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Cancer Gene Therapy: A Novel Strategy for Cancer Treatment

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INTRODUCTION

Cancer is currently the most common cause of death in Korea,[1] and as average life expectancy is rising, cancer must be consistently one of the biggest medical problems over the next decades. With advances in diagnostic tools, early detection of cancer is much increased, however, the outcome of treatment is still dismal. Despite significant advances in diagnosis and management of cancer, complete cure can be expected only when the disease has been localized within the organs. For patients with advanced, metastatic or recurrent cancer, chemotherapy, hormone or immunotherapy as a systemic treatment remains the mainstay of therapy. Although conventional systemic therapies may result initially in significant improvement and responses, the response duration is brief and, in most patients, disease progressions are inevitable.

Because no curative treatment is currently available for most advanced cancers, it is important to develop new therapeutic approaches to improve overall cure rate for advanced cancers. One novel therapeutic strategy under current development is gene therapy. Cancer is a good model for application of novel strategies such like gene therapy because advanced cancer is refractory to conventional therapies including chemotherapy, radiotherapy and hormonal therapy. With recent increment of our knowledge for genetic background in human diseases, current recombinant DNA technologies allow us to manipulate the disease in molecular level. Gene therapy as a therapeutic modality can be defined as an introduction of a normal or modified genetic code into target cells of patient to reverse a genetic or acquired disease. With respect to cancer, the strategy is to prevent, treat, or cure by using the therapeutic information encoded in the treatment DNA sequences.

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GENE DELIVERY SYSTEMS

For a successful gene therapy strategy, three treatment decisions must be considered; therapeutic genes for insertion, the appropriate target cells and gene delivery systems. Numerous methods are currently available for delivering genes into the desired target cells. Delivery methods can be generally divided into those engineered from a pathogenic virus, which has been attenuated for gene transfer, and synthetic or physical systems of gene transfer (Table 1).

Viral vectors

Most early gene therapy trials have used retroviral vectors as a gene delivery system, because this vector has several advantages for transfer of foreign genes into target cells.[2,3] Retroviral vectors for gene therapy have been constructed by substituting the treatment gene in place of the viral replication regions, thus these viruses become replication-incompetent vectors of high efficiency and low toxicity.[4] With these retroviral vectors, high infectivity and expression into target cells can be achieved. Retroviruses are able to integrate desired genes stably into the chromosomal DNA of the target cell. But, these retroviral vectors have some disadvantages. They are not capable of carrying large genes, restricted to 10 kb. Retroviruses can functionally integrate genes upto 70% of

Table 1. Gene delivery systems for cancer gene therapy

Vector	Size	Advantages	Disadvantages
Viral vectors			
Retrovirus	10 Kb	chromosomal integration long-term stability	low transduction efficiency target only dividing cells random DNA insertion risk of replication
Adenovirus	30 Kb	large capacity target nondividing cells high transduction efficiency	Immunogenicity transient delivery
AAV	5 Kb	Integration target nondividing cells	small capacity risk of replication
Vaccina virus	25 Kb	large capacity	high immunogenicity, toxicity
Herpes simplex virus	40~50 Kb	large capacity neuronal tropism latency expression	toxicity
Non-viral vectors			
Naked DNA		no viral genes no limitation on size	inefficient unstable
Liposomes		no viral genes no limitation on size	inefficient

target cells *ex vivo*, but, *in vivo*, transfer efficiency is markedly diminished. For viral DNA integration, retroviral vectors can only infect actively dividing cells,[5] and this makes it difficult to apply retrovirus to gene therapy for slow growing tumors, like prostate cancer. Retroviruses integrate their DNA randomly into host chromosomes, so it might inactivate essential host genes or alter genes, thus, causing neoplastic change, insertional mutagenesis. And there is a possibility of generating replication-competent retrovirus by recombinating between retrovirus and helper plasmid sequences in the packaging cell line. It can be pathogenic in a severely immunocompromized host.[7]

E1 region of adenovirus is responsible for viral replication.[8] After placing desired gene to deleted E1 region, these replication deficient adenoviral vectors can be produced at high titer ($>10^{11}$ pfu/ml) with packaging cell line 293 cells (human embryonic kidney cell line containing adenovirus E1 DNA).[8,9] These adenoviral vectors provide significant tropism to epithelial cells,[10] and adenoviral infection causes no serious illness, but causes only cold-like symptom to human. These vectors can transfer larger DNA sequences compared with retroviral vectors. Adenoviruses are capable of entering cells through a receptor mediated endocytosis and transferring genes into target cells at any stage of the cell cycle. In addition, these viral vectors have potential advantages of high titer production and transfer efficiency. Adenoviruses do not integrate the genes transferred into the host genome, thus do not cause insertional mutagenesis. But, these vectors could induce anti-adenoviral antibodies and T-cell responses, potentially limiting prospects for long-term intermittent therapy in sensitized individuals.[11] Currently, to overcome this obstacle, many studies are being carried out; for instance, coadministration of adenoviral vectors and immunosuppressive agent.

Several other viral vectors are being investigated for gene transfer. Adeno-associated viruses (AAV) need helper-virus for replication and these viruses cause no illness to human being.[12,13] As retroviruses, AAVs have the property of mediating gene transfer via stable integration of the treament gene into the host chromosome,[14] thus gene expression with these vectors can be durable. But, AAVs have difficulty in producing large amount of high titer. Vaccina viruses[15] have large DNA insert capacity and do not need packaging cell lines for replication. These vectors replicate within the cytoplasm of target cells, but, gene expression with these vectors is transient.

Non-viral vectors

Various gene delivery systems using chemical and physical methods have been introduced. Physical method using calcium phosphate coprecipitation[16] is the earliest

method for gene transfer, but, this method has poor transfer efficiency. Some physical methods of gene delivery are currently available. These include direct transfer of the DNA into target cells by microinjection,[17] gene gun that shoots gold beads coated with DNA,[18] high voltage current pulses to make pores in the cell membrane,[19] and encapsulation of DNA into liposomes.[20]

Although, non-viral vector systems result in transient gene expression and lower transfer efficiency in comparison with viral vectors, these vector systems have some promising advantages of large DNA insert capacity, ease of production and safety to host.

CANCER GENE THERAPY STRATEGIES

Gene therapy for human cancer can be categorized into one of two entirely different therapeutic strategies for the transfer of treatment genes into target cells; corrective gene therapy and cytoreductive gene therapy.

Corrective gene therapy for cancer involves preventing or reversing pathophysiology of the cancer by insertion of a wild-type gene into preneoplastic or neoplastic cells. Cytoreductive gene therapy for cancer involves the therapeutic strategy that kills malignant cells by inducing tumor specific immune response or using recombinant DNA gene transfer *in vivo* like cytotoxic drugs.

Corrective gene therapy

With recent dramatic advances in molecular biology, our informations of cancer initiation and progression has been explosively expanded. Cancer is the disease of an accumulation of multiple genetic alterations. These genetic abnormalities include overexpression of oncogenes and functional loss of tumor suppressor genes. Ultimately, these alterations result in excessive cellular proliferation and cancer cell accumulation through loss of programmed cell death (apoptosis). Corrective gene therapy for cancer involves the replacement of normal tumor suppressor genes, such as p53, or using antisense complementary to oncogenes.

The inactivation of tumor suppressor genes may result in the initiation or progression of cancer.[21] Several tumor suppressor loci are inactivated during multistep genetic alterations in carcinogenesis. The best known tumor suppressor genes is p53 genes. p53 mutations are common in a wide spectrum of human malignancies.[22] p53 is 53 kD cellular protein localized on chromosome 17p13, and this regulates cellular responses to DNA damage, cell cycle progression and genomic stability.[23] Major functions of p53 include the G_1/S checkpoint to DNA damage and apoptosis induced by radiation or cytotoxic drugs.[23,24] Recent researches for roles of p53 in human carcinogenesis have revealed that wild-type p53 gene can suppress cell transformation and neoplastic cell growth.[25~28] It has been demonstrated that transient transfection of wild-type p53 can suppress the growth of human prostate cancer cell lines containing mutated p53 gene.[29] Thus, corrective gene therapy approach targeting p53 has been pursued enthusiastically by many research groups. Wild-type p53 gene replacement using a retroviral p53 expression vector can suppress the growth of both human lung cancer cells *in vitro* and xenograft mouse model containing mutant p53.[30,31] Wild-type p53 restoration with adenoviral vectors inhibits cell growth, induces apoptosis of prostate cancer cells *in vitro*, and inhibits tumor growth in nu/nu mouse xenograft model.[32] Ko et al demonstrated that overexpression of wild-type p53 using adenoviral vector inhibited cell growth *in vitro*, and intratumoral administration of wild-type p53 caused long lasting tumor necrosis in androgen-independent, metastatic tumors that express low level of p53 protein.[33] Asgari et al demonstrated the cell growth-inhibitory effects of adenovirus-mediated wild-type p53 in primary cultures of tumor from radical prostactomy specimen as well as the anti-tumorigenic effects after single injection of AdWT-p53 to pre-established subcutaneous tumors of nude mouse models.[34] Delivery of wild-type p53 into chemo-resistant tumor can induce synergistic tumor regression with anti-neoplastic agents.[35,36] In addition, the presence of a wild-type p53 gene is speculated to be useful in accelerating induction of apoptosis caused by cytotoxic agents like cisplatin.[36] These evidences suggest that gene therapy with p53 may benefit in synergistic manner with other conventional therapies. Ultimately, corrective gene therapy for wild-type p53 restoration could have a role as a potential modality to improve the therapeutic index of conventional cancer treatments.

The inactivation of Rb gene is also important in cancer progression.[37] Retrovirus-mediated introduction of wild-type Rb can suppress the tumorigenicity of DU145 cells

that have non-functional truncated Rb protein.[38,39] Thus, wild-type Rb transfection appears to be a potent candidate for corrective gene therapy of cancer.

In prostate cancer gene therapy, glutathione-S-transferase (GST) π gene is a very attractive target gene for corrective gene therapy. The promotor of the GST π gene is located on chromosome 11q13. Recent research for GST π gene showed that methylation of this region was detected in every one of 30 prostate cancer DNAs examined. On the other hand, no methylation was detected in any normal or hyperplastic prostate tissue.[40] This inactivation in prostate cancer is the most common genomic alteration and may occur as early as PIN.[40] GST π gene acts a key role of detoxifying potential carcinogens, thus the inactivation of GST π gene could result in increment of the susceptibility of prostate tissue to DNA damage and accumulation of mutation in the DNA of the stem cells of the prostate epithelium. Conceptually, the reintroduction of a GST π gene that detoxifies potential carcinogens to the prostate epithelial cells could serve as a cancer prevention strategy employing corrective gene therapy.

CAMs (cell adhesion molecules) can also be a candidate as a therapeutic gene for potential delivery *in vivo*; they have important roles in regulating cell growth and differentiation. E-cadherin protein levels have been found to be reduced or absent in cancers.[41] Inactivation of E-cadherin has a strong correlation with metastatic and/or invasive potential of cancer.[42] Importantly, loss of E-cadherin is a powerful predictor of poor outcomes in cancer. C-CAM acts as a tumor suppressor in cancer.[43] Adenoviral C-CAM transfection into a tumorigenic prostate cancer cell line (PC-3) appears to reduce growth rate *in vitro* and decrease tumor take rate and tumor growth *in vivo*.[44,45]

Antioncogene therapy includes complementary or antisense oligonucleotides to target oncogenes and ribozymes. By annealing antisense sequences to specific oncogenes, this strategy results in blocking the transcription or translation of oncogenes, thus eliminating expression of oncogenic proteins. Retroviral vector carrying antisense sequences to c-myc is being developed for intraprostatic injection to block prostate cancer growth.[46] The ras genes are potential targets for antisense strategy as they are frequently mutated oncogenes in human cancer development. Antisense DNA oligonucleotides to ras mRNA has been shown to block the production of ras mRNA and reduce the growth of human lung cancer *in vitro* and *in vivo*.[47~49] The bcl-2 gene is proto-oncogene which decreases the rate of cell death. Disruption of the process involving apoptosis is critical event resulting in cancer development and progression. Overexpression of bcl-2 induces inhibition of apoptosis in variable cancers.[50] Furthermore, overexpression of bcl-2 is correlated with poor outcome and resistance to treatment in several cancers.[51~53] A strategy to block bcl-2 expression induces facilitating of apoptosis. With immunohistochemical stainings, recent reports reveal that bcl-2 is higher in androgen-independent prostate cancer than in androgen-dependent prostate cancer.[52] Specific ribozyme against bcl-2 mRNA can be used for inducing apoptosis in androgen-independent prostate cancer.[53] Bcl-xs, functionally known as an inhibitor of bcl-2, enhances apoptotic signals in cells that express bcl-2. Adenoviral vector expressing bcl-xs selectively induces apoptosis in primary carcinoma cells and cell lines of solid tumors.[54]

Cytoreductive gene therapy

Tumor cell vaccines

Widely used protocol of the earliest trials for cancer gene therapy was the ex vivo introduction of cytokine genes into autologous tumor cells. These strategies aim the augmentation of anti-tumor immune response against malignancy by vaccinating cancer patients with genetically modified tumor cells as vaccines. Genetically modified tumor cell vaccines provide constant local secretion of immune stimulatory genes and stimulate anti-tumor immunity via transfected tumor.

An autologous tumor cell for immune manipulation can easily be obtained from patient's tumor at surgery. Immunestimulatory cytokine genes are introduced into

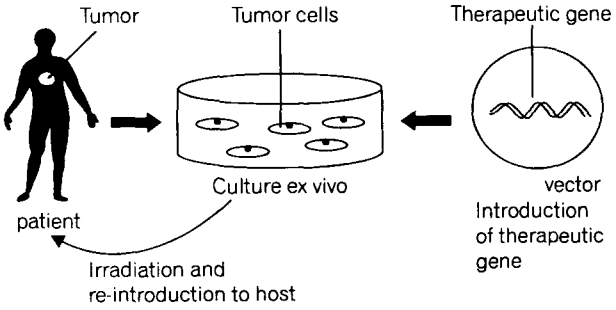

Fig. 1. Tumor cell vaccine therapy.

tumor cells ex vivo, and the tumor cells are irradiated to eliminate malignant activity and re-injected into the host (Fig. 1). Cytotoxic T cells recognize tumor-specific antigens on the transduced tumor cell surface. Immune effector cells including T-cells, B-cells, NK cells and antigen presenting cells are activated and destroy tumor cells.[55]

However, tumor cells for vaccine are not always available from patients, and even if available, the transduced cells may not be satisfactory to express the cytokine genes. Currently available alternative approaches use genetically engineered fibroblasts or allogenic tumor cells to elaborate the cytokine genes.

Suicide gene therapy

Suicide gene therapy is one strategy to transfect virus-directed enzyme selectively into tumor cells and systemically administer prodrugs (Fig. 2). A gene encoding a non-mammalian enzyme in tumor cells can convert a non-toxic prodrug into a potent toxic metabolite. Normal mammalian cells lack herpes simplex virus thymidine kinase (HSV-tk): This enzyme converts non-toxic ganciclovir (GCV) into phosphorylated compounds, ganciclovir triphosphate, that is toxic to cancer cells through its high affinity for DNA polymerase. Therefore, ganciclovir triphosphate can terminate DNA synthesis and finally kill cancer cells in S-phase of the cell cycle.[56] This approach is attractive with the "by-stander effect" which produces cell death of innocent nearby cells not transfected with an enzyme. The mechanisms of this unique antitumor effect by suicide gene therapy include transfer of toxic metabolites via gap junctions, endocytosis of apoptotic vesicles and up-regulation of an immune response. This bystander effect is able to amplify the low efficiency of actual gene transfer *in vivo* into measurable shrinkage of solid tumors in rodent models. Adenovirus-mediated HSV-tk gene in conjunction with GCV in prostate cancer has been shown to produce growth suppression *in vitro* and significant regression of tumor growth and of spontaneous metastasis in animal models.[57,58]

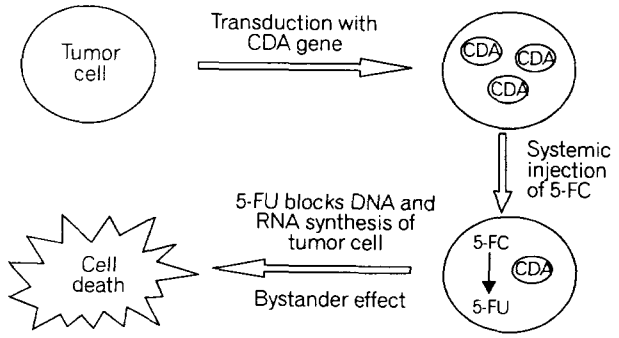

Fig. 2. Concept of suicide gene therapy.

Other suicide substrate genes, for example, cytosine deaminase (CDA) and purine nucleoside phosphorylase (PNP), are also currently being investigated both preclinically and clinically. CDA which is present in bacteria and fungi but not in mammalian cells, deaminase the non-toxic antifungal drug 5-fluorocytosine (5-FC) to toxic chemotherapeutic agent 5-fluorouracil (5-FU). 5-FU is a drug of choice for many human carcinomas. In addition, this virus-directed CDA/5-FC gene therapy produces a significant therapeutic efficacy because tumor cells expressing CDA produce 5-FU which diffuses into the non-transfecting neighboring cells.[59] 5-FU is membrane-permeant toxin, thus this toxic metabolite is readily diffusible from one cell to another. Recent report shows significant tumor regression *in vivo* in colorectal cancers when only 2% of the tumor mass contains CDA expressing cells.[59] PNP in eukaryotic cells converts the prodrug 6-methylpurine-deoxyriboside to membrane permeant toxin 6-methylpurine. This PNP/prodrug strategy also has a significant bystander effect.[60]

Unfortunately, HSV-tk, CDA and other cytotoxic prodrug activating enzyme genes may have limitations to some cancers for clinical application, since only a very small population of the cells are in the S-phase reflected by the fact that cancers are resistant to many S-phase dependent chemotherapeutic agents.

Other strategies

Drug resistance gene therapy for bone marrow rescue

P-glycoprotein, encoded by MDR1 gene, transport chemotherapeutic drugs to the outside of cells, using ATP energy as a cellular efflux pump. Thus, this protein is responsible for drug resistance of tumors to several potent chemotherapeutic agents (doxorubicin, actinomycin D, vinblastine, etoposide and taxol). Gene therapy aiming at transferring the MDR1 gene into bone marrow stem cells is to protect bone marrow during chemotherapy. This strategy allows to deliver higher doses of chemotherapeutic agents in the treatment of drug sensitive tumors

with less bone marrow toxicities. Human clinical trials are currently underway with transferring MDR1 gene into bone marrow cells to diminsh toxicity of chemotherpeutic agents.[61] However, some cancers are not chemo-sensitive tumors, therefore, higher dose of chemotherapeutic agents may not lead to higher response rate. Thus, this strategy of bone marrow rescue using MDR1 gene has an advantage only to apply to the treatment of chemo-sensitive cancers.

Antiangiogenesis gene therapy

Angiogenesis is essential for development and progression of malignancy. Several angiogenic activators for neovasculature of tumors include basic fibroblast growth factor (FGF), acidic FGF and vascular endothelial growth factor (VEGF). Recently, many studies show that angiogenesis inhibitors regress the tumor growth.[62~65] Prolonged delivery of angiogenesis-inhibitor, platelet factor 4, using retroviral and adenoviral vectors selectively inhibits endothelial cell proliferation *in vitro*, and results in hypovascular tumors *in vivo*.[66] In addition, this antiangiogenic strategy inhibits tumor angiogenesis and prolongs survival of animal models.

CONCLUSIONS

Cancer is the most common cause of death in Korea, however, the therapeutic outcome of recurrent or metastatic cancers has been disappointing in spite of advanced techniques of diagnosis and treatment. For the treatment of these merciless diseases, exploration of new and effective therapeutic strategies are imperative in order to overcome current obstacles of cancer therapies. Currently gene therapy as a novel strategy for cancer treatment is in the early stage of development. Even though a number of clinical trials of gene therapy as potential and promising therapeutic strategies for variable cancers are under evaluation, further efforts are the utmost importance to improve transduction efficacy and target specificity.

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