Genetically engineered stem cells expressing cytosine deaminase and interferon-β migrate to human lung cancer cells and have potentially therapeutic anti-tumor effects

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Received February 3, 2011; Accepted April 20, 2011

DOI: 10.3892/ijo.2011.1126

Abstract. Recent studies have shown that genetically engineered stem cells (GESTECs) produce suicide enzymes that convert non-toxic pro-drugs to toxic metabolites which selectively migrate toward tumor sites and reduce tumor growth. In the present study, we evaluated whether these GESTECs are capable of migrating to lung cancer cells and examined the potential therapeutic efficacy of gene-directed enzyme pro-drug therapy against lung cancer cells *in vitro*. A modified transwell migration assay was performed to determine the migratory capacity of GESTECs to lung cancer cells. GESTECs [i.e., HB1.F3.CD or HB1.F3.CD.interferon-β (IFN-β)] engineered to express a suicide gene, cytosine deaminase (CD), selectively migrated toward lung cancer cells. Treatment of a human non-small cell lung carcinoma cell line (A549, a lung carcinoma derived from human lung epithelial cells) with the pro-drug 5-fluorocytosine (5-FC) in the presence of HB1.F3.CD or HB1.F3.CD.IFN-β cells resulted in the inhibition of lung cancer cell growth. Based on the data presented herein, we suggest that GESTECs expressing CD may have a potent advantage for selective treatment of lung cancers. Furthermore, GESTECs expressing fusion genes (i.e., CD and IFN-β) may have a synergic antitumor effect on lung cancer cells.

Introduction

Lung malignancy is the second most frequent type of tumor in men and women, and a major problem in human health (1). Despite intense research efforts, the mechanism(s) of transformation and development of lung malignancies are not well understood. Conventional therapies such as surgical removal (pneumonectomy and lobectomy with lymph node dissection), diverse radiotherapies, and chemotherapies using cyclophosphamide or methotrexate are the main treatments for lung tumors. Since conventional treatments or therapies that have low selectivity or specificity for human tumors are used clinically and result in toxicity to normal and healthy tissue, novel therapeutic strategies are critically needed to enhance selectively therapeutic efficiency and effectiveness to treat lung cancer. For this, novel strategies for targeting drugs to lung tumors are absolutely necessary.

Since gene/pro-drug systems can be designed to more selectively target tumor cells than normal cells (2,3), the application of enzyme/pro-drug systems to minimize side-effects has received much attention. The cytosine deaminase (CD)/5-fluorocytosine (5-FC) system (4-8), one of the gene-directed enzyme/pro-drug therapies (GEPT), metabolically converts non-toxic 5-FC into the toxic metabolite 5-fluorouracil (5-FU) (9,10) and inhibits DNA synthesis in cancer cells (11,12). The CD/5-FC GEPT system has been used to experimentally treat several types of cancers including clinical trials for colorectal and prostate cancers (13-15). The application of the pro-drug seems to reduce toxicity in normal tissues, but there are potential problems with exogenous enzyme delivery in selectively targeting tumor cells.

Stem cells have recently received a great deal of attention for their clinical and therapeutic potential in treating human cancers. For instance, neural stem cells (NSCs) may have great tropic and therapeutic potential for treating human malignant tumors such as medulloblastomas and gliomas (16-18), suggesting that enzyme/pro-drug therapy using NSCs can serve as a potent delivery system to target and eradicate tumor cells specifically following systemic pro-drug administration (Fig. 1). HB1.F3, a new cell line of human NSCs immortalized using a retroviral vector carrying v-myc, has been generated from fetal telencephalon cells (19). This clonally isolated, multi-potent human NSC line has the ability to self-renew and to differentiate into cells of neuronal and glial lineages both *in vivo* and *in vitro* (20). In addition to the therapeutic potential of these NSCs to treat brain disorders, their inherent migratory

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Key words: lung cancer, tumor tropism, therapeutic stem cells, cytosine deaminase, interferon-β

and tumor-tropic properties represent a novel and potentially powerful approach for the treatment of invasive tumors. As a delivery vehicle for targeting tumor cells and to disseminate therapeutic gene products throughout tumor sites, these therapeutic NSCs may overcome major obstacles facing current gene therapy strategies by selectively infiltrating tumor masses. Human NSCs generated from a single clone can be engineered to stably express the therapeutic CD gene to activate the pro-drug 5-FC because they are homogeneous and can be expanded to large numbers *in vitro*. HB1.F3 cells, the parental cell line of HB1.F3.CD cells, migrate to subcutaneous xenografts of diverse solid tumors including prostate tumors, breast tumors, melanoma, glioma, and neuroblastoma, indicating that these cell lines do not possess a tissue-specific homing tendency but could be useful therapeutically due to their ability to migrate to tumor tissues in general (17).

In this study, we investigated whether GESTECs [HB1.F3.CD and HB1.F3.CD.interferon-β (IFN-β)] have a significant migrating capacity for selectively targeting human lung cancers as well as the therapeutic value of an enzyme/pro-drug system in lung cancer therapy.

Materials and methods

Cell culture. The human non-small cell lung carcinoma cell line A549 (Korean Cell Line Bank, Seoul, Korea) was cultured in RPMI (PAA Laboratories, Austria) supplemented with 10% FBS (HyClone Laboratories, Inc.. Logan, UT, USA), 1% penicillin G and streptomycin (Cellgro Mediatech, Inc., Manassas, VA, USA), 1% anti-fungal HEPES (Invitrogen Life Technologies, Calsbad, CA, USA), and 0.1% antimycoplasmal plasmocin (Invivogen, San Diego, CA, USA) at 37˚C in a humidified atmosphere of 5% CO_2-95% air. HB1.F3 is an immortalized human NSC (hNSC) line derived from human fetal brain at 15 weeks of gestation by an amphotropic, replication-incompetent retroviral vector v-myc (19,21). The clonal HB1.F3.CD line was derived from parental HB1.F3 cells (obtained from University of British Columbia, Vancouver, Canada) and transducted to *E. coli* CD gene. Also the clonal HB1.F3.CD.IFN-β line was derived from the parental HB1.F3.CD cells and this cell line expressed *E. coli* CD and human IFN-β genes. HB1.F3, HB1.F3.CD, HB1.F3.CD.IFN-β and the bovine fibroblast (Bovine FB) cell line (obtained from Chungbuk National University, Cheongju, Korea) were cultured in DMEM (Hyclone Laboratories Inc.) supplemented with 10% FBS, 1% penicillin G and streptomycin, 1% HEPES, and 0.1% plasmocin at 37˚C in a humidified atmosphere of 5% CO_2-95% air. Cells were trypsinized with 0.05% trypsin/0.02% EDTA (PAA Laboratories) in Mg^{2+}/Ca^{2+}-free HBSS.

Confirmation of chemoattractant ligands and receptors. Recent studies suggest that the tumor-targeting behavior of NSCs is mediated by chemoattractant molecules and their respective receptors including stem cell factor (SCF)/c-Kit (22), CXC chemokine receptor 4 (CXCR4) (23), and vascular endothelial growth factor (VEGF)/VEGF receptor (VEGFR)-1 and VEGFR2 (24). Therefore, the expression of these chemoattractant ligands and their associated receptors was examined in A549 cell by reverse transcription PCR (RT-PCR).

Total cellular RNA was prepared using the TRIzol reagent (Invitrogen Life Technologies). Single-stranded cDNA was prepared from 1 μg of total RNA using random primer M-MLV RT (iNtRON Biotechnology, Sungnam, Kyeonggido, Korea). cDNA from the reverse transcription reaction was used in the subsequent PCR reactions in the presence of 0.2 μmol/l of each 5' and 3' primers, 2.5 U of Taq polymerase (iNtRON Biotechnology), 1.5 mmol/l $MgCl_2$, 0.2 mmol/l deoxynucleotide mix (iNtRON Biotechnology), and 10X PCR buffer (iNtRON Biotechnology). PCR for these chemoattractant factors (ligands and receptors) and GAPDH, as a positive control, was carried out for 30 cycles using PTC-100 (MJ Research Inc., Waltham, MA, USA). The PCR reactions were denaturated at 95˚C for 30 sec, annealed at 58˚C for 30 sec, and extended at 30 sec. The products were analyzed on a 2.5% agarose gel stained with ethidium bromide. The sense and antisense primers and the predicted sizes of the RT-PCR reaction products are shown in Table I.

Cell growth assay. To investigate the effect of 5-FC and 5-FU in lung cancer cells, A549 cells (4,000 cells/well) were seeded in 96-well plates and cultured in 0.1 ml medium with 5% FBS. After a 24-h pre-incubation, HB1.F3, HB1.F3.CD, or HB1.F3.CD.IFN-β cells were added to the cultures in medium containing 5% FBS and incubated for 24 h before treatment with 5-FC or 5-FU. On the day of treatment, 5-FC and 5-FU (Sigma-Aldrich Corp., St. Louis, MO, USA) were serially diluted with PBS (final concentration 100, 200, 300, 400, and 500 μg/ml) and the cells were treated for 4 day. MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide) assays were performed to measure cell viability on day 7. MTT solution (10 μl; 5 mg/ml) was added to each well in the plates and incubated for 4 h at 37˚C. Supernatants were 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. Optical densities were measured at 540 nm using an ELISA reader (VERSA man, Molecular Devices, CA, USA). MTT assays were carried out in duplicate.

In vitro migration assay. To investigate whether GESTECs are capable of migrating to lung cancer cells, A549 cells ($1x10^5$ cells/well) were plated in 24-well plates and incubated in DMEM contained 10% FBS for 24 h at 37˚C. The cells were then incubated with new serum-free media and incubated for 24 h. Transwell plates (8 μm; BD Biosciences, Franklin Lakes, NJ, USA) coated with fibronectin (250 μg/ml; Sigma-Aldrich Corp.) were placed in the 24-well plates and incubated overnight. Using a general protocol, chloromethylbenzamido-1,1'-dioctadecyl-3,3,3'-tetramethylindocarbocyanine perchlorate (CM-DiI; Invitrogen Life Technologies) was used to labeled HB1.F3.CD or HB1.F3.CD.IFN-β cells ($1x10^5$ cells/well) that were plated in the upper chambers of the transwell plates and cultured in serum-free medium for 24 h at 37˚C. A549 cells were stained by adding DAPI (200 ng/ml, 4',6-diamidino-2-phenylindole; Invitrogen, Ltd.) and the plate was incubated for 20 min at 37˚C. Each well was washed with PBS and the upper side of the transwell membrane was then scraped to remove cells that had not migrated into the membrane. The cells stained with CM-DiI and DAPI were examined by fluorescence microscopy (IX71 Inverted Microscope, Olympus, Japan).

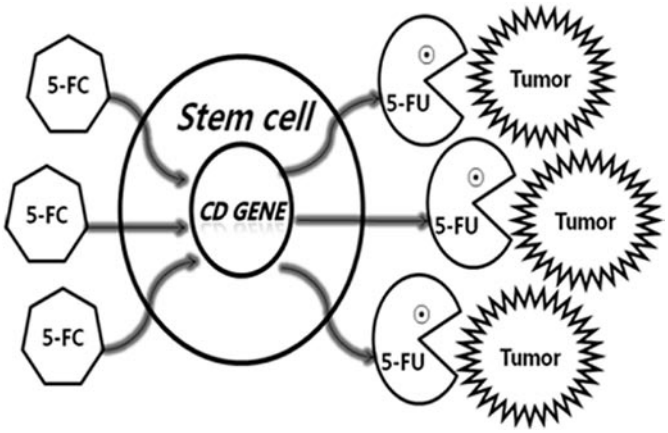


Figure 1. Strategy using GESTECs to selectively target human non-small cell carcinoma cells (lung cancer). Genetically engineered stem cells (GECTECs) have the *E. coli* cytosine deaminase (CD) gene for producing suicide enzymes. CD converts the non-toxic pro-drug 5-fluorocytosine (5-FC) to toxic 5-fluorouracil (5-FU). Application of the pro-drug seems to reduce toxicity in normal tissues, but there are potential problems with exogenous enzyme delivery in targeting tumor cells selectively. GESTECs have significant migrating capacity to selectively target human lung cancers, as well as the therapeutic value of a suicide/prodrug system in lung cancer therapy.

Statistical analysis. The results of all cell growth assay experiments are presented as the mean ± SD. Statistical analysis was performed with a one-way ANOVA test. $P<0.05$ was considered statistically significant.

Results

Confirmation of CD and IFN-β genes expression in GESTECs. Confirmation of CD and IFN-β genes expression in HB1.F3.CD and HB1.F3.CD.IFN-β cells was performed by RT-PCR. CD mRNA (559 bp) was detected in HB1.F3.CD and HB1.F3.CD.IFN-β cells (Fig. 2). In addition, the RT-PCR results showed that HB1.F3.CD.IFN-β cells contained human IFN-β mRNA (296 bp) but the HB1.F3.CD cells did not (Fig. 2). GAPDH was used to positive control. GAPDA size was predicted to 351 bp in HB1.F3.CD and HB1.F3.CD.IFN-β cell lines and confirmed expression through 2.5% agarose gel electrophoresis.

In vitro cell migration assay. A modified transwell migration assay was performed to measure the ability of GESTECs to migrate toward lung cancer cells and observed to use fluorescence microscopy. At results, DAPI-stained cancer cell, A549, significantly increased cell migration of CM-DiI stained stem cell, HB1.F3.CD and HB1.F3.CD.IFN-β, compared to of DAPI-stained bovine FB cells (Fig. 3A and B). Additionally, we counted the number of migrated bovine FB and engineered stem cells in order to determine whether only stem cells migrate toward lung cancer cells and each migrated cell number is presented in Fig. 3C.

Confirmation of chemoattractant ligands and receptors. To examine whether lung cancer cells express chemoattractant

Table I. The oligonucleotide sequences of the primers used in this study and the predicted sizes of the PCR products.

mRNA		Oligo-sequences (5'-3')	Expected size (bp)
IFN-β	Sense	AAAGAAGCAGCAATTTTCAG	296
	Antisense	TTTCTCCAGTTTTTCTTCCA	
CD	Sense	GCGCGAGTCACCGCCAGCCACACCACGGC	559
	Antisense	GTTTGTAATCGATGGCTTCTGGCTGC	
SCF	Sense	ACTTGGATTCTCACTTGCATTT	505
	Antisense	CTTTCTCAGGACTTAATGTTGAAG	
c-Kit	Sense	GCCCACAATAGATTGGTATTT	570
	Antisense	AGCATCTTTACAGCGACAGTC	
CXCR4	Sense	CTCTCCAAAGGAAAGCGAGGTGGACAT	558
	Antisense	AGACTGTACACTGTAGGTGCTGAAATCA	
VEGF	Sense	AAGCCATCCTGTGTGCCCCTGATG	377
	Antisense	GCTCCTTCCTCCTGCCCGGCTCAC	
VEGFR2	Sense	ACGCTGACATGTACGGTCTAT	438
	Antisense	GCCAAGCTTGTACCATGTGAG	
GAPDH	Sense	ATGTTCGTCATGGGTGTGAACCA	351
	Antisense	TGGCA GGTTT TTCTA GACGG CAG	

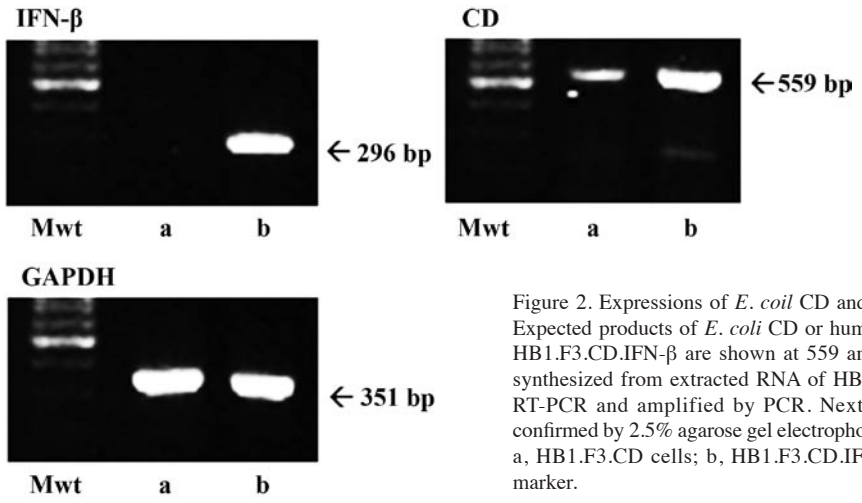
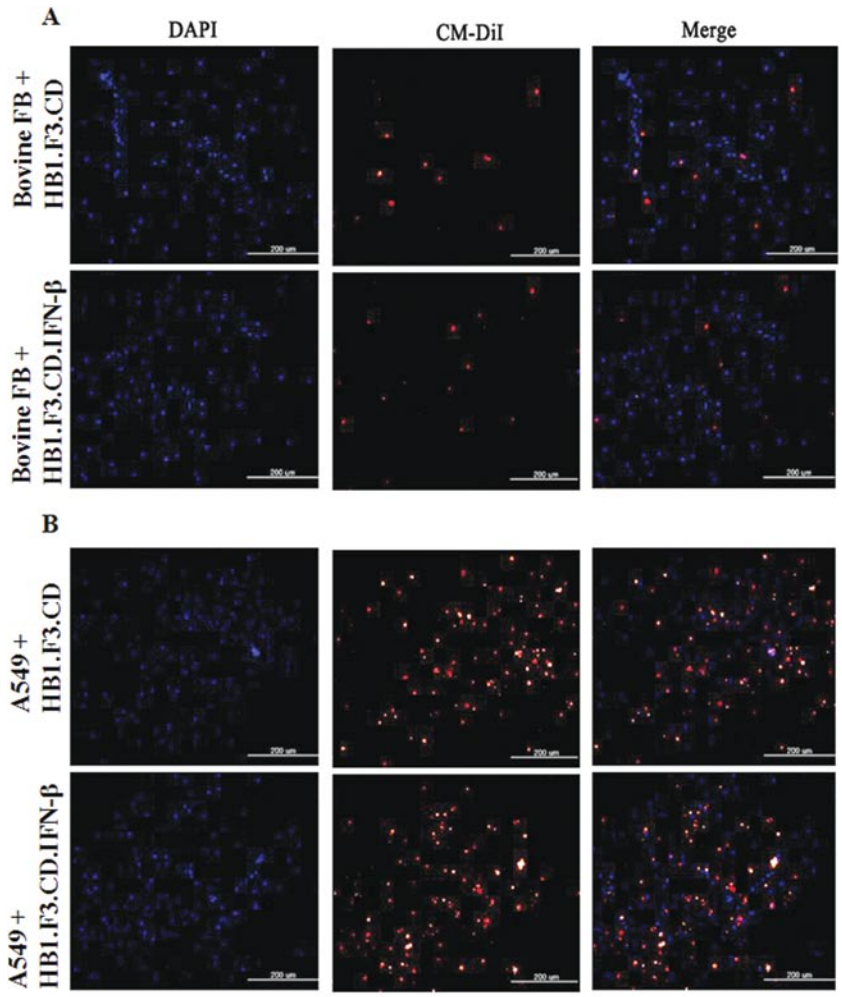


Figure 2. Expressions of *E. coil* CD and human IFN-β genes in GESTECs. Expected products of *E. coli* CD or human IFN-β genes in HB1.F3.CD and HB1.F3.CD.IFN-β are shown at 559 and 296 bp, respectively. cDNA was synthesized from extracted RNA of HB1.F3.CD and HB1.F3.CD.IFN-β by RT-PCR and amplified by PCR. Next, sizes of the PCR products were confirmed by 2.5% agarose gel electrophoresis. GAPDH was used as a control. a, HB1.F3.CD cells; b, HB1.F3.CD.IFN-β cells; Mwt, molecular weight marker.



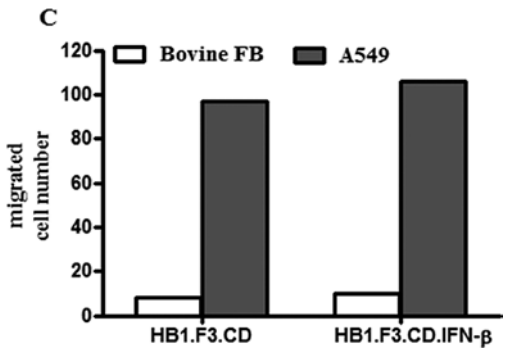


Figure 3. *In vitro* migration of HB1.F3.CD and HB1.F3.CD.IFN-β toward lung cancer cells. The migratory capacity of HB1.F3.CD and HB1.F3.CD.IFN-β cells were assessed by a modified transwell migration assay. The 24-well plates were pre-coated with fibronectin and HB1.F3.CD or HB1.F3.CD.IFN-β cells were added to the upper chamber after placing A549 and bovine FB cells in the lower transwell chamber. (A) Bovine FB cells (100,000 cells/well, blue stained) were placed in the lower well of 24-well plates, and HB1.F3.CD and HB1.F3.CD.IFN-β cells (100,000 cells/well, red stained) were seeded in the upper well of the insert. The inserts were collected and stained as previously described. (B) Migrated cell number of A549 cells. (C) The number of the cells migrating into the membrane were counted using a fluorescent microscopy.

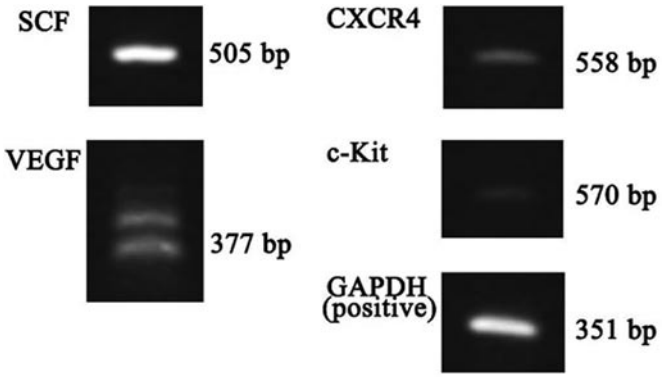


Figure 4. Expression of factors potentially involved in chemoattraction or cell growth in lung cancer. PCR products of GAPDH, SCF, CXCR4, VEGF, VEGFR2 and c-Kit were obtained by RT-PCR. After cDNA synthesis, these products were separated by 2.5% agarose gel electrophoresis. GAPDH was used as a positive control.

factors, the expression of several chemoattractant ligands and their associated receptors was examined in A549 cells by RT-PCR. As seen in Fig. 4, chemoattractant ligands and receptors (i.e., SCF, CXCR4, VEGF, and c-Kit) were expressed in the A549 cells.

Effect of 5-FC/5-FU on lung cancer cells and GESTECs. To confirm the cytotoxic effects of HB1.F3, HB1.F3.CD, and HB1.F3.CD.IFN-β cells, cell viability studies were done in a co-culture system using an MTT assay. After 5-FC (200 μg/ml) treatment, viability of A549 cells co-cultured with the HB1.F3.CD cells decreased by 77.2%, and HB1.F3.CD.IFN-β cells decreased by 63.7% whereas HB1.F3 cells did not decreased (Fig. 5A).

To determine the effect of 5-FC on lung cancer cells, A549 cells were seeded and treated with 5-FC at increasing concentrations (100, 200, 300, 400, and 500 μg/ml), and cell viability was measured. We also examined the cytotoxic effect of 5-FC on HB1.F3.CD and HB1.F3.CD.IFN-β cells. As seen in Fig. 5B, treatment with 5-FC led to a reduction in cell viability in HB1.F3.CD cells and a more significant reduction in cell viability in HB1.F3.CD.IFN-β cells. Treatment with 5-FC resulted in a dose-dependent inhibition of cancer cell growth in HB1.F3.CD and HB1.F3.CD.IFN-β cells. To confirm the cytotoxic effects of the active drug in A549 cells, cells were treated with 5-FU (the active form), which resulted in a significant decrease of cell proliferation (Fig. 5C).

To investigate whether treatment with 5-FC induces anti-proliferative effects in lung cancer cells cultured in the presence of HB1.F3.CD or HB1.F3.CD.IFN-β cells, A549 cells (4,000 cells/well) were co-cultured with different numbers of HB1.F3.CD or HB1.F3.CD.IFN-β cells ($8x10^3$, $1.6x10^4$, or $2.4x10^4$ cells/well) and treated with 5-FC (500 μg/ml). Treatment with 5-FC appeared to decrease A549 cell growth in the presence of a high density of HB1.F3.CD ($2.4x10^4$ cells/well) or HB1.F3.CD.IFN-β cells (Fig. 5D). These results demonstrate that HB1.F3.CD or HB1.F3.CD.IFN-β cells induce cell growth inhibition of lung cancer cells in the presence of a non-toxic pro-drug, 5-FC, following conversion of the pro-drug to the 5-FU active form.

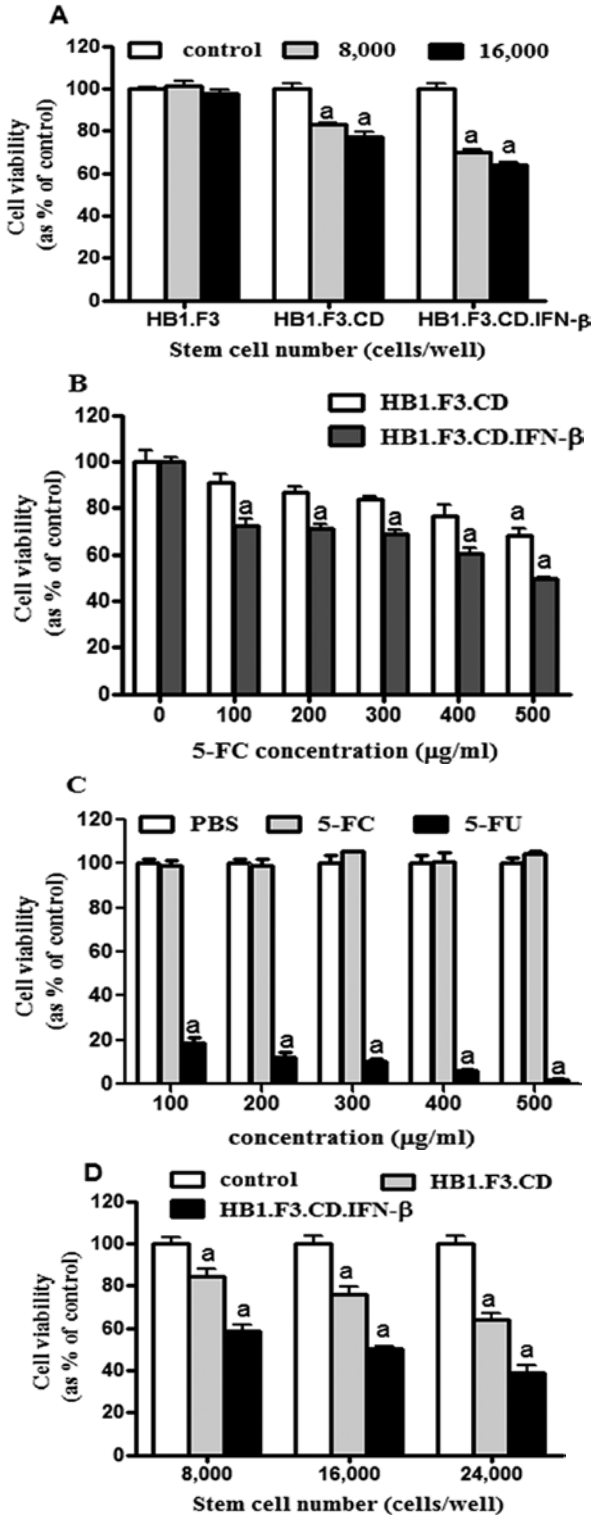


Figure 5. Effect of 5-FC/5-FU on cell viability. Cell viability at each concentration of 5-FC or 5-FU are expressed as relative fold-change compared to the controls. (A) A549 cells ($4x10^3$ cells/well) were seeded in 96-well plates and HB1.F3, HB1.F3.CD, or HB1.F3.CD.IFN-β cells ($1.6x10^4$ cells/well) were seeded and treated with 5-FC (200 μg/ml). (B) A549 cells ($4x10^3$ cells/well) and HB1.F3.CD or HB1.F3.CD.IFN-β cells ($8x10^3$ cells/well) were seeded and treated with 5-FC at increasing concentrations (100, 200, 300, 400, and 500 μg/ml), and the cell viability was measured. (C) A549 cells were treated with increasing concentrations of 5-FU or 5-FC (100, 200, 300, 400, and 500 μg/ml) (D) A549 cells ($4x10^3$ cells/well) were seeded in 96-well plates. Following incubation for 24 h, increasing numbers of HB1.F3.CD or HB1.F3.CD.IFN-β cells ($8x10^3$, $1.6x10^4$, and $2.4x10^4$ cells/well) cells were seeded. After 24 h, the cells were treated with 5-FC at a concentration of 500 μg/ml for 4 days. Values are the mean ± SD for three independent experiments. [a]P<0.05 compared to the control.

Discussion

Recent studies have found that immortalized GESTECs have advantages that may be useful for gene therapy and cell replacement therapeutic approaches for the treatment of neurological diseases and injuries (20,25-30). These GESTECs selectively migrate to brain tumors and reduced tumor growth both *in vitro* and *in vivo* (17,18). A previous study demonstrated that when HB1.F3.CD cells expressing *E. coli* CD gene and 5-FC were treated, tumor size was decreased in an animal model but did not show any toxicity in an animal model when treated only with 5-FC or HB1.F3.CD cells (18). Also another recent study reported that GESTECs expressing fusion gene (HB1.F3.CD.IFN-β) had an anti-tumor effect rather than the parental cell line, HB1.F3 (31).

The CD/5-FC-derived GEPT system has been tested as a potential therapy for some types of cancers including those in breast, prostate, and colon (8,15,32), but the effect of GESTECs on many other types of cancer cells remains largely unknown. Thus, in this study we examined whether GESTECs expressing CD/CD.IFN-β can be a used to selectively treat lung cancer. First, we performed a modified transwell migration assay to examine whether these GESTECs expressing CD/CD.IFN-β are capable of migrating to lung cancer cells. The migration ability of GESTECs expressing CD/CD.IFN-β cells increased in A549 cells compared to bovine fibroblast cells, suggesting that these GESTECs likely respond to factors secreted by lung cancer cells. These results are in agreement with a previous report demonstrating that GESTECs migrate to brain tumors in an animal model (33). Additionally, HB1.F3 cells, the parent cell line of the HB1.F3.CD and HB1.F3.CD.IFN-β cell lines, migrate toward subcutaneous xenografts of diverse solid tumors including prostate tumors, breast tumors, melanoma, glioma, and neuroblastoma. This result indicates that this cell line does not possess a tissue-specific homing tendency, but might be exploited for therapeutic use in the treatment of many types of cancer (17).

The result of migration assay indicated that the lung cancer cells may contain chemoattractant factors which accelerate the migration of the HB1.F3.CD and HB1.F3.CD.IFN-β cells, thus enhancing the delivery of a therapeutic enzyme to human tumors *in situ*. Although several factors such as SCF, SDF-1, and VEGF play a chemoattractive role (22-24,34,35) in tumor cells, the molecular mechanism of the tumor-tropism of GESTECs is not clearly understood. Thus, we further examined the expression of these genes in A549 lung cancer cells and found that chemoattractant ligands and receptors (i.e., SCF, CXCR4, VEGF, and c-Kit) were expressed in these cells. Thus, these chemoattractant molecules and their respective receptors may play a role in the tumor-tropic effects that enable GESTECs to selectively deliver a suicide enzyme to the tumor. These factors also play important roles in the biology of lung cancer (36-38). Further study is required to confirm the role of these genes in the mechanisms underlying tumor cell recognition and/or tumor tropism by GESTECs.

Although 5-FU, a potent inhibitor of thymidylate synthetase (39), has been used in treatment of cancer patients over the past few decades, the systemic application of 5-FU has been limited by its toxic effects, including myelosuppression and stomatitis, that can occur prior to achieving a therapeutic response. Therefore, the non-toxic metabolite 5-FC, which is converted to 5-FU by *E. coli* CD, has received much attention in the last few years (18,40). In the present study, treatment with 5-FC resulted in decreased cell growth with increasing numbers of HB1.F3.CD cells co-cultured with A549 cells, indicating that the application of the CD/5-FC GEPT system may have potential as a therapeutic modality for the treatment of lung cancer. Since an earlier report showed that a small number of CD-transfected cells can induce anti-tumor effects through a bystander effect (41), we examined whether the number of GESTECs is important in modulating the proliferation of lung cancer cells. Different numbers of GESTECs were co-cultured with A549 cells and treated with 5-FC at various doses. HB1.F3.CD cells expressing the CD gene and HB1.F3.CD.IFN-β cells expressing a CD.IFN-β fusion gene maximized the decrease of A549 cell growth in cultures containing a 1:6 ratio of A549 cells: GESTECs, suggesting that the number of GESTECs in the enzyme/pro-drug system needs to be considered in order to maximize their therapeutic benefit. To our knowledge, this is the first report of GESTECs-induced anti-proliferation in lung cancer cells using a GEPT system.

Our goal for adopting the CD.IFN-β fusion gene was to maximize the anti-tumor effect. The individual therapeutic actions of CD and IFN-β are different; thus, we could expect a synergic effect with the fusion gene. CD acts as a pro-drug-activating enzyme (18) and IFN-β can enhance anti-angiogenic effects and immune responses (42). As the results from our study showed, HB1.F3.CD.IFN-β cells show a maximum effect in the cell viability assay and migration assay compared to HB1.F3.CD cells. In our previous study, treatment of a human epithelial ovarian cancer cell line (SKOV-3) with the pro-drugs 5-FC or camptothecin-11 (CPT-11) in the presence of HB1.F3.CD or HB1.F3.CE cells resulted in the inhibition of ovarian cancer cell growth, suggesting that GESTECs expressing CD/rabbit carboxyl esterase (CE) may be used to selectively treat ovarian cancers (43).

In conclusion, the results of the present study have shown that GESTECs expressing CD or CD.IFN-β genes may selectively migrate toward lung cancer cells. Moreover, this GEPT system resulted in an anti-proliferative effect on lung cancer cells, suggesting that GESTECs expressing suicide genes combined with the application of pro-drugs may have therapeutic potential for selectively treat lung cancers. Furthermore, GESTECs expressing the CD.IFN-β fusion gene have a synergic anti-tumor effect compared to GESTECs expressing CD alone.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant (no. 2010-0003093) funded by the Korea government (MEST).

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