Cancer Gene Therapy: Chemosensitization by an Enzyme-Prodrug Activation Strategy

Injae Chung, Ph.D.
Duksung Women's University

Recent development of human genetics and techniques of gene transfer and expression have opened the way for investigating novel approaches based on the genetic modification of cells to treat both inherited and acquired diseases. This approach is referred to as gene therapy.

Over the past few years, gene therapy has moved from the laboratory to phase I clinical trials. Although the clinical performance of gene transfer experiments is still in an early phase of development, the NIH of Health Recombinant DNA Advisory Committee (RAC) has approved more than 150 protocols that involve gene transfer or putative gene therapy procedures in clinical settings. Many sectors of society in United States have participated in the design and formulation of these clinical trials through local Institutional Review Boards, the National Institutes of Health (NIH) RAC, the Chemotherapy Evaluation Program of the National Cancer institute, and the FDA. Currently, clinical trials involving gene modification are under way at many medical centers throughout the United States. The goals of these trials are as follows. (1) The design should be directed to short-term achievable goals. (2) Each clinical trial is best considered as an intermediate step in a multistep process. (3) The design should identify evaluable proximate endpoints for toxicity and for efficacy, (4) The potential benefits and possible risks for patients participating in these trial should be defined.

The delivery systems for gene correction process that have been approved by the NIH RAC include retrovirues, adenoviruese, liposomes, adeno-associated virus, herpes simplex virus type I, naked DNA and adenovirus coat protein. Retroviral vectors are the most widely used and most developed gene transfer systems and are used in more than 80% of the gene therapy clinical trials conducted to date.

Most trials involve gene transfer therapy in human cancer. A minority of RAC-approved trials focus on single gene deficiencies, of which more than 50% involve protocols to treat cystic fibrosis. A still negligible number of trials are devoted to the treatment of AIDS, vascular disease, or rhematoid disorders. Although in most cases the exact genes responsible for malignant transformation are not known, recent advances in the understanding of growth factors, molecular oncology, and tumor immunology have provided the rationale for several strategies for treatment of cancer: *genetic immunopotentiation, mutation compensation, chemoprotection, chemosensitization.*

A number of approaches based on immunopotentiation have been developed including (a) introduction of various cytokine genes into tumor cells with the intent of stimulating T-cell and natural killer cell proliferation and activity, (b) introduction of genes to augment MHC class I antigen presentation or direct introduction of foreign MHC class I molecules, (c) introduction of genes that provide

co-stimulatory signals to augment T-cell proliferation, and (d) use of agents that inhibit the production of suppressive factors. As the molecular basis of malignancies becomes more clear, it may be possible to specifically correct or alter the oncogenes (e.g. K-ras, ErbB2) or tumor suppressor genes (e.g. p53) that contribute to the malignant phenotype. Investigators have approached some malignancies by the introduction of genes that may protect normal tissues from chemotherapy or radiation. Examples include transfer of multidrug resistance (MDR-1) gene to bone marrow stem cells. One promising approach to the treatment of cancer is the use of an enzyme-prodrug activation system, in which a gene that encodes an enzyme is transferred to tumor cells. The enzyme then converts a normally nontoxic agent into a toxic substance, which eradicates the tumor cells.

Ovarian cancer, when it is found to be recurrent following initial surgical resection and subsequent conventional dose salvage chemotherapy, is ultimate predictive of an 80 % mortality rate. Simple escalation of chemotherapy to dose ranges which require exogenous hematopoietic reconstitution, has not yet been associated with a curable benefit to the patients. Similarly, delivery of chemotherapy directly into the intraperitoneal space has not resulted in routine eradication of peritoneal implants of ovarian cancer.

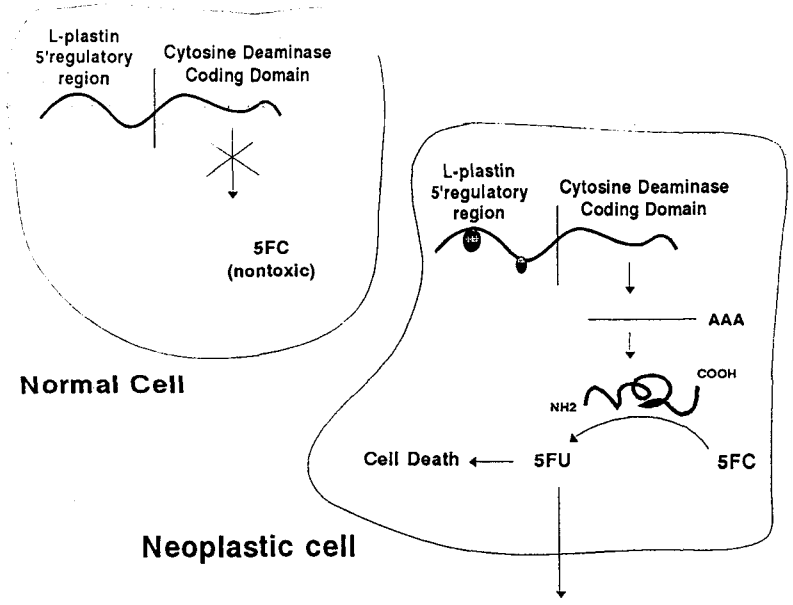
Adenoviral vectors have become the most widely used vector in the field of gene therapy. However, one of the limitations of this vector system for cancer gene therapy may be its broad cellular host range, which results in infection of both the tumor cells as well as of surrounding normal cells. One way to circumvent this limitation would be the use of a tumor or tissue-specific promoter which is active in the target tumor cells but not in the normal cells. Comparative examination studies in the laboratory of John Leavitt of protein synthesis in normal versus neoplastic human fibroblasts led to the discovery of L-plastin, a gene which codes for an actin binding protein, which is expressed at high levels in human epithelial cancer cells but not in normal cells. L-plastin was found in these studies to be a marker expressed at high levels in the majority of human cancer cells of nonhematopoietic origin. A survey of SV-40-transformed human fibroblasts and human sarcoma cell lines as well as human carcinoma cell has demonstrated that the L-plastin gene was transcriptionally activate, although at widely varying degrees, in nearly all human cancer cells tested. In particular, high levels of L-plastin synthesis are found in the neoplastic cells of female reproductive organs. In addition, the L-plastin gene appears to be transcriptionally regulated through the 5.1 kb of the 5-regulatory region. Based on these findings, we proposed to use the L-plastin promoter in an adenoviral vector to restrict the expression of therapeutic gene to the L-plastin positive carcinoma cells, so as to avoid the expression in normal cells.

In an attempt to establish a new direction for the therapy of ovarian cancer, we have proposed the use of an adenoviral vector which carries the cytosine deaminase prodrug activation transcription unit driven by the L-plastin transcription promoter. We first gnerated a replication-deficient adenoviral vector, Ad.LP.LacZ. Ad.LP.LacZ is an adenoviral type 5-based vector from which the E1 and E3 genes have been deleted. Ad.LP.LacZ vector contains the human L-plastin promoter and the *E.*

coli LacZ gene which codes for -galactosidase in E1 region.

We demonstrated *in vitro* that the 2.4 kb of L-plastin promoter could direct a heterologous gene expression preferentially in neoplastic cell lines, and that the level of promoter activity is compatible strength of transcriptional activity of the CMV promoter in neoplastic epithelial cell lines. This indicates that the breast and ovarian cancer cell lines are infectable by the adenoviral vectors and also support the activity of the L-plastin promoter at a high level. In contrast, when established cell lines of hematopoietic origin were exposed to the vectors, neither of the vector transgenes were active in the cell. This indicated that the cell lines were not infectable by the adenoviral vectors. This data is coincident with studies of hematopoietic cell sat early stages of differentiation. Thus, the pattern of expression of the L-plastin promoter driven adenoviral vector transcription units depend both on the infectability of the cells by the adenoviral vectors, as well as the activity of the promoter within the regulatory environment of the cells.

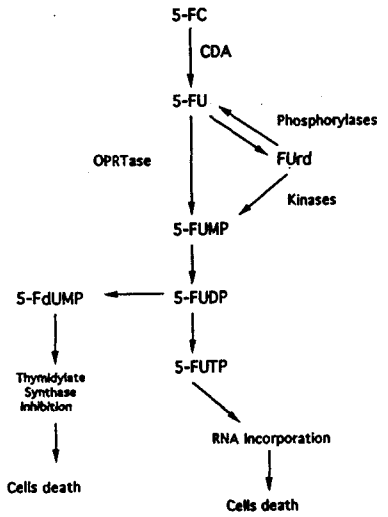
Interestingly, when primary normal mesothelial cells, obtained as an incidental part of therapeutically indicated surgical procedures, were exposed to adenoviral vectors carrying transcription units driven by either the CMV or the L-plastin promoters, the cells were positive for the LacZ transgene activity when the transcription unit was driven by the CMV promoter but not when the LacZ gene was under the control of the L-plastin promoter. This suggests that adenoviral vectors carrying therapeutic genes, such as the cytosine deaminase, a pro-drug activation gene, could sensitize the cancer cells but not the normal mesothelial cells to the effects of 5-fluorocytosine chemotherapy (Figure 1).



Cytosine deaminase is an enzyme present in some bacteria and fungi but

absent in animal cells. This enzyme catalyzes the demination of cytosine to uracil as well as the convertion of innocuous prodrug, 5-fluorocytosine (5FC), into the cytotoxic agent, 5-fluorouracil (5FU). If the intracellular concentrations of 5FU are sufficiently high and the conversion of 5FU into phosphorylated 5FU is sufficiently fast, then cycle independent cell death will result from incorporation of the 5FU into RNA, which disrupts the production of functional protein (Figure 2).

INTERCONVERSION OF 5-FC INTO 5-FU AND ITS METABOLITES AND INDUCTION OF CELL DEATH



We generated an adenovirus vector (Ad.LP.CD) carrying the *E. coli* cytosine deaminase gene driven by the human L-plastin promotor. Infection of ovarian adenocarcinoma cells, i.e., OVCAR3 and SKOV3, by Ad.LP.CD at M.O.I. (multiplicity of infection) of 100 have resulted in 55 % and 12 % of conversion rate of 5FC to 5FU respectively. Subsequently, infection of Ad.LP.CD vector efficiently suppressed OVCAR3 cell growth *in vitro* in the presence of 5FC (95-100% of growth inhibition). These results implicate that the L-plastin promoter could be used to drive the expression of adenoviral pro-drug activation transcription units for intraperitoneal therapy of ovarian cancer when present at the microscopic level. We are currently under the way to evaluate the efficacy of Ad.LP.CD in tumor xenograft in nude mice.

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