a 24-well plate (1x10^5 cells/500 μl/well) with culture media containing 10% FBS and incubated for 24 h. Next, HB1.F3.CD and HB1.F3.CD.IFN-β cells (1x10^5 cells/300 μl/well) stained with CM-DiI were added to each Transwell chamber and incubated for 24 h. GESTECs migration toward the PANC-1 cells or HDFs was compared under a microscope. Blue, DAPI-stained PANC-1 cells or HDFs. Red, HB1.F3.CD or HB1.F3.CD.IFN-β stained with CM-DiI.
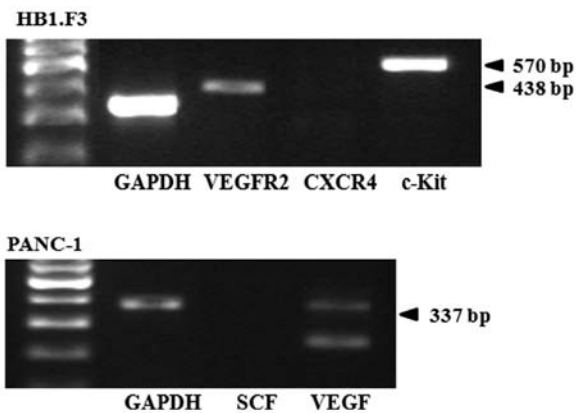

Figure 3. Identification of chemoattractant factors expressed by the GESTECs and PANC-1 cells. After total RNA was extracted from the GESTECs (HB1.F3) and PANC-1 cells, cDNA was synthesized and PCR was performed to confirm the expression of chemoattractant factors in these cells. In the HB1.F3 cells, expression of the ligands VEGFR2 and c-KIT was detected. In the PANC-1 cells, expression of the receptor VEGF was observed. GAPDH was used as an internal control.
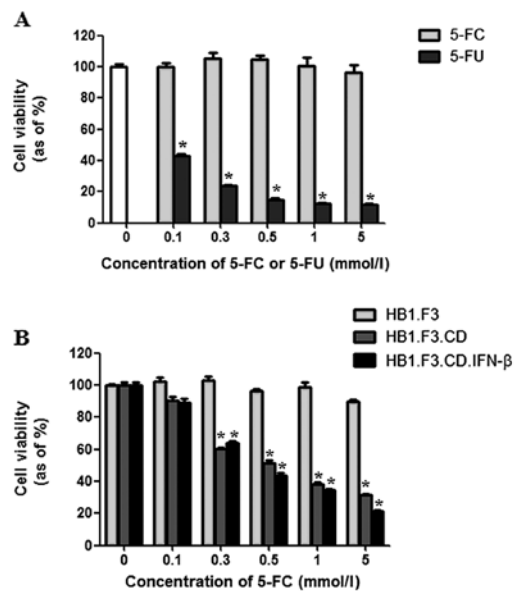

Figure 4. Anticancer effects of GESTECs *in vitro*. PANC-1 cells were seeded in 96-well plates and co-cultured with HB1.F3, HB1.F3.CD or HB1.F3.CD. IFN-β cells (5x10^3 cells/well) for 24 h. The cells were then treated with different concentrations of 5-FC or 5-FU (0, 0.1, 0.3, 0.5, 1 and 5 mmol/l). After 3 days, cell viability was measured with an MTT assay. (A) The cytotoxic effect of 5-FC or 5-FU at different concentrations was measured in the PANC-1 cells. (B) PANC-1 cells (1.25x10^3 cells/well) were co-cultured with GESTECs (3.75x10^3 cells/well) in the presence of increasing concentrations of 5-FC (0, 0.1, 0.3, 0.5, 1 and 5 mmol/l). *P<0.05 vs 5-FC or HB1.F3.

immunotherapy are being developed. One of these modalities, stem cell therapy using a GEPT system, has received much attention as an innovative cancer therapy (30). The CD/5-FC system is a GEPT (14-18) involving the conversion
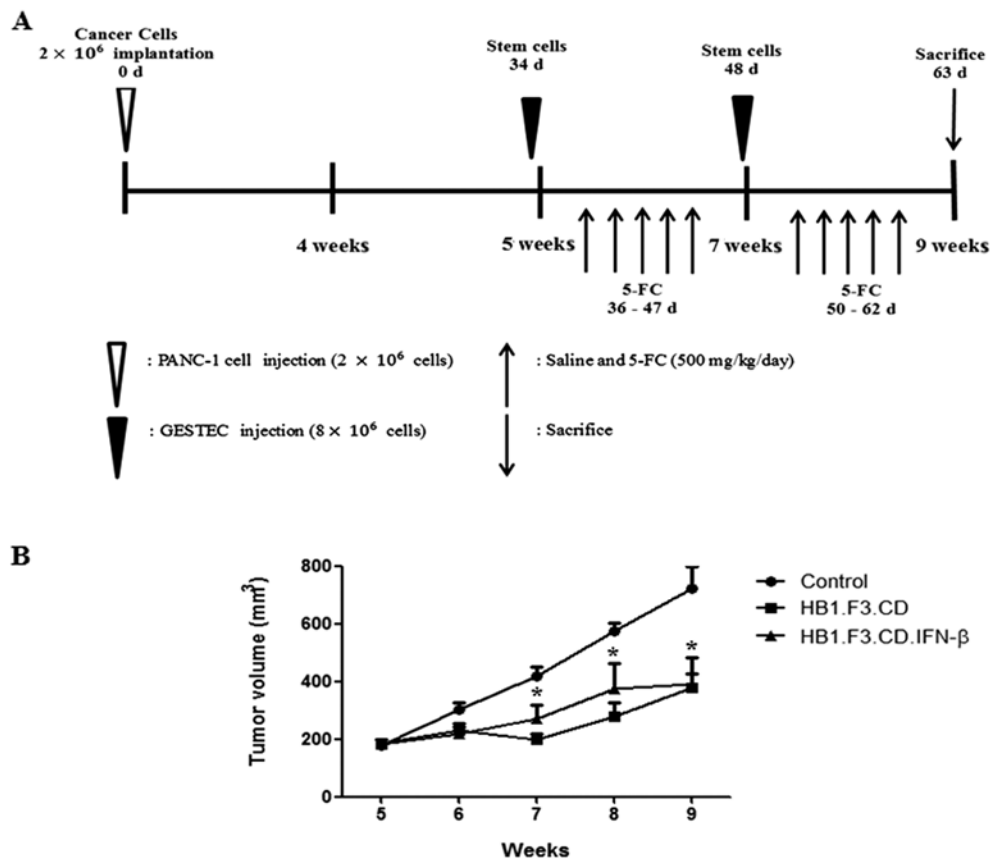
Figure 5. *In vivo* therapeutic effect of GESTECs. A xenograft mouse model was established by injecting PANC-1 cells ($2x10^6$ cells/mouse) into the subcutis of male BALB/c nude mice. Thirty-four days after cancer cell injection, CM-DiI-stained GESTECs ($8x10^6$ cells/mouse) were injected close to the tumor sites. After 2 days, 5-FC was injected into the intraperitoneum every day (500 mg/kg/day), and after 2 weeks from GESTEC injection, the same procedure was repeated 2 times. $^*P<0.05$ vs control.

of a non-toxic prodrug 5-FC into a cytotoxic metabolite, 5-FU (19,20). 5-FU inhibits DNA synthesis in cells, resulting in cytotoxicity (21,22). This CD/5-FC GEPT system has been experimentally tested against several types of tumors including colorectal and prostate lesions (23-25). However, GEPT systems have not yet been used clinically for treating pancreatic cancer since the efficacy of gene delivery vectors (i.e., adenovirus and lentivirus) remains questionable.

Recent studies have found that immortalized GESTECs have advantages that may be useful for gene therapy and neurological cell replacement therapeutic approaches for treating neurological diseases and injuries (12). These GESTECs were shown to selectively migrate toward brain tumors and reduced tumor growth both in *in vitro* cell models and *in vivo* mouse models (9,10). A previous study demonstrated that when HB1.F3.CD cells expressing the *E. coli* CD gene are administered along with 5-FC in an mouse model, tumor size is decreased but this effect was not observed with 5-FC or HB1.F3 CD cells alone (10). Another study reported that GESTECs expressing a fusion gene (HB1.F3.CD.IFN-β) have antitumor effects unlike the HB1.F3 parental cell line (31).

In the present study, tumor-tropic effect of HB1.F3.CD and HB1.F3.CD.IFN-β cells toward PANC-1 cells was verified by a migration assay. The therapeutic effects of our GEPT system specific for pancreatic cancer were examined *in vitro*. Based on the results of our *in vitro* experiments, the therapeutic effect of the GEPT system was evaluated in a xenograft mouse model injected with pancreatic cancer cells.

In a modified Transwell assay, GESTECs migrated toward PANC-1 cancer cells but not non-tumorigenic HDFs. Although the molecular mechanism underlying the tumor-tropism of GESTECs has not been clearly elucidated, a major factor appears to be chemoattractant molecules secreted by the pancreatic cancer cells. Our results showed that the chemoattractant ligands and their corresponding receptors, i.e., VEGF and VEGFR2 were expressed in PANC-1 cells and GESTECs, suggesting a migratory capacity of therapeutic stem cells toward human pancreatic cancer cells. These chemoattractants are presumed to enable GESTECs to selectively migrate toward pancreatic cancer cells and deliver therapeutic genes.

Expression of CD and IFN-β genes in the HB1.F3.CD and HB1.F3.CD.IFN-β cells was confirmed. These cells were found to exert a high cytotoxic effect on the PANC-1 pancreatic cancer cells in the presence of 5-FC. But no significant difference in cell viability between HB1.F3.CD and HB1.F3.CD.IFN-β was observed. In a similar study of liver cancer, HB1.F3.CD.IFN-β cells were found to have a greater inhibitory effect on cancer growth compared to the HB1.F3.CD cells, indicating that HB1.F3.CD.IFN-β cells have a synergistic cytotoxic effect in the CD/5-FC system (26,32). However, PANC-1 cells may not express IFN receptors. In this case, HB1.F3.CD.IFN-β cells expressing CD and IFN-β would not be likely to have a synergistic effect (33).
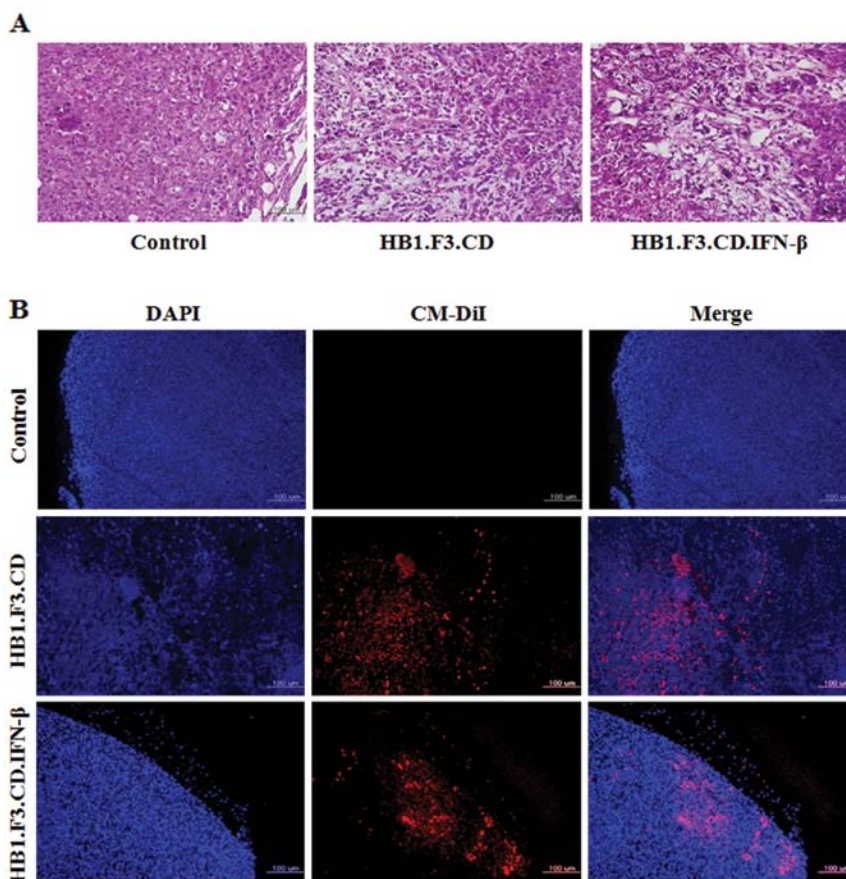
Figure 6. Histopathological and fluorescence microscopic analysis of pancreatic cancer masses from the mouse xenograft model. Pancreatic cancer tissues were excised from the xenograft mice and fixed in a 10% normal formalin solution. The fixed tumor tissues were cut into 4- to 6-μm sections, embedded in paraffin, then cut again into 3-μm sections. (A) Prepared tumor tissues were stained with H&E (x200 magnification). (B) GESTECs were stained with CM-DiI before injection into mice. DAPI counter-staining was performed on the tumor section slides. Stained slides were viewed under a fluorescence microscope (x100 magnification). Red, CM-DiI-stained GESTECs, Blue, DAPI-stained pancreatic cancer cells and GESTECs.

Finally, the therapeutic efficacy of GESTECs was verified in a xenograft mouse model bearing tumors resulting from injection with PANC-1 pancreatic cancer cells. Treatment with HB1.F3.CD or HB1.F3.CD.IFN-β cells resulted in the inhibition of pancreatic tumor growth up to 50% in the presence of 5-FC compared to the control. However, no differences between mice receiving HB1.F3.CD and HB1.F3.CD.IFN-β cell were observed similar to the *in vitro* experimental results. Fluorescence microscopy was used to observe the migration of GESTECs toward the pancreatic tumor mass. Notably, HB1.F3.CD and HB1.F3.CD.IFN-β cells were found to have tumor-tropic migration capabilities *in vivo*. According to the histopathological analysis, typical tumor formation and morphology were observed in the mice without GESTECs. In contrast, the mice treated with HB1.F3.CD or HB1.F3.CD.IFN-β cells showed extensive tumor necrosis associated with pyknosis and destruction of cell morphology in the presence of the prodrug 5-FC. This result implies that GESTECs effectively migrated toward the pancreatic tumor mass and exerted their anticancer effects through our GEPT system.

In summary, this present study demonstrated that GESTECs expressing CD and/or the IFN-β gene can selectively migrate toward human pancreatic cancer cells in *in vitro* and mouse xenograft models. This GEPT system using therapeutic stem cells had an anti-proliferative effect on pancreatic cancer cells *in vitro*. Furthermore, treatment of xenograft mice with GESTECs expressing the CD suicide gene and IFN-β in the presence of the prodrug 5-FC had a significant antitumor effect. Taken together, these results suggest that stem cell therapy using a GEPT system expressing a suicide gene and IFN-β may have potential as a clinical therapeutic tool for treating patients suffered from pancreatic cancer.

Acknowledgements

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