

Review

Potential Tumor-tropic Effect of Genetically Engineered Stem Cells Expressing Suicide Enzymes to Selectively Target Invasive Cancer in Animal Models

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Abstract. Stem cells have recently received a great deal of attention for their clinical and therapeutic potential to treat human disease and disorders. For instance, neural stem cells expressing a suicide gene which can convert prodrugs to their active metabolites may have great tropic and therapeutic potential for brain tumors, i.e., medulloblastoma and glioma. We are currently interested in therapeutic potential of these genetically engineered stem cells (GESTECs) to selectively target invasive tumors, i.e. ovarian, endometrial, breast, and lung cancer which can have a great impact on human and animal health. Thus, in this review we summarize the therapeutic potential of GESTEC, developed by us, and the putative mechanism(s) underlying their therapeutic and tropic potential in expressing suicide genes which can convert prodrugs to their active metabolites and in selectively targeting invasive tumors.

Gene-directed enzyme prodrug therapy (GEPT) shows much promise as a strategy for improving the selectivity of conventional chemotherapeutics. This approach involves improving selectivity by delivering 'suicide' genes to cancer cells, enabling them to convert non- or low-cytotoxic prodrugs to cytotoxic drugs. Using GEPT, human tumors can be targeted

and specifically treated to enhance efficacy and reduce side-effects of biological drugs. Examples of GEPT include: gene/prodrug to drug; cytosine deaminase (CD)/5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU); carboxylesterase (CE)/camptothecin (CPT-11) to SN-38; and herpes simplex virus 1-thymidine-kinase (HSV1-TK)/ganciclovir (GCV) to its active metabolite. To successfully deliver these suicide genes to gynecologic malignancies, a specific and selective mediator or delivery system is crucial to enhance the selective effectiveness of these prodrugs in gynecologic malignancies.

Stem cells are multi-pluripotent cells which are capable of self-renewal and generate differentiated progeny for organ development. Stem cells have recently received a great deal of attention for their clinical and therapeutic potential to treat human cancer, for instance, neural stem cells (NSCs) may have great tropic and therapeutic potential for human malignant tumors, such as brain tumors including medulloblastoma and glioma. Recently, a continuously dividing immortalized cell line of NSCs, HB1.F3, was developed by introducing the myelocytomatosis viral oncogene homolog, *v-myc*. In addition to potential therapy for brain disorders by these NSCs, by virtue of their inherent migratory and tumor-tropic properties, they represent a novel and potentially powerful approach for the treatment of invasive tumors. As a delivery vehicle to target and disseminate therapeutic gene products using HB1.F3.CD (NSC with *CD* gene) or HB1.F3.CE (NSC with *CE* gene) throughout tumor sites, these therapeutic NSCs may overcome major obstacles facing current gene therapy strategies by selectively infiltrating tumor masses. These human NSCs are homogeneous, as they were generated from a single clone, can be expanded to large numbers *in vitro*, and can be engineered to stably express the therapeutic

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suicide genes CD or CE, and to activate prodrugs 5-FC or CPT-11. It is of interest to note that HB1.F3 cells, a parental cell line of the HB1.F3.CD/CE cell lines, migrate to subcutaneous xenografts of diverse solid tumors, prostate and breast cancer, melanoma, glioma and neuroblastoma, indicating that these cell lines do not present tissue-specific characteristic for therapeutic use (1).

Neural Stem/Progenitor Cells

Stem cells are multi-pluripotent cells which can self-renew and generate differentiated progeny for organ development (2). There are two types of mammalian pluripotent stem cells: i) embryonic stem (ES) cells derived from the inner cell mass of blastocysts and ii) embryonic germ (EG) cells obtained from post-implantation embryos, which can give rise to diverse organs and tissues *in vivo* (3-6). In addition to these ES and EG stem cells, adult stem cells found in adult tissues and cord blood stem cells from the umbilical cord have been clinically tested as a potential therapeutic method (7-9). Tissue-specific stem cells can be isolated from various tissues of more advanced developmental stages, such as bone marrow mesenchymal stem cells, hematopoietic stem cells and neural stem cells. Multipotent NSCs have been found in developing and adult rodent central nervous system (CNS) tissues, which grow indefinitely and have multipotent potential to differentiate into three major cell types of CNS, neurons, astrocytes and oligodendrocytes (10, 11). In humans, the existence of multipotent NSCs has also been reported in embryonic and adult human brains (12-14).

NSCs have great therapeutic potential for treatment of injury and disease in human brain and other organs (2, 15-19). Recently, the continuously dividing immortalized cell lines of NSCs were generated by introducing viral oncogenes. Primary cultures of fetal human telencephalon cells (at 15 weeks gestation) were infected with a retroviral vector carrying the *v-myc* oncogene and several clones with continuously dividing NSCs were selected (20). These NSC cell lines can be expanded to large numbers in culture in a short time (24–36 h doubling time) and are homogeneous since they were generated from a single clone, thus stable expression of therapeutic genes can be achieved (20-23). In addition, these immortalized NSC lines have advantageous characteristics for basic studies on neural development and cell replacement therapy or gene therapy studies. Immortalized NSCs have also emerged as a highly effective source of cells for genetic manipulation and gene transfer; they were genetically manipulated *in vitro*, survive, integrate into host tissues and differentiate into both neurons and glial cells after transplantation to intact or damaged brain (2, 11, 13, 23).

More recently, a new cell line of immortalized human NSCs using a retroviral vector carrying *v-myc*, HB1.F3, has

been generated from fetal telencephalon cells. This clonally isolated, multipotent 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* (24, 25). These cells showed a normal human karyotype of 46,XX. In addition, cell type-specific markers, *i.e.*, nestin, musashi-1, and neurofilament (NF) for NSCs, and glial fibrillary acidic protein (GFAP) for astrocytes, are expressed in the HB1.F3 human NSC line.

Gene-directed Enzyme/Prodrug Therapy of Human Cancer

Conventional cancer treatments are hindered by a lack of selectivity and specificity to *de novo* tumors, resulting in toxicity to normal and healthy tissue. GEPT is a suicide gene therapeutic approach that specifically aims to improve the selectivity of conventional chemotherapy by enabling cancer cells to convert non- or low-cytotoxic prodrugs to cytotoxic drugs (26-28). As described above, CD/5-FC to 5-FU, CE/CPT-11 to SN-38, and HSV1-TK/GCV to active metabolite are good examples of GEPT. Some of these treatments have already been tested in clinical trials. A key component of GEPT is a foreign enzyme that is expressed selectively at the tumor site where it can convert a prodrug into its cytotoxic metabolite *in vivo* (28). The gene encoding the prodrug-activating enzyme needs to be expressed selectively and efficiently in tumor cells in order not to expose normal tissue to damage. Substantial efforts have been made to develop gene therapy vectors that are capable of specifically targeting cancer cells. A large number of gene delivery systems have been identified for GEPT, but viral vectors are the most advanced (26, 27).

The therapeutic effect of a polymerase-chain reaction (PCR) vector carrying the yeast *CD* gene, which converts the nontoxic prodrug 5-FC into the cytotoxic 5-FU, was demonstrated after delivery by infusion into the regional circulation in a multifocal hepatic metastasis model of colon cancer. This shows that a novel delivery of a suicide gene by a PCR vector infused into the portal circulation results in progressive transduction of multiple tumor foci in the liver, which can achieve significant inhibition of tumor growth (29). Combined cancer gene therapy using human tumor necrosis factor- α (hTNF α) and the *CD* suicide gene has been evaluated in two human breast adenocarcinoma cell lines. A significant increase in apoptotic cells and decrease of cell proliferation in human breast cell lines was observed when using combined treatment with hTNF α expression plus the CD/5-FC suicide system (30). In addition, CD-expressing breast tumor cells were highly sensitive to treatment with 5-FC prodrug, and were shown to have increased therapeutic potential in these cells for radiotherapy *in vivo* (31).

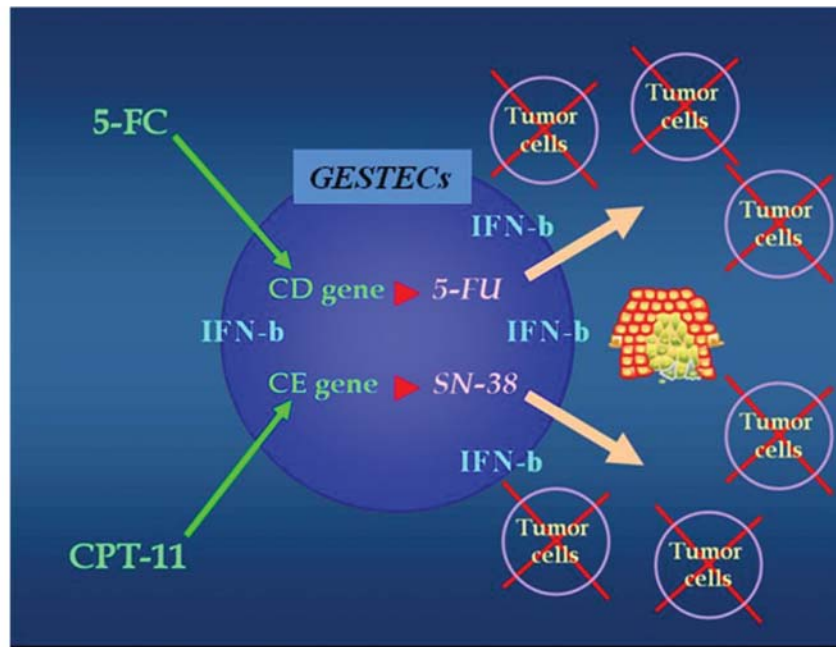


Figure 1. Potential bystander effect of genetically modified stem cells (GESTEC) expressing suicide enzymes which can convert prodrugs to their active forms to selectively target invasive tumors; CD: cytosine deaminase, CE: carboxy esterase, 5-FC: 5-fluorocytosine, 5-FU: 5-fluorouracil.

Further exploring a multi-pronged approach, treatment with endostatin-CD, an angiogenesis inhibitor, provided stronger tumor growth suppression and increased mean survival rate of the mice, compared with the treatment of endostatin alone or CD alone. The endostatin-CD therapeutic system significantly inhibited the growth of endothelial cells and preferentially induced tumor cell apoptosis, indicating that this endostatin-CD fusion would be a good therapeutic approach to treat human metastatic cancer with enhanced angiogenesis for cancer-targeting therapy (32).

Tropism of Neural Stem/Progenitor Cells to Human Cancer and Disorders

Transplantation of NSCs into the brain of animal models of focal ischemia (33, 34), intracerebral hemorrhage (35), Huntington's disease (36, 37), Parkinson's disease (38) and lysosomal storage disease MPS VII (39) showed that F3 human NSCs successfully integrated into host brain parenchyma and provided functional recovery in these experimental animals. As a delivery vehicle to target and disseminate therapeutic gene products throughout tumor sites, NSCs may overcome major obstacles facing current gene therapy strategies by the selective infiltration of tumor masses. Clearly, in addition to the high therapeutic potential to treat brain disorders, NSCs, by virtue of their inherent migratory and tumor-tropic properties, represent a novel and potentially powerful approach for the treatment of invasive tumors.

A previous study showed that when murine NSC cell lines carrying oncolysis-promoting prodrug activating enzyme CD were grafted into glioma-bearing animals and prodrug 5-FC was injected systemically, there was an 80% reduction in the tumor mass (40, 41). Recently Bello *et al.* at Harvard University have demonstrated that human NSCs carrying the *PEX* gene, a fragment of human metalloproteinase-2 which acts as an inhibitor of glioma proliferation, migration and angiogenesis (42), surround the invading glioblastoma tumor cells, chasing down infiltrating tumor cells, and attack and kill tumor cells, resulting in a 90% reduction in tumor volume (17). When human NSC line HB1.F3.CD was transplanted intracranially at distant sites from the tumor, the donor NSCs migrate through normal tissue and selectively 'homed in' on the glioblastoma tumor mass, whereupon an 80-85% reduction in tumor volume was observed after administration of prodrug 5-FC (18, 41, 43). Histological analyses showed that NSCs migrate to the tumor boundary, leading to the reduction of tumor volume in the treated group, suggesting the effectiveness of inherently migratory NSCs as a delivery vehicle for targeting therapeutic genes to refractory, migratory and invasive brain tumors, such as glioblastoma, medulloblastoma and melanoma (18, 44, 45). It is postulated that these NSC cells may have a bystander effect *via* which toxic prodrugs and their metabolites travel across gap junctions as well as through interstitial space to surrounding cells, selectively killing dividing tumor cells, as shown in Figure 1.

Another strategy using the tumor-tropic property of HB1.F3 cells has been developed to deliver an effective therapeutic agent selectively to metastatic tumors. Following immortalization, an HB1.F3.CE cell line carrying activating enzyme rabbit carboxylesterase (rCE) has been developed. These cells have been shown to replicate *in vitro* to form identical daughter cells, or they can also be induced to differentiate into other cells of neuronal lineage, thereby exhibiting characteristics of both stem cells and progenitor cells (1). Intravenous administration of HB1.F3.CE cells expressing irinotecan (CPT-11)-activating enzyme CE significantly increased the antitumor effect of tolerated doses of CPT-11 in mice bearing disseminated neuroblastoma tumors. This indicates that it may be possible to exploit the tumor-tropic property of stem or progenitor cells to mediate effective tumor-selective therapy for metastatic tumors, for which no tolerated curative treatments are currently available. The approach described may also be adapted for patients with other types of metastatic solid tumors, as suggested (1). Since no effective treatments are available for most metastatic tumors, it would be highly significant if these stem or progenitor cells of fetal or adult origin could be used to improve the prognosis of patients with such cancer.

The molecular basis of tumor-tropism of HB1.F3 parental cells, HB1.F3.CD/CE cell lines or other stem/progenitor cells, has not been clearly elucidated, however, biological factors, *i.e.*, stromal cell-derived factor-1 (SDF-1), scatter factor (SCF; hepatocyte growth factor), vascular endothelial growth factor (VEGF) and macrophage chemotactic protein-1 (MCP-1) expressed by tumor cells appear to play a role in chemotaxis to human tumors (46-53). In addition, recent *in vitro* results indicated extracellular matrix (ECM) proteins preferentially expressed in invasive areas of brain tumors, *i.e.*, glioma, may provide a permissive environment for the tropism of these NSC lines to disseminated tumor cells (54). Although the mechanism by which HB1.F3.CD/CE cells target neuroblastoma cells is largely unknown, it is clear that this tropism is not cell line-specific since these cells migrated to liver metastases, minimal bone marrow disease, and macroscopic bone marrow tumors (1). Evaluation for the mechanism of tumor cell recognition or tumor tropism by stem/progenitor cells is still ongoing. In addition, HB1.F3.CE cells migrate to subcutaneous xenografts of diverse solid tumors including prostate and breast cancer, melanoma, glioma, and neuroblastoma (unpublished results (1)), suggesting that undifferentiated cells of neural origin may be preferable to mesenchymal stem cells and that HB1.F3.CE cells may have significant utility for the treatment of metastatic tumors of different types (18).

Clinical Impact and Gene Therapy of Ovarian, Endometrial and Breast Cancer

Although ovarian, endometrial and breast cancer significantly impact on women's health (55), the mechanism(s) of transformation and development of these lethal diseases remains undiscovered. Conventional therapies like surgical removal, chemotherapy using cisplatin or paclitaxel, and radiotherapy, are the main treatments for gynecologic malignancies. In addition, the preventive removal of these organs, as in ovariectomy (oophorectomy), hysterectomy (removal of the uterus and surrounding tissue) or bilateral salpingo-oophorectomy (removal of the fallopian tubes and ovaries) is appropriate through major surgical procedures after certain ages. Although a conventional treatment or therapy which has a low selectivity or specificity human tumors, resulting in toxicity to normal and healthy tissue may be in clinical use, novel therapeutic strategies are absolutely necessary to enhance therapeutic efficacy in treating these gynecologic cancers.

Epithelial Ovarian Cancer

Ovarian cancer represents the primary and most lethal cause of death from gynecological malignancies in the Western world (56, 57). Annually, 2,400 new cases are diagnosed and 1,715 women die from the disease in Canada alone (55). This high fatality rate may be due to the lack of effective screening methods and the absence of signs and symptoms in early stages of the disease (58-60). Despite intense research efforts, the mechanism of transformation and development of ovarian cancer is not well elucidated. Epithelial ovarian carcinomas, which comprise over 90% of human ovarian cancer cases, arise in the ovarian surface epithelium (OSE) (57). The etiology and early events in the progression of these carcinomas are poorly understood as there are no appropriate animal models, and methods to culture OSE have only recently become available (57, 61, 62).

Surgical removal, chemotherapy using cisplatin, and radiotherapy are the main treatments of this disease, however inhibitors of topoisomerase I, an isomerase enzyme that acts on the topology of DNA, have been approved recently. These inhibitors have the most promising therapeutic effects in human cancer overall in recent years. Clinically prospective trials and retrospective examinations have involved agents including cisplatin, paclitaxel, topotecan, and doxorubicin (63), which have been previously approved by the American Food and Drugs Administration (FDA) for use in ovarian cancer, and the development of new regiment strategies for approved drugs such as docetaxel, irinotecan (CPT-11 and SN-38, an active metabolic form of CPT-11 a second-generation topoisomerase I inhibitor) (64), and bevacizumab. Furthermore, it can be anticipated that future studies

involving novel approved agents will further expand the oncologist's armory against ovarian cancer. CPT-11 has recently been approved for treatment of ovarian carcinomas, and is likely one of the most active chemotherapeutic agents for the treatment of this disease (65). A combination of chemotherapy and an immune derived factor, overexpression of inositol hexakisphosphate kinase 2 (IHPK2), enhanced apoptotic effects of IFN-beta (IFN- β), and Apo2L/TRAIL expression and nuclear localization of IHPK2 were both observed and required for the induction of apoptosis by IFN- β in ovarian carcinoma (66).

In our previous study, we evaluated whether these GESTECs are capable of migrating to human ovarian cancer cells and examined the potential therapeutic efficacy of the gene-directed enzyme prodrug therapy against ovarian cancer cells *in vitro* (67). In the study, GESTECs (HB1.F3.CD or HB1.F3.CE cells) engineered to express a suicide gene (CD or CE) selectively migrated toward ovarian cancer cells, and treatment of human epithelial ovarian cancer cell line (SKOV-3) with the prodrugs 5-FC or CPT-11 in the presence of HB1.F3.CD or HB1.F3.CE cells resulted in the inhibition of ovarian cancer cell growth. Taken together, these results imply that GESTECs expressing CD/CE may have a potent advantage to selectively treat ovarian cancer (67).

Endometrial Cancer

Endometrial carcinoma is the most common neoplasm of the female genital tract, accounting for nearly one half of all gynecologic cancer in the Western world. It is estimated that approximately 4,100 new cases of endometrial cancer are diagnosed annually and about 755 women die of this disease in Canada per year (55, 68). Although intensive research on pathological phenomena of endometrial cancer is on-going, the exact cause and biological aspects of this disease have not been well elucidated. Two different clinicopathological cancer subtypes are recognized: i) estrogen-related (type I, endometrioid) and ii) non-estrogen related (type II, non-endometrioid) with specific genetic alterations such as microsatellite instability or mutations in *PTEN*, *PI3K*, *K-Ras*, and beta-catenin in type I, and *p53* mutations or chromosomal instability in type II (69, 70).

A combination of cisplatin plus doxorubicin for clinical therapeutic treatments for endometrial cancer is the most common therapy, however, low toxicity therapeutic effectiveness of carboplatin plus paclitaxel was observed in advanced or recurrent endometrial cancer (71, 72). In addition, the inhibitors of mammalian target of rapamycin (mTOR), a serine/threonine protein kinase involved in cell growth, survival, protein synthesis, and transcription, represents a promising therapeutic strategy for endometrial cancer (72). Anti-HER-2/neu-targeted therapy

might be a novel and attractive therapeutic alternative in patients with uterine serous papillary carcinoma or clear cell carcinoma. Taken together, more efforts in better understanding the signal transduction pathways in endometrial carcinogenesis may allow the development of novel selectively targeting inhibitors (71, 72). Treatment with paclitaxel followed by cisplatin resulted in synergistic effects and simultaneous treatment with SN-38 and cisplatin resulted in synergistic effects to all endometrial cancer cell lines, indicating that quantitative data analysis for synergism provides a rational design for clinical protocols for combination chemotherapy using SN-38 and other chemotherapeutic agents in patients with endometrial cancer (73).

Breast Cancer

As a gene therapy, mesenchymal stem cells (MSC) have been employed as a systemic delivery vehicle for therapeutic genes against breast cancer, and their combined ability to home in on tumor sites and evade the host immune response has been assessed (74). The use of suicide gene (CD) and 5-FC system was employed to treat breast cancer *in vitro* using different gene delivery mediators, but efficacy of these delivery systems is questionable (75, 76). Taken together, these current studies indicate that a specific and selective mediator or delivery system is crucial to enhance selective effectiveness of these prodrugs to *de novo* breast tumors.

Impact of Lung Cancer on Human Health

Pathology of lung cancer. Lung cancer is a devastating disease that presents a challenge to basic research to provide new therapeutic advances because lung cancer is the most common cause of cancer mortality in men and women. The majority of lung cancer is attributable to use of tobacco products, tobacco smoking, and, in some cases, other environmental risk factors (77). Worldwide, more than a million deaths are due to lung cancer. Tobacco smoking is the major causal agent, being responsible for about 85% of the lung cancer incidence. Other respiratory exposure to occupational or environmental carcinogens, such as asbestos or radon, and as yet unknown genetic factors contribute to the remaining 15% (78). Lung cancer has two major histopathological groups: non-small-cell lung cancer (NSCLC) (79) and small cell lung cancer (SCLC) (80). About 80% of lung tumors are NSCLC, which are subdivided into adenocarcinomas, squamous cell, bronchioalveolar, and large-cell carcinomas (81). SCLC and NSCLC have major differences in histopathologic characteristics that can be explained by the distinct patterns of genetic lesions found in both tumor classes (82).

Responsiveness to treatment with chemotherapy and/or radiation also differs significantly between NSCLC and SCLC and has a dramatic effect on clinical treatment outcome. The overall 5-year survival rate for lung cancer is only about 14% (83); for SCLC alone it is even worse, at about 5% (84).

Experimental models of NSCLC. Spontaneous lung tumors in mice are similar in morphology, histopathology, and molecular characteristics to human adenocarcinomas. Mouse models for lung cancer can thus serve as a valuable tool both for understanding the basic lung tumor biology and for the development and validation of new tumor intervention strategies as well as for identification of markers for early diagnosis. In fact, extensive preclinical testing of lung cancer therapeutics has been performed using xenograft models in which human lung cancer cell lines have been subcutaneously grafted in immunodeficient mice. An important disadvantage of this approach is that xenograft models do not behave as lung tumors because they are placed in the wrong environment. Moreover, xenograft models have a poor record of accurately predicting the clinical efficacy of antitumor drugs (85). Therefore spontaneous lung tumor models based on conditional mutations in genes known to be critical for lung tumorigenesis are better models of choice for preclinical lung cancer therapy testing.

The requirement of continuous oncogene stimulation, such as *K-ras*, for adenocarcinoma development in an NSCLC model (86) is good news because it indicates that the tumor remains critically dependent on activity of the RAS pathway, an important prerequisite for developing targeted therapeutics. Approximately 30% of human tumors carry *RAS* gene mutations. Of the three members of RAS family, *K-RAS*, *N-RAS* and *H-RAS*, *K-RAS* is found to be the most frequently mutated member in human tumors, including lung adenocarcinomas (25-50%) (87). Mice carrying such mutations are highly predisposed to a range of tumor types and exhibit short latency and high penetrance (88). Now that more sophisticated murine lung cancer models have been made available, much progress of lung cancer treatment/prevention has been achieved (89-93). With this information at hand, murine NSCLC models could become a most valuable preclinical tool, and it will be important to see whether these models are also refractory to chemotherapy and/or radiation therapy as is so frequently observed for NSCLC. Since conventional treatments or therapies that have a low selectivity or specificity towards human lung tumors are in clinical use and result in toxicity to normal and healthy tissue, novel therapeutic strategies are crucial. Taken together, current studies indicate that a specific and selective mediator or delivery system is crucial to enhance selective effectiveness of prodrugs to *de novo* lung tumors.

Current Research Using GESTECs Selectively to Target Invasive Tumors in Animal Models

As potentially successful therapeutics to gynecological tumors, the NSCs HB1.F3.CD and HB1.F3.CE, by virtue of their inherent migratory and tumor-tropic properties, represent a novel and potentially powerful approach for the treatment of invasive gynecological tumors. Since no effective treatments are available for most metastatic tumors, these stem/progenitor cells with suicide genes, *CD* or *CE*, have the potential to improve the prognosis of patients with fatal metastatic cancer including ovarian, endometrial, breast and lung cancer. Using xenograft and genetic mouse models for diverse human cancer types, we are currently examining whether therapeutic GESTECs are the highly effective source of the cells for genetic manipulation and gene transfer into metastatic malignancies. Although the accurate molecular mechanism of tumor-tropism of NSCs has not been clearly elucidated, the tumor-tropic activities of HB1.F3.CD and HB1.F3.CE can be an excellent delivery mediator to specifically and selectively target human metastatic cancer. Tropic biological factors, such as SDF-1, SCF, VEGF and MCP-1 expressed by tumor cells which play a role in chemotaxis to human tumors, are being carefully scrutinized. In addition, immunodeficient and some transgenic or knockout mouse models are being employed to elucidate the mystery of the therapeutic usefulness of these GESTECs.

Overall Significance

A number of issues should be clarified before the therapeutic use of GESTECs can be widely accepted in clinical medicine for stem cell replacement or gene therapy in patients with metastatic human cancer. Since neurons can be derived not only from NSCs, but also from ES cells, EG cells, bone marrow mesenchymal stem cells, or umbilical cord blood hematopoietic stem cells, the next question would be which cell types provide the best strategy for treating human tumors (2). Although the debates whether embryonic stem cells would be an appropriate application to treat human diseases or disorders are on-going, the current research indicates that embryonic stem cells appear to be more potent and reliable for therapeutic application than adult stem cells. For this reason, the use of embryonic or fetal tissues will continue, although the use of immortalized cell lines of human NSCs as generated by introduction of *v-myc* does have its advantages for therapeutic application of a cell replacement and gene therapy as it reduces religious and ethical concerns (2). These human GESTECs are homogeneous, since they were generated from a single clone, can be expanded to large numbers *in vitro*, and stably express the therapeutic suicide genes, *CD* or *CE*, to activate prodrugs 5-FC or CPT-11.

Thus, these GESTECs have emerged as highly effective source of cells for genetic manipulation and gene transfer into metastatic tumors including ovarian, endometrial, breast and lung cancer. There is the consideration that the use of these GESTECs may exhibit possible side-effects due to *v-myc*, a viral oncogene, which was used for immortalizing neural stem cells, even though a human MYC gene was not effective to immortalize them. To address this concern, a floxed HB1.F3.CD or CE will be generated to remove *v-myc* gene by a conditional inactivation system, Cre-loxP.

Dr. KS Aboody's group (Division of Hematology and Hematopoietic Cell Transplantation, City of Hope National Medical Center, Duarte, CA, USA) has proposed a clinical trial using HB1.F3.CD for treatment of brain tumor, *i.e.*, gliomas. This research group recently suggested that HB1.F3 cells, the parental cell line of HB1.F3.CD/CE, migrate to subcutaneous xenografts of diverse solid tumors, prostate, breast, melanoma, glioma and neuroblastoma, indicating that these cell lines do not present tissue-specific characteristics for therapeutic use (1). Furthermore, these NSC cell lines appear not to differentiate brain cells *in vitro* nor *in vivo* and do not replicate *in vivo* and undergo apoptosis in normal tissues as discussed (1, 39, 40). These facts indicate the feasibility of the current study and provide the opportunity for a novel and selective therapeutic application using these GESTECs for clinical use to treat human invasive tumors.

Future Work

To augment the use of immunodeficient mouse model *i.e.*, SCID and NOD/SCID, and to evaluate these GESTEC lines to treat invasive cancer, the genetically engineered mouse models will be further employed in future studies. For instance, the genetically engineered transgenic mouse models of ovarian carcinogenesis generated by us (94) and others (95-98) will be employed to evaluate the therapeutic activities of these stem cells for selectively targeting these tumors. Thus, this strategy to employ the transgenic mice would compensate the use of immunodeficient mice to evaluate therapeutic activities of these GESTECs. In addition to the genetically engineered mouse models, a clinical trial using these GESTEC lines will be further proposed for clinical application and use in collaboration with medical doctors to treat human invasive tumors if these NSCs have selective and potential therapeutic effect to treat those tumors.

A parental cell line of HB1.F3.CD/CE has been shown to migrate to subcutaneous xenografts of diverse solid tumors, prostate, breast, melanoma, glioma and neuroblastoma, suggesting that engineered NSC lines do not present a tissue-specific characteristics for therapeutic use as described above. To overcome the challenge of putative tissue-specific tropic effect of the engineered NSC lines, HB1.F3.CD and HB1.F3.CE, the MSC lines originated from adipose tissue or

amniotic fluid are being established in our laboratories for therapeutic use (99, 100). Thus, in addition to engineered NSC lines, engineered MSC lines with *CD* and *CE* genes will be employed to selectively target human tumors for therapeutic use in future studies.

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