The Journal of Applied Pharmacology, 13, 143-149(2005)

Chemosensitization of Human Ovarian Carcinoma Cells by a Recombinant Adenoviral Vector Containing L-plastin Promoter Fused to Cytosine Deaminase Transcription Unit

Injae CHUNG*

College of Pharmacy, Duksung Women's University, Seoul, Korea

(Received July 26, 2005; Accepted August 19, 2005)

Abstract – We have demonstrated previously on a replication incompetent recombinant adenoviral vector, AdLPCD, in which the expression of cytosine deaminase (CD) gene is driven by the tumor-specific L-plastin promoter. The object of this study was to evaluate the efficacy of AdLPCD together with 5-fluorocytosine (5-FC) in suppression of the growth of established human tumor cells of ovary. Consistent with the knowledge that infection of OVCAR-3 cells with AdLPCD resulted in expression of a functional intracellular CD enzyme capable of converting 5-FC to 5-fluorouracil (5-FU) (Chung and Deisseroth, 2004), statistically significant differences in cytotoxicity were observed when AdLPCD infected cells were also exposed to 5-FC for 6 days ($p=0.05$), 9 days ($p<0.0005$) and 12 days ($p<0.005$), compared to 5-FC exposure alone. These results indicate that the CD gene delivered by adenoviral vector could efficiently sensitize OVCAR-3, otherwise non-toxic 5-FC. On the other hand, SKOV-3 cells, an ovarian carcinoma cell line, were more resistant to the CD/5-FC strategy compared with OVCAR-3 cells under the same condition. The results of present study suggest that the replacement of 5-FU with CD/5-FC in combination chemotherapy would be less toxic and much greater cytotoxicity than the conventional combination chemotherapy in some patients.

Keywords □ L-plastin promoter, tumor-specific promoter, adenoviral vector, gene therapy, ovarian cancer, cytosine deaminase

INTRODUCTION

The infection of normal as well as tumor cells by the adenoviral vector has represented a disadvantage for adenoviral vectors, since the expression of the therapeutic transgenes in the normal cells generates toxicity. In an attempt to direct the transgene expression in a tumor cell-specific way, we have proposed to use an adenoviral vector system that carries the L-plastin promoter in a transcription. Therefore, the [1]AdLP- vector system carrying a prodrug activation chemotherapy sensitization transcription unit may have significant utility in the chemosensitization of the ovarian cancer cells present as implants on the peritoneal surface or as ascites to intraperitoneal chemotherapy, because the L-plastin promoter was very active in ovarian cancer cell lines and in fresh ascitic ovarian cancer cells but was not active in cell lines of mesodermal origin or in normal peritoneal mesothelial cells unit (Chung *et al.*, 1999).

Most of the existing chemotherapy regimens for advanced carcinoma of the ovarian include 5-fluorouracil (5-FU) as a basic component. When 5-FU is administered systemically at the maximal doses with safety, it is usually considered to be toxic only for proliferating cells due to incorporation of the 5-FU into DNA and binding of the 5-FU to thymidylate synthase (Armstrong *et al.*, 1986). The failure of existing 5-FU based chemotherapy in most of advanced cancer patients may be due in part to the fact that less than 10% of epithelial origin cancer cells are proliferating at any given time. If it were possible to safely increase the levels of 5-FU to those at which RNA is sufficiently substituted with 5-FU in order to suppress protein synthesis (Armstrong *et al.*, 1986), then one could kill all cancer cells regardless of the status of cell proliferation. However, the dose of systemically administered 5-FU that would be needed to prevent protein synthesis would generate unacceptable levels of toxicity to the normal cells of the bone marrow and gastrointestinal tract.

The *E. coli* or yeast cytosine deaminase (CD) gene (Ander-

*Corresponding author
Tel: 02-901-8522, Fax: 02-901-8386
E-mail: ijchung@duksung.ac.kr

son *et al.*, 1989; Moolten, 1994) converts the harmless drug, 5-fluorocytosine (5-FC), into the cytotoxic agent, 5-FU (Austin and Huber, 1993; Huber *et al.*, 1993). It has been reported that the levels of 5-FU which are generated by the CD/5-FC system within tumor cells is much higher than those possible when 5-FU is systemically administered (*e. g.*, Garcia-Sanchez *et al.*, 1998).

The L-plastin promoter (Lin *et al.*, 1993a, 1993b; Leavitt, 1994) has been shown to drive the expression of reporter gene, when delivered as a recombinant adenoviral vector, in tumor cells but not in normal cells (Chung *et al.*, 1999). In regard of ovarian cancer, an adenoviral vector with a non-specific cytomegalovirus (CMV) promoter expressed the *LacZ* gene in ascitic ovarian cancer cells as well as in normal peritoneal mesothelial cells. However, an adenoviral vector with the L-plastin promoter (*i.e.*, AdLPLacZ) has directed the expression of *LacZ* gene in ovarian cancer cells but not in the normal mesothelial cells obtained from a single patient at the time of a therapeutically indicated surgical procedure (Chung *et al.*, 1999).

Based on these findings we have hypothesized that use of L-plastin promoter as a transcription unit of adenoviral vector would render the transcription of CD gene to be restricted to L-plastin-positve ovarian carcinoma cells. It would not be expressed in normal cells including cells belonging to the hematopoietic system due to the inefficient transduction of these cells by the adenoviral vector. Therefore, only ovarian carcinoma cells expressing the CD will catalyze the metabolic activation of 5-FC to 5-FU intracellulary, which would then be further metabolized by cell enzymes, ultimately leading to suppression of cells growth, and cell death. Subsequently, a replication-incompetent adenoviral vector in which CD is driven by the L-plastin promoter (AdLPCD) has been generated. Infection of 293 cells by AdLPCD produced the functional CD protein as measured by HPLC analysis for the conversion of 5-FC to 5-FU. HPLC analysis in conjunction with counting radioactivity for [6-3H]-5FC and [6-3H]-5FU demonstrated vector dose-dependent conversion of 5-FC to 5-FU in AdLPCD infected ovarian cancer cells (Chung and Deisseroth, 2004).

The present study is directed to evaluate the efficacy of AdLPCD vector together with 5-FC in suppression of the growth of established human tumor cells of ovary. The results of present and previous studies (Chung *et al.*, 1999; Peng *et al.*, 2001; Akbulut *et al.*, 2003; Chung and Deisseroth, 2004) suggest that the use of the AdLPCD/5-FC system may be of value in the treatment of microscopic ovarian cancer in the peritoneal cavity in some patients.

MATERIALS AND METHODS

Adenoviral vectors

AdLPCD (Chung and Deisseroth, 2004) is an E1a-, partial E1b-, partial E3-, linear double stranded adenovirus vector based on the Ad5 genome. The AdLPCD vector contains the expression cassette consisting of 2.3 kb of the human L-plastin promoter (Chung *et al.*, 1999) and the *E. coli* CD gene in E1 region. AdCMVCD (generous gift of Dr. Crystal at Cornell Medical Center, U.S.A.) is as same as AdLPCD except for cytomegalovirus (CMV) promoter instead of L-plastin promoter in the expression cassette.

The replication-incompetent adenoviral vectors, AdLPCD (Chung and Deisseroth, 2004), AdLPLacZ (Chung *et al.*, 1999) and AdCMVCD were propagated in 293 cells and recovered 36 hr after infection by five cycles of freezing/thawing of the infected cells. All viral preparations were purified by CsCl density centrifugation, dialyzed, and stored in dialysis buffer (10 mM Tris-HCl, pH 7.4, 1 mM $MgCl_2$) at -70°C prior to use. Titers of the viral stocks were determined by plaque assay using 293 cells by standard methods (Graham and Prevec, 1991). The titer of vector was in the range between 2 X 10^{10} and 1 X 10^{11} pfu/ml. The organization of AdLPCD and AdLPLacZ recombinant adenoviral vectors are shown in Figure 1.

Cell culture

The cells were maintained in IMEM (Biofluids, Rockville,

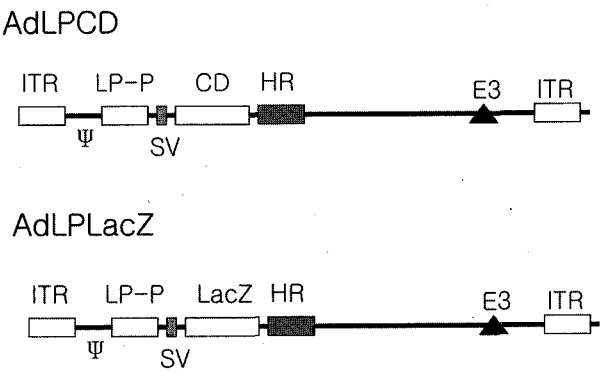

Fig. 1. The map of replication-incompetent adenoviral vectors used in the study. In the AdLPCD vector, the CD gene is driven by the 2.4 kb of L-plastin promoter. AdLPLacZ vector contains *E. coli* β-galactosidase gene under the control of L-plastin promoter. ITR, adenoviral inverted terminal repeat; Ψ, packaging signal; LP-P, L-plastin promoter; SV, SV40 splice donor/acceptor site; HR, homologous in sequence to those present in the pBHG10 adenoviral vector plasmid; E3, early transcription unit 3.

MD) supplemented with 10 % fetal bovine serum (FBS) and 2 mM glutamine (in the case of the 293 cell line), in DEME/F12 (GIBCO BRL) supplemented with 10 % FBS (in the case of OVCAR-3 and SKOV-3).

Chemosensitization assays

Cells were plated in triplicate monolayer a day before the infection. Cells were infected with 100 multiplicity of infection (MOI) of AdLPCD vector in medium containing 2% FBS for 90 min, followed by the addition of 5-FC at 100 μM. Controls included 5-FC (100 μM) alone, AdLPCD alone, or no treatment. Cells were further maintained in culture for various times. The half of media were removed and replaced with complete fresh media containing 100 μM 5-FC on every 3 days dung cell culturing. Viable cell number was assessed using 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT). The data are expressed as percent control of no treatment cells at each time point.

Statistical analysis

Significance levels for comparison between treatment groups were determined using the unpaired two-tailed Student's *t* test.

RESULTS

Cytotoxic effect of AdLPCD/5-FC strategy on OVCAR-3 cells

Adenovirus transduction of ovarian carcinoma cells such as SKOV-3, PA-1, and OVCAR-3 *in vitro* has been tested using AdLPLacZ previously. As demonstrated by X-gal staining or flow cytometry analysis of β-galactosidase activity, AdLPLacZ was able to transduce these cells and to express LacZ gene with varied efficiencies (Chung *et al.*, 1999). Furthermore, the expression of the CD gene in OVCAR-3 cells after infection of AdLPCD vector was demonstrated by evaluating culture media for the conversion of [6-^{3}H]-5-FC to [6-^{3}H]-5-FU in a dose-dependent manner. There was a 31 % and 54 % conversion of 5-FC to 5-FU at a MOI of 10 and 100, respectively, increasing to 55 % conversion at a MOI 250 (Chung and Deisseroth, 2004). Previous study (Chung *et al.*, 1999) also have shown that the L-plastin promoter was active in ovarian cancer cell line as well as in fresh ascitic ovarian cancer cells, but inactive in samples of normal peritoneum. These observations suggested that the AdLPCD vector combined with 5-FC administration may have significant utility in the chemosensitization of ovarian cancer carcinoma which have metastasized into the intraperitoneal cavity.

To accomplish to this therapeutic goal, we determined whether introduction of the *E. coli* cytosine deaminase gene would render OVCAR-3 ovarian carcinoma cells susceptible to killing by 5-FC. After exposure of cells to AdLPCD viral vector for 90 min at MOI of 100, 5-FC was introduced at the concentration of 100 μM. The MOI of 100 of AdLPCD had resulted in the most effective conversion of 5-FC to 5-FU in previous study. Cytotoxic effects were evaluated by MTT test at day 6 after initiation of AdLPCD/5-FC treatment. The results are shown in Table I. As shown in Table I, consistent with the knowledge that infection of the OVCAR-3 cells with AdLPCD resulted in expression of a functional intracellular CD enzyme capable of converting 5-FC to 5-FU, the growth of OVCAR-3 cells which has been infected with AdLPCD and exposed to 5-FC was suppressed when compared to all treatments ($p<0.05$). Whereas, no such synergistic cytotoxic effect was observed in the cells which have been infected with AdLPLacZ vector and exposed to 5-FC. This indicates that cytotoxic effects did arise from generation of active metabolite, 5-FU, which have been enforced by expression of bacterial CD in AdLPCD-transduced cells.

To quantify growth of the OVCAR-3 cells in the presence or in the absence of AdLPCD, the cells were plated in monolayers, infected with AdLPCD (MOI 100), and exposed to the 5-FC at 100 μM. The cytotoxicity was determined by MTT test on 3, 6, 9, and 12 days after initiation of infection (Fig. 2). While 5-FC treatment had little impact on cell growth during the time course, the introduction of AdLPCD had resulted in time-dependent inhibition of cell growth, *i. e.*, approximately

Table I. Study of the cytotoxicity of the CD vector versus the control LacZ vector in OVCAR-3 cells.

Treatment	Relative cell survival (%)
Control	100 ± 15.9
5-FC	104 ± 4.1
AdLPCD	45 ± 13.8
AdLPCD + 5-FC	18 ± 0.9[a]
AdLPLacZ	57 ± 3.8
AdLPLacZ + 5-FC	52 ± 3.8

Cells (4×10^4 cells/well on 24 well plate) were infected by either 100 MOI of AdLPCD or 100 MOI of AdLPLacZ for 90 min. Another set of the experiments were set up and following infection with the same vector were incubated in 0.5 ml of media containing 100 μM 5-FC for 6 days. The percentage of surviving cells was measured by MTT test. Value represent the mean ± SD. [a]$P<0.05$ compared with the percentage of cell survival treated with AdLPCD vector only.

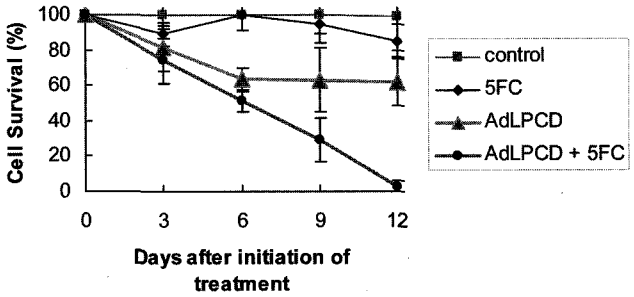

Fig. 2. Chemosensitzation OVCAR-3 cells to 5-FC by the infection AdLPCD vector. A day after plating the cells (8 X 10^4 cells/well) on 24 well plate, 100 MOI of AdLPCD vector were introduced for 90 min followed by the addition of 1 ml of media containing 100 μM 5-FC. On every 3 days, half of media were replaced with fresh media containing 100 μM 5-FC. The percentage of surviving cells was measured by MTT test. Statistically significant differences observed; when AdLPCD infected cells were also exposed to 5-FC at day 9 ($p<0.0005$) and day 12 ($p<0.005$), compared to 5-FC alone.

70% and 97% inhibition of cell growth at day 9 and 12, respectively, when compared with the cells which had never been exposed neither 5-FC nor AdLPCD. At 12 days after initiation of AdLPCD infection followed by a prodrug administration, hardly any cells were remained. Statistically significant differences of cytotoxic effects were observed when AdLPCD infected cells were also exposed to 5-FC at 6 day ($p=0.05$), at 9 day ($p<0.0005$) and day 12 ($p<0.005$), compared with 5-FC alone. These results indicate that the CD gene delivered by adenoviral vector could efficiently sensitize an ovarian cancer cell, OVCAR-3, to otherwise non-toxic prodrug 5-FC.

Cytotoxic effect of AdLPCD/5-FC strategy on SKOV-3 cells

On the other hand, SKOV-3 cells, an ovarian carcinoma cell line, were more resistant to the CD/5-FC strategy compared with OVCAR-3 cells under the same condition (Table II). No statistically significant differences in synergistic cytotoxic effect were seen when the cell survival rate of AdLPCD/5-FC treatment was compared with those of either AdLPCD vector treatment or 5-FC treatment only at day 6 and 9.

This observation had led us to test another vector, AdCMVCD, which is as same as AdLPCD except for a nonspecific strong CMV instead of L-plastin promoter to drive CD expression, in SKOV-3 cells. As shown in Table III, at least, there was a statistically significant inhibition of cell growth in cells which have been infected with AdCMVCD and exposed to 5-FC for 9 days when compared with cells treated with AdCMVCD vector only ($p<0.05$), indicating that SKOV-3 cells have some

Table II. Chemosensitization of SKOV-3 cells to 5-FC after infection of AdLPCD vector.

Days after treatment	Treatment	Relative cell survival (%)
6 Days	Control	100 ± 7.6
	5-FC	85 ± 8.1
	AdLPCD	88 ± 2.2
	AdLPCD + 5-FC	81 ± 7.7
9 Days	Control	100 ± 15.7
	5-FC	77 ± 10.5
	AdLPCD	79 ± 14.5
	AdLPCD + 5-FC	74 ± 11.1

A day after plating the cells (2×10^4 cells/well) on 24 well plate, 100 MOI of AdLPCD vector were introduced for 90 min followed by the addition of 0.5 ml of media containing 100 μM 5-FC. On every 3 days, media were replaced with fresh media containing 5-FC. The percentage of surviving cells was measured by MTT test. Value represent the mean ± SD.

Table III. Chemosensitization of SKOV-3 cells to 5-FC after infection of AdCMVCD vector

Days after treatment	Treatment	Relative cell survival (%)
6 Days	Control	100 ± 5.0
	5-FC	82 ± 4.2
	AdCMVCD	106 ± 21.9
	AdCMVCD + 5-FC	86 ± 6.4
9 Days	Control	100 ± 14.8
	5-FC	85 ± 9.8
	AdCMVCD	99 ± 7.2
	AdCMVCD + 5-FC	71 ± 5.8[a]

A day after plating the cells (2×10^4 cells/well) on 24 well plate, 100 MOI of AdCMVCD vector were introduced for 90 min followed by the addition of 0.5 ml of media containing 100 μM 5-FC. The percentage of surviving cells was measured by MTT test. Value represent the mean ± SD.
[a]$P<0.05$ compared with the percentage of cell survival treated with AdCMVCD vector only.

degree of sensitivity to cytotoxin 5-FU. However, almost 70 % of SKOV-3 cells with AdCMVCD/5-FC were still survived by day 9. On the other hand, the infection of L-plastin promoter-driven CD adenoviral vector (AdLPCD) combined with 5-FC have resulted in 30 % of OVCAR-3 cell survival at day 9 (see Fig. 2).

DISCUSSION

Ovarian cancer, when it is found to be recurrent following initial surgical resection and subsequent conventional dose salvage chemotherapy, is 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. Similarly, delivery of chemotherapy directly into the intraperitoneal space has not resulted in eradication of peritoneal implants of ovarian cancer (Cannistra, 1995). Therefore, new chemotherapy drugs or combinations of chemotherapy with other modalities are needed to prolong the survival of patients with advanced stages of ovarian cancer. There have been many attempts to combine recombinant biologicals with chemotherapy. For an example, Akbulut *et al.*, (2004) compared the *in vivo* efficacy of the genetic combination therapy with the conventional combination chemotherapy with Ad-LpCDIRESE1A vector. Ad-LpCDIRESE1A vector is conditionally replication competent adenoviral vector that carries the L-plastin promoter-driven transcription unit encoding the CD gene linked to the E1A gene by an internal ribosomal entry site element. Because of the tumor-specificity of L-plastin promoter, Ad-LpCDIRESE1A vector replicates only in tumor cells. The replacement of 5-FU in 5-FU-based combination chemotherapy with the Ad-LpCDIRESE1A/5-FC reduced toxicity and increased efficacy of chemotherapy in a mouse colon cancer model.

5-FU has been the one of major chemotherapeutic agent in the adjuvant therapy of ovarian carcinoma. The main mechanisms of action of 5-FU include inhibition of thymidylate synthase resulting in a rise of DNA damage, and incorporation into DNA as a false nucleotide. However, additional way to cause cell growth inhibition could be achieved if the intracellular concentrations of 5-FU are sufficiently high and the conversion of 5-FU into phosphorylated 5-FU is sufficiently fast, which is cell cycle independent cell death by incorporation of the 5-FU into RNA (Grazer and Lloyd, 1982; Kufe and Major, 1981; Pritchard *et al.*, 1997). Previous studies suggested that the higher concentration of 5-FU could be obtained by the use of the adenoviral vector carrying the CD gene/5-FC system on various tumor cell lines and *in vivo* models (Hirschowitz *et al.*, 1995; Kievit *et al.*, 2000). However, the infectivity of normal as well as tumor cells by the adenoviral vectors has represented a disadvantage for adenoviral vectors, since the expression of the therapeutic trangenes in the normal cells generates toxic side effects. We have shown that it is possible to use of L-plastin promoter for the regulated expression of an adenoviral exogenous gene in a tumor cell specific manner and spare the normal cells of the body, resulting in the increase of therapeutic index (Chung *et al.*, 1999; Peng *et al.*, 2001; Zhang *et al.*, 2002; Akbulut *et al.*, 2003; Chung and Deisseroth, 2004). Among L-plastin promoter containing recombinant adenoviral vectors, AdLPCD/5-FC system (Chung and Deisseroth, 2004) has been tested in ovary, breast and colon carcinoma so far and shown effective chemosensitization with different extents (Peng *et al.*, 2001; Zhang *et al.*, 2002; Akbulut *et al.*, 2003). Therefore, AdLPCD viral vector has been suggested to increase 5-FU within tumor cells that are not possible to achieve using systemic administration of 5-FU chemotherapy, thereby increasing therapeutic outcome.

In this study, AdLPCD vector has been evaluated for its efficacy of chemosensitization of ovarian cancer cells to 5-FC. In spite of the fact that OVCAR-3 and SKOV-3 cell lines are capable for adenoviral transduction judged by LacZ reporter gene analysis, two cell lines demonstrated quite different sensitivities toward ADLPCD/5-FC system. In OVCAR-3 cells, at 6 days after infection of AdLPCD followed by exposure to 5-FC resulted in the suppression of cell growth with statistical significance ($p<0.05$). In addition, we also have shown that synergistic cell death seen in Table I is not attributable to the adenoviral backbone but to the action of the CD protein and 5-FC. The presence of AdLPCD started to cause OVCAR-3 cells be sensitive enough to 5-FC, thereby a statistically significant time-dependent growth suppression to occur from day 6 after initiation of treatment, reaching at day 12 when hardly any cells were remained (Fig. 2). These data indicated that the CD gene delivered by adenoviral vector could efficiently sensitize an ovarian carcinoma, OVCAR-3 cells to otherwise non toxic prodrug 5-FC. On the other hand, SKOV-3 cells, an ovarian carcinoma cell line, were more resistant to the CD/5-FC strategy compared with OVCAR-3 cells under the same condition (Table II). No statistically significant differences in synergistic cytopatic effect were seen when the cell survival rate of AdLPCD/5-FC treatment was compared with those of either AdLPCD vector treatment or 5-FC treatment at day 6 and 9. The higher level of cell killing in SKOV-3 cells has been reported when no media changes were conducted after initiation of infection with AdLPCD vector and exposure to 5-FC (Peng *et al.*, 2001). However, as explained, the situation of continuous exposure to 5-FU would probably not be seen *in vivo* because of the removal of 5-FU by blood flow and the metabolic degradation. Thus, the current experiment with replacement of half of media containing 5-FC on every 3 days is more likely reflecting *in vivo* condition.

The presence of different sensitivities to AdLPCD/5-FC system between OVCAR-3 and SKOV-3 cell lines could be attributable to differences in the infectivity of cell lines, the sensitivity of cell lines to 5-FU, the expression level of endog-

enous L-plastin transcripts, or the expression level of the L-plastin promoter-driven CD transcription units in the OVCAR-3 or SKOV-3, etc. At least, the higher transduction efficiency of AdLPLacZ vector to OVCAR-3 cells than SKOV-3 cells was observed in previous study, comparing the expression of LacZ gene in 30-95 % of OVCAR-3 cells *versus* 20-70% of SKOV-3 cells measured by FACS-gal analysis (Chung *et al.*, 1999). Based on the detection of Lacz gene expression after infection with AdLPLacZ vector, we assumed that the endogenous L-plastin gene expression was occuring in both cell lines. However, it would be very interesting to check the levels of endogenous L-plastin transcripts in both cell lines to see if direct correlation with the efficacy of AdLPCD/5-FC exits. Two steps necessary for the transduction of adenoviral vector are proposed and the proteins responsible for these steps are identified. The attachment of fiber proteins of adenovirus to CAR (coxakivirus and adenovirus receptor) on target cells (Bergelson *et al.*, 1997) followed by the internalization of virus, which is mediated by interactions between penton bases of adenovirus and subsets of integrin such as αvβ3 and αvβ5 on cell surface (Wickham *et al.*, 1993). In addition, recent findings suggested more significant involvement of αvβ5 integrin in the process of adenovirus interalization to the cells (Wickahm *et al.*, 1994; Goldman and Wilson, 1995). The level of intrinsic sensitivity of tumor to active compound would be a factor, which determines an outcome of enzyme-prod rug activation strategy. Among the molecules, which have been searched for relating to 5-FU sensitivity, the status of p53 was found to be the case in many cancer cell types (Pritchard *et al.*, 1997; Higashiyama *et al.*, 1998; Lenz *et al.*, 1998). Some of enzyme/prodrug strategies require additional endogenous enzymatic modification to make once modified prodrug be final active species. After phosphorylation of GCV mediated by herpes simplex virus (HSV)-thymidine kinase, cellular kinases add second and third phosphoryl group to the drug. The availability of cellular enzymes, which are involved in multiple step of drug modification, would therefore determine the extent of achievement of prodrug activation strategy. Finally, the extent of bystander effects also affect to the efficacy of enzyme prodrug activation strategy.

A similar result, that is inefficient chemosensitization by AdLPCD/5-FC system in human hepatocellular carcinoma HepG2 cells, was observed in our laboratory. HepG2 cells also exhibited LacZ expression by the infection of AdLPLacZ viral vector and contains endogenous L-plastin transcript (data not shown). Combined with present data suggest that the selection of potential patient based on the screening results of AdLPLacZ could result in the failure of therapeutic outcome. Therefore, we are currently undergoing studies to investigate factors that would determine the sensitivity to AdLPCD/5-FC treatment.

The results of these experiments suggest that the replacement of 5FU with CD/5FC in combination chemotherapy would be less toxic and much greater cytotoxicity than the conventional combination chemotherapy in some ovarian cancer patients.

ACKNOWLEDGMENTS

This study was supported by the grant of Duksung Women's University (2004).

REFERENCES

Akbulut, H., Zhang, L., Tang, Y., and Deisseroth, A. (2003). Cytotoxic effect of replication-competent adenoviral vectors carrying L-plastin promoter regulated E1A and cytosine deaminase genes in cancers of the breast, ovary and colon. *Cancer Gene Ther.* **10**, 388-395.

Akbulut, H., Tang, Y., Maynard, J., Zhang, L., Pizzorno, G., and Deisseroth, A. (2004). Vector targeting makes 5-fluorouracil chemotherapy less toxic and more effective in animal models of epithelial neoplasms. *Clin. Cancer Res.* **10,** 7738-7746.

Anderson, L., Kilstrup, M., and Neuhard, J. (1989). Pyrimidine, purine and nitrogen conrol of cytosine deaminase synthesis in Escherichia coli K12. Involvement of the glnG and purR genes in the resgulation of codA expression. *Arch. Microbiol.* **152,** 115-118.

Armstrong, R. D., Lewis, M., Stern, S. G., and Cadman, E. C. (1986). Acute effect of 5-fluorouracil on cytoplasmic and nuclear dihydrofolate reductase messenger RNA metabolism. *J. Biol. Chem.* **261**, 7366-7371.

Austin, E. A., and Huber, B. E. (1993). A first step in the development of gene therapy for colorectal carcinoma: cloning, sequencing, and expression of E. coli cytosine deaminse. *Mol. Pharmacol.* **43**, 380-387.

Bergelson, J. M., Cunningham, J. A., Droguett, G., Kurt-Jones, E. A., Krithivas, A., Hong, J. S., Horwitz, M. S., Crowell, R. L., and Finberg, R. W. (1997). Isolation of a common receptor for cosackie B viruses and adenoviruses 2 and 5. *Science.* **275,** 1320-1323.

Cannistra, S. A. (1995). Cancer of the ovary. *N. Engl. J. Med.* **329**, 1550-1559.

Chung, I., Schwartz, P. E., Crystal, R. G., Pizzorno, G., Leavitt, J. L., and Deisseroth, A. B. (1999). Use of L-plastin promoter to develop adenoviral system which confers transgene expression in ovarian cancer cells but not in normal mesothelial cells. *Cancer Gene Ther.* **6**, 99-106.

Chung, I., and Deisseroth, A. B. (2004). Recombinant adenoviral vector containing tumor-specific L-plastin promoter fused to cytosine deaminase gene as a transcription unit: generation and functional test. *Arch. Pharm. Res.* **27**, 633-639.

Garcia-Sanchez, F., Pizzorno, G., Fu, S. Q., Nanakorn, T., Krause, D. S., Liang, J., Adams, E., Leffert, J. J., Yin, L. H., Cooperberg, M. R., Hanania, E., Wang, W. .L., Won, J. H., Peng, X. Y., Cote, R., Brown, R., Burtness, B., Giles, R., Crystal, R., and Deisseroth, A. B. (1998). Cytosine deaminase adenoviral vector and 5-fluorocytosine selectively reduce breast cancer cells 1 million-fold when they contaminate hematopoietic cells: a potential purging method for autologous transplantation. *Blood* **92**, 672-682.

Glazer, R. I., and Lloyd, L. S. (1982). Association of cell lethality with incorporation of 5-fluorouracil and 5-fluoridine into nuclear RNA in human colon carcinoma cells in culture. *Mol. Pharmacol.* **21**, 468-473.

Goldman, M. J., and Wilson, J. M. (1995). Expression of αvβ3 and αvβ5 integrin is necessary for efficient adenovirus-mediated gene transfer in the human airway. *J. Virol.* **69**, 5951-5958.

Graham, F. L., and Prevec, L. (1991). Manipulation of adenovirus vectors, Methods in molecular biology, Vol. 7, p109-128, E. J. Murray, The Human Press Inc., Clifton, NJ.

Higashiyama, M., Kodama, K., Yokouchi, H., Takami, K., Doi, O., Kobayashi, H., Tanisaka, K., and Minamigawa, K. (1998). Immunohistochemical p53 protein status in nonsmall cell lung cancer is a promising indicator in dtermining in vitro chemosensitivity to some anticancer drugs. *J. Surg. Oncol.* **68**, 19-24.

Hirschowitz, E. A., Ohwada, A., Pascal, W. R., Russi, T. J., and Crystal, R. G. (1995). In vivo adenovirus-mediated gene transfer of the Escherichia coli cytosine deaminase gene to human colon carcinoma-derived tumors induces chemosensitivity to 5-fluorocytosine. *Hum. Gene Ther.* **6**, 1055-1063.

Huber, B. E., Austin, E. A., Good, S. S., Knick, V. C., Tibbels, S., and Richards, C. A., (1993). In vivo antitumor activity of 5-fluorocytosine on human colorectal carcinoma cells genetically codified to express cytosine deaminase. *Cancer Res.* **3**, 4619-4626.

Kievit, E., Nyati, M. K., Ng, E., Stegman, L. D., Parsels, J., Ross, B. D., Rehemtulla, A., and Lawrence, T. S. (2000). Yeast cytosine deaminase improves radiosensitization and bystander effect by 5-fluorocytosine of human colorectal cancer xenografts. *Cancer Res.* **60**, 6649-6655.

Kufe, D. W., and Major, P. P. (1981). 5-Fluorouracil incorporation into human breast carcinoma RNA correlates with cytotoxicity. *J. Biol. Chem.* **256**, 9802-9805.

Leavitt, J. (1994). Discovery and characterization of two novel huamn cancer-related proteins using two-dimensional gel electrophoresis. *Electrophoresis* **15**, 345-357.

Lenz, H. J., Hayashi, K., Salonga, D., Danenberg, K. D., Danenberg, P. V., Metzger, R., Banerjee, D., Bertino, J. R., Groshen, S., Leichman, L. P., and Leichman, C. G. (1998). p53 point mutations and thymidylate synthase messenger RNA levels in disseminated colorectal cancer: an analysis of response and survival. *Clin. Cancer Res.* **4**, 1243-1250.

Lin, C.-S., Chen, Z. P., Park, T., Ghosh, K., and Leavitt, J. (1993a). Characterization of the human L-plastin gene promoter in normal and neoplastic cells. *J. Biol. Chem.* **268**, 2793-2801.

Lin, C.-S., Park, T., Chen, Z. P., and Leavitt, J. L. (1993b). Human plastin genes. *J. Biol. Chem.* **268**, 2781-2792.

Moolten, F. L. (1994). Drug sensitivity (suicide) genes for selective cancer chemotherapy. *Cancer Gene Ther.* **1**, 279-287.

Peng, X. Y., Won, J. H., Rutherford, T., Fujii, T., Zelterman, D., Pizzorno, G., Sapi, E., Leavitt, J., Kacinski, B., Crystal, R., Schwartz, P., and Deisseroth, A. (2001). The use of the L-plastin promoter for adenoviral-mediated, tumor-specific gene expression in ovarian and bladder cancer cell lines. *Cancer Res.* **61**, 4405-4413 .

Pritchard, D. M., Watson, A. J., Potten, C. S., Jackman, A. L., and Hickman, J. A. (1997). Inhibition by uridine but not thymidine fo p53-dependent intestinal apoptosis initiated by 5-fluorouracil: evidence for the involvement of RNA perturbation. *Proc. Natl. Acad. Sci. USA* **94**, 1795-1799.

Wickham, T. J., Mathias, P., Cheresh, D. A., and Nemerow, G. (1993). Integrins αvβ3 and αvβ5 promote adenovirus internalization but not virus attachment. *Cell.* **73**, 309-319.

Wickham, T. J., Filardo, E. J., Cheresh, D. A., and Nemerow, G. R. (1994). Integrin αvβ5 selectively promotes adenovirus mediated cell membrane permeabilization. *J. Cell. Biol.* **127**, 257-264.

Zhang, L., Akbulut, H., Tang, Y., Peng, X., Pizzorno, G., Sapi, E., Manegold, S., and Deisseroth, A. (2002). Adenoviral vectors with E1A regulated by tumor-specific promoters are selectively cytolytic for breast cancer and melanoma. *Mol. Ther.* **6**, 386-393.