Arch Pharm Res Vol 27, No 6, 633-639, 2004

Archives of Pharmacal Research
http://apr.psk.or.kr

Recombinant Adenoviral Vector Containing Tumor-Specific L-Plastin Promoter Fused to Cytosine Deaminase Gene as a Transcription Unit : Generation and Functional Test

Injae Chung and Albert B. Deisseroth[1]

College of Pharmacy, Duksung Women's University, Seoul, Korea and [1]Sidney Kimmel Cancer Center, San Diego, California, U.S.A.

(Received January 10, 2004)

The expression of therapeutic transgenes in recombinant adenoviral vectors is a major cause of toxicity in dividing cancer cells as well as non dividing normal cells. To solve the problem of toxicity to normal cells, we have reported on a recombinant adenoviral vector system (AdLP-) in which the expression of the transgene is directed by the tumor-specific L-plastin promoter (LP) (Chung *et al.*, 1999). The object of this study was to generate a recombinant adenoviral vector system which would generate tumor cell specific expression of cytosine deaminase (CD) gene. We report the construction of a replication-incompetent adenoviral vector in which CD is driven by the L-plastin promoter (AdLPCD). Infection of 293 cells by AdLPCD generated the functional CD protein as measured by HPLC analysis for the conversion of 5-Fluorocytosine (5-FC) to 5-Fluorouracil (5-FU). HPLC analysis in conjunction with counting radioactivity for [6-^{3}H]-5FC and [6-^{3}H]-5FU demonstrated vector dose-dependent conversion of 5-FC to 5-FU in AdLPCD infected ovarian cancer cells. The results from present and previous studies (Peng *et al.*, 2001; Akbulut *et al.*, 2003) suggest that the use of the AdLPCD/5-FC system may be of value in the treatment of cancer including microscopic ovarian cancer in the peritoneal cavity.

Key words: L-Plastin promoter, Tumor-specific promoter, Cytosine deaminase, AdLPCD, Adenoviral Vector

INTRODUCTION

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).

CD/5-FC prodrug activation gene therapy has became a promising approach to cancer treatment. Cytosine deaminase is an enzyme present in some bacteria and fungi but absent in animal cells. This enzyme catalyzes the deamination of cytosine to uracil (Anderson *et al.*, 1989; Moolten, 1994) as well as the conversion of the innocuous prodrug, 5-FC, into the cytotoxic agent, 5-FU (Austin and Huber, 1993; Huber *et al.*, 1993). 5-FU is a pyrimidine antimetabolite cytotoxin with multiple mechanisms of action, which include inhibition of thymidylate synthase resulting in a rise of DNA damage, incorporation into DNA as a false nucleotide, and incorporation into RNA. If the intracellular concentrations of 5-FU are sufficiently high and the conversion of 5-FU into phosphorylated 5-FU is sufficiently fast, then cycle independent cell death will result from incorporation of the 5-FU into RNA, which disrupts the production of functional protein (Grazer and Lloyd, 1982; Kufe and Major, 1981; Pritchardn *et al.*, 1997).

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

Correspondence to: Albert B. Deisseroth, Sidney Kimmel Cancer Center, 10835 Altman Row, San Diego, California 92121, U.S.A.
Tel: 1-858-410-4205, Fax: 1-858-623-2740
E-mail: adeisseroth@skcc.org

attempt to direct the transgene expression in a tumor cell-specific way, we have proposed the use of an adenoviral vector which carries the L-plastin promoter in a transcription unit (Chung *et al.*, 1999). Plastins are a family of human actin-binding proteins which are abundantly expressed in all normal replicating mammalian cells. One isoform, L-plastin, is constitutively expressed at high levels in hematopoietic cell types while T-plastin is constitutively expressed in all non-hematopoietic cells of solid tissues that have replicative potential. L-plastin is, however, constitutively synthesized in many types of malignant human cells of solid tissues indicating that its expression is induced at the time of transformation. Moreover, the activation of L-plastin expression during tumorigenesis appeared to be governed by 5.1 kb of DNA sequence containing promoter and flanking region (Lin *et al.*, 1993). Based on these reports, we utilized 2.4 kb of L-plastin 5'-flanking region as a transcription unit of adenoviral vector to achieve transcriptional targeting for cancer cells. AdLPLacZ included a 2.4 kb instead of a 5.1 kb fragment of L-plastin 5'-flanking region, because of limited capacity of adenoviral vector, and the *E. coli* LacZ gene. The results of screening of cell lines by AdLPLacZ showed the expression of β-galactosidase gene in a tumor-specific manner in cell lines and patient samples collected from ascites but not in normal mesothelial cells (Chung *et al.*, 1999). On the basis of experiments in cell lines, both in our laboratories and in those of Dr. John Leavitt of Palo Alto Medical Research Institute (Lin *et al.*, 1993a, 1993b; Leavitt, 1994), it is likely that the L-plastin promoter is active in epithelial neoplastic cells but inactive in peritoneal cells. If this is true, then a totally specific approach to the therapy of ovarian cancer could result.

Based on these findings, we hypothesize that use of L-plastin promoter as a transcription unit of adenoviral vector will render the transcription of CD gene to be restricted to L-plastin-positve ovarian carcinoma cells. It will 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, replication-incompetent recombinant adenoviral vector, AdLPCD which contains the L-plastin promoter and the *E. coli* CD gene was constructed. Experiments with the AdLPCD/5-FC system have recently shown a tumor-specific suppressive effect to breast, ovarian, bladder and colon cancers (Peng *et al.*, 2001; Akbulut *et al.*, 2003). However, none of those studies described on the construction and functional test of AdLPCD vector, which would be a rationale to use. Therefore, we now report on the construction of the AdLPCD vector and the production of functional CD in AdLPCD infected 293 as well as OVCAR-3 ovarian cancer cells. Functional test of CD was carried out by HPLC analysis in conjunction with counting radioactivity for [6-^{3}H]-5FC and [6-^{3}H]-5FU.

MATERIALS AND METHODS

Generation of AdLPCD

AdLPCD 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. The AdLPCD recombinant virus vector was generated by homologous recombination of a shuttle plasmid, pLP.CD. shuttle and the adenovirus type 5 backbone plasmid, pBHG10 (Microbix Biosystems Inc., Ontario, Canada), in human embryonic kidney cell line 293 which supplies the missing E1 protein in trans (Fig. 1).

The shuttle plasmid, pLP.CD.shuttle was constructed as follows. We obtained 1.5 kb of *E. coli* CD gene by the digestion of pCMV-CD, which was given to us by Dr. Ronald Crystal (The New York Hospital-Cornell Medical

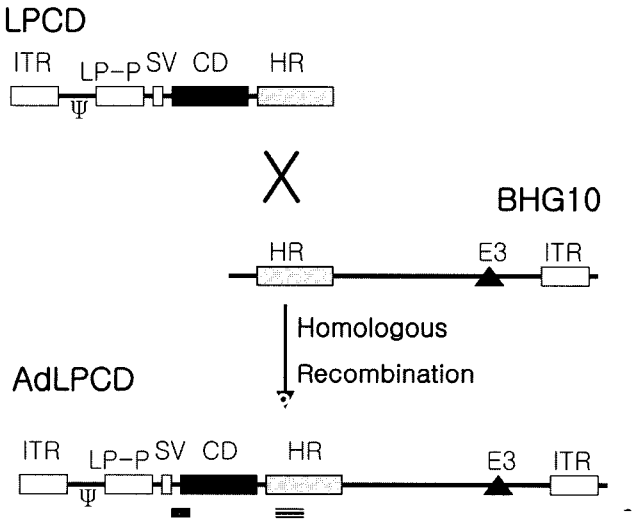

Fig. 1. Assembly of the AdLPCD adenoviral vector by the homologous recombination method. LPCD is a shuttle vector that contains an adenoviral inverted terminal repeat (ITR) and packaging signal (ψ), the CD gene driven by the 2.4 kb of L-plastin promoter, SV40 splice donor/acceptor (SV) site and some adenoviral vector sequences that are homologous in sequence (HR) to those present in the pBHG10 adenoviral vector plasmid. These HR sequences, when combined with the pBHG10 adenoviral vector plasmid, produced a complete replication-incompetent adenoviral vector named AdLPCD. AdLPCD contains the CD gene under the control of the L-plastin promoter.

Center, New York, NY), with Not 1 restriction enzyme. After Not I digestion of pLPLacZshuttle (Chung *et al.*, 1999), which is a shuttle vector containing 2.4 Kb of L-plastin promoter and *E. coli* β-galactosidase gene as a transcription unit, the 9.3 Kb fragment was saved and ligated with Not 1 digested-1.5 Kb of *E. coli* CD gene to generate the pLP.CD.shuttle. The adenoviral vector AdLPCD was then produced by homologous recombination using standard techniques (Rosenfeld *et al.*, 1992). For homologous recombination to occur, pBHG10 (Microbix Biosystems Inc., Ontario, Canada) and pLP.CD.shuttle were co-transfected into 293 cells by the calcium phosphate co-precipitation method.

PCR assays of AdLPCD vector

Individual plaques were screened and the identity of each as AdLPCD was verified (see Fig. 2) by amplifying part of the sequences of the CD gene and Ad5 by PCR. The DNA sequence of primers used for screening for infected cells is described in the following paragraphs. The forward primer of Ad5, 5'-TCGTTTCTCAGCAGCT-GTTG-3'; and the reverse primer of Ad5, 5'-CATCTGA-ACTCAAAGCGTGG-3', were located at 11 and 13.4 map unit of the Ad5 genome, respectively. A sequence amplification using these primers generated 860 bp PCR product as previously published (Zhang *et al.*, 1993).

The primer set for the amplification of part of the SV 40 splice donor/accepter and *E. coli* CD gene in the shuttle vector were designed as follows. The forward primer of the SV40 splice donor/acceptor sequence included from 657 to 676 bp, 5'-CCAGAAAGTTAACTGGTAAG-3'; the reverse primer of *E. coli* cytosine deaminase gene, from 238 to 219 bp, 5'-CCGGACTGATTCCAGTTCGG-3', which generated a 390 bp PCR product. The sequence information on the SV 40 splice donor/accepter site and *E. coli* CD were obtained from pCMV-β plasmid sequence provided by Clontech and the paper published by Austin and Huber (1993), respectively.

Production of replication-incompetent adenoviral vector

The replication-incompetent adenovirual vectors, AdLPCD and AdLPLacZ (Chung *et al.*, 1999), 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 MgCl2) 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 2×10^{10} and 1×10^{11} pfu/mL. The organization of the L-plastin-CD adenoviral vector is shown in Fig. 1.

Cell culture

The cells were maintained in IMEM (Biofluids, Rockville, 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).

HPLC analysis for cytosine deaminase activity from AdLPCD infected 293 cells

A day after plating 293 cells on 6 cm^2 dish, the cells were infected by AdLPCD or AdLPLacZ, for 90 min at 50 MOI (multiplicity of infection). The latter was included as a control. Cells were further incubated for 24 h in 5 mL of medium which contained 150 μM 5-FC and 20 μL of [6-^{3}H]-5FC (specific activity: 20 Ci/mmol, Moravek Biochemicals, Brea, CA). After dilution of an aliquot of the medium at 1:10, the conversion of 5-FC to 5-FU was evaluated by HPLC (high performance liquid chromatography) on a Microsorb C18 reverse phase column, 25 cm×4.6 mm i.d. (Rainin, Inc., Woburn, MA), eluted with 50 mM potassium phosphated monobasic (KH_2PO_4), pH 3.0 at room temperature. The retention times for 5-FC and 5-FU were approximately 6.10 min and 8.70 min, respectively. Fractions were collected at 1 minute intervals followed by counting radioactivity after addition of scintillation fluid.

HPLC analysis for cytosine deaminase activity from AdLPCD Infected OVCAR-3 cells

A day after plating 2×10^6 OVCAR-3 cells on 6 cm^2 dish, the cells were infected by either 10, 100, and 250 MOI of AdLPCD or 100 MOI of AdLPLacZ, for 90 min. The latter was included as a control. Cells were further incubated for 3 days in 5 mL of medium which contained 100 μM 5-FC and 10 μL of [6-^{3}H]-5FC (specific activity: 20 Ci/mmol, Moravek Biochemicals, Brea, CA). HPLC analysis was conducted as described as above.

Statistical analysis

Significance levels for comparison between treatment groups were determined using the two-sided Student's t test for paired samples.

RESULTS

Generation of AdLPCD

We have generated a replication-incompetent recombinant adenoviral vector, AdLPCD, in which the *E. coli* CD gene is placed downstream of a 2.4 kb DNA sequence (-2263 to +118 bp with respect to the transcription start site) which was taken from the human L-plastin 5'-transcriptional regulatory region (Fig. 1). Recombinant adenovirus was produced by co-transfecting 293 cells with pLP.CD.shuttle and a plasmid, pBHG10, containing

the adenovirus genome. The pLP.CD.shuttle was prepared by insertion of the *E. coli* CD gene into the plasmid pLP.LacZ.shuttle (Chung *et al.*, 1999) to replace the LacZ gene. The 293 cells are transformed human kidney cells that contain the E1 region of the adenovirus genome. When 293 were co-transfected with pLP.CD.shuttle and pBHG10, replication-defective adenovirus was produced by homologous recombination.

DNA was isolated from plaques resulting from calcium phosphate transfection of the LPCD shuttle vector and the pBHG10 adenoviral vector with a deletion in the E1. The homologous recombination event would take place such that the L-plastin/CD gene would be inserted into the adenoviral vector in the E1 region. PCR was performed on this DNA as described in Materials and Methods. Fig. 2 shows the gel electrophoresis of PCR products which have been amplified from the supernatants of individual plaques for the part of the sequences of the CD gene and Ad5. The adenoviral vector strain 5 primers were synthesized to generate an 860 bp fragment. The part of the SV40 splice donor/acceptor and CD primers generate a 390 bp fragment.

Lanes 3 to 6 contain the PCR products of recombinant vectors. The 860 bp and 390 bp bands indicate the adenoviral genome and the CD insert, respectively. In other words, primers to amplify the part of Ad5 generated an 860 bp PCR product as expected. The forward primer of the SV 40 splice donor/acceptor sequence and the reverse primer of *E. coli* CD gene generated a 390 bp PCR product as expected. We conclude that the desired recombinant vector was obtained and designated as AdLPCD.

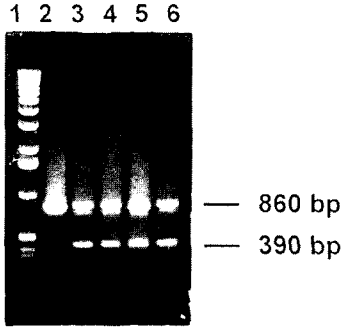

Fig. 2. PCR analysis of recombinant adenoviral vector with L-plastin promoter and CD gene. DNA was isolated from plaques resulting from calcium phosphate transfection of the LPCD shuttle vector and the pBHG10 adenoviral vector with a deletion in the E1. The homologous recombination event would take place such that the L-plastin /CD gene would be inserted into the adenoviral vector in the E1 region. PCR was performed on this DNA as described in Materials and Methods. The adenoviral vector strain 5 primers generate an 860 bp fragment, which is indicated in Fig. 1 as (═══). The part of SV40 splice donor/acceptor and CD primers generate a 390 bp fragment, which is indicated in Fig. 1 as (▬). Lane 1, molecular weight markers; lane 2, control for the adenoviral vector; lanes 3-6, recombinant vector. We conclude that the desired vector was obtained and designated as AdLPCD.

HPLC analysis to detect the conversion of 5-FC to 5-FU in AdLPCD infected 293 cells

To test if the AdLPCD infected cells produce a functional CD protein, the media of 293 cells that have been infected by either AdLPCD or AdLPLacZ followed by the addition of 5-FC were subjected to HPLC analysis. The 293 cell line has been included in the study because of its ability to amplify the replication-incompetent adenoviral vector. It is a human embryonic kidney cell line which supplies the missing E1 protein of adenovirus 5 *in trans*. A replication-incompetent adenoviral vector carrying the *E. coli* β-galactosidase gene under control of the L-plastin promoter (AdLPLacZ) was produced previously (Chung *et al.*, 1999) and used as a control vector. The AdLPLacZ vector containing the *E. coli* β-galactosidase gene consists of the same DNA sequence as AdLPCD except for the cytosine deaminase gene.

As seen in standard (STD) of Fig. 3, the retention times for 5-FC and 5-FU were about 6.10 min and 8.70 min, respectively. Addition of 5-FC in uninfected 293 cells generated only the 5-FC prodrug peak (no virus). The media from the cells infected with the AdLPCD vector showed conversion of [6-^{3}H]-5-FC to [6-^{3}H]-5-FU, whereas no such conversion was detected from cells which have been infected with a control vector, AdLPLacZ. The result indicates that AdLPCD is able to produce the CD enzyme which catalyzes the deamination of 5-FC, thereby producing 5-FU.

HPLC analysis for cytosine deaminase activity in the AdLPCD infected OVCAR-3 cells

To see if replication-incompetent AdLPCD vector is able to produce detectable amounts of functional CD in ovarian cancer cells, we carried out HPLC analysis in the AdLPCD infected OVCAR-3 cells followed by the treatment with 5-FC. The OVCAR-3 cell line was selected for study because of its ability to express reporter gene, LacZ, driven by the L-plastin promoter (Chung *et al.*, 1999). The CD activity was determined by conversion of [6-^{3}H]-5FC to [6-^{3}H]-5FU. To establish the AdLPCD-dependent conversion ratio of 5-FU/5-FC, HPLC fractions were collected at 1 minute intervals followed by counting radioactivity after the addition of scintillation fluid.

OVCAR-3 cells were infected by either 10, 100, and 250 MOI of AdLPCD or 100 MOI of AdLPLacZ, for 90 min. The latter was included as a control. Cells were further incubated for 3 days in 5 mL of medium which contained 100 μM 5-FC and 10 μL of [6-^{3}H]-5FC. The results are shown in Table I. There is a dose-dependent increases of the conversion rate in AdLPCD infected cells.

Infection of the cells with 100 MOI of AdLPCD vector resulted in conversion of 53.8% of inert prodrug 5-FC to the active cytotoxic 5-FU. Significant differences were observed in conversion rate between 100 MOI of AdLPCD infected- and 100 MOI of AdLPLacZ control vector infected. The results suggest 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.

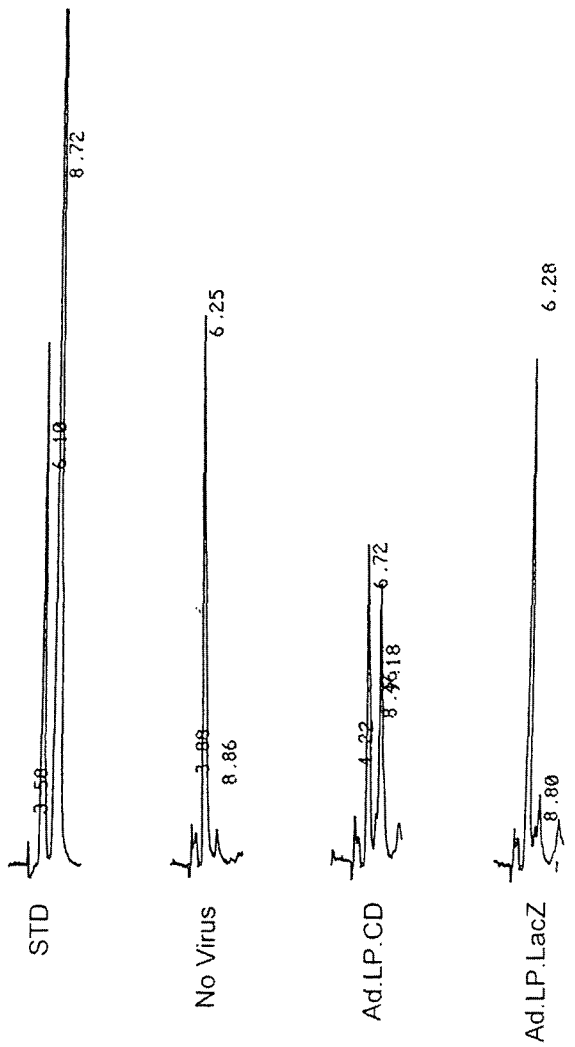

Fig. 3. Production of functional cytosine deaminase in 293 cells which have been infected with AdLPCD. Conversion of 5-FC to 5-FU, which was mediated by CD, was detected by the HPLC analysis. STD: standard peaks for 5-FC and 5-FU. The retention times for 5-FC and 5-FU were about 6.10 min and 8.70 min, respectively. No virus: Uninfected 293 cells were treated with only with 5-FC. AdLPCD: 293 cells were infected with AdLPCD vector followed by the addition of 5-FC. AdLPLacZ: 293 cells were infected with AdLPLacZ control vector prior to 5-FC treatment.

Table I. Conversion rate of [6-^{3}H]-5FC to [6-^{3}H]-5FU in OVCAR-3 cells in which either AdLPCD or AdLPLacZ viral vector has been infected

Vector	AdLPCD			AdLPLacZ
MOI	10	100	250	100
Conversion rate (%)	*30.6±0.07*	*53.8±0.42*[a]	*55.0±2.12*	*9.4±0.21*

Value represent the mean±SD.
[a]P<0.001 compared with the conversion rate of the cells infected with 100 MOI AdLPLacZ control vector.

DISCUSSION

We previously developed an adenoviral vector containing a transcription unit in which the transgene expression is under the control of 2.4 kb of the human L-plastin promoter. The pattern of â-galactosidase expression was tumor-specific in various cell lines when the LacZ gene had been placed down stream of the L-plastin promoter in an adenoviral vector. Moreover, the L-plastin promoter drove the expression of β-galactosidase not in normal mesothelial cells obtained from a patient biopsy, but in the microscopic ovarian cancer samples which were isolated from patient ascitic fluids. This observation had led us to use this adenoviral vector to generate the expression of the *E. coli* CD gene in L-plastin positive carcinoma cells but not in normal cells. The use of a tumor-specific promoter as a part of the adenoviral vector would increase the therapeutic index when it is used as a mean of delivery of the cytotoxic gene or chemosensitizing gene such as the CD gene.

In this report, successful assembly of a replication-incompetent recombinant adenoviral vector which carries the L-plastin promoter and CD gene of *E. coli* as a transcription unit was described.

To confirm that the increased sensitivity to 5-FC in AdLPCD infected cells was the result of the conversion of 5-FC to 5-FU, cells were cultured in the presence of [6-^{3}H]-FC after infection with the vector. Analysis demonstrated that there was selective conversion of 5-FC to 5-FU in AdLPCD infected cells in a dose-dependent manner.

The level of transgene expression would also affect the efficacy of combinatorial strategy of enzyme-prodrug activation. In addition, toxicity could be generated from the nonselective expression of therapeutic gene, thereby decreasing the therapeutic index. General and strong promoters such as the heterologous cytomegalovirus (CMV) promoter was the choice at the early stage of developing viral vectors for cancer treatment. However, tissue- or tumor- specific promoters have been adopted in transcription units (Miller and Whelan, 1997) to achieve safe transgene expression. One such is the L-plastin promoter which has been selected for targeted expression in ovarian cancer. The L-plastin promoter was incorporated in the expression cassette of the adenoviral vector. This

resulted in a pattern of transgene expression, such as that of the *E. coli* LacZ gene, preferentially in neoplastic cells which included ovarian cancer cell lines and fresh ascitic ovarian cancer cells. Moreover, the level of promoter activity is comparable to that of the CMV promoter (Chung *et al.*, 1999). The selection of a promoter which would direct therapeutic genes to express a level high enough to be effective and in a selective enough manner to be safe is an important step towards success in cancer gene therapy.

Since no currently available gene transfer vectors are capable of transferring a gene to all tumor cells comprising a solid tumor, successful application of enzyme-prodrug activation strategy relies on a bystander effect. The active agent diffuses from the tumor cell in which it was produced to neighboring uninfected malignant cells in sufficient concentrations to suppress growth. Therefore the size and half-life of each prodrug and its metabolite would affect the bystander effect. In a colon carcinoma cells, the expression of CD in 10% of cells was associated with a bystander effect when combined with 5-FC (Hirschowitz *et al.*, 1995).

Since the L-plastin promoter in our previous study was active in an ovarian cancer cell line and in fresh ascitic ovarian cancer cells, but inactive in samples of normal peritoneum, the AdLPCD vector combined with 5-FC administration may have significant utility in the chemosensitization of ovarian cancer carcinoma metasized into intraperitoneal cavity.

FOOTNOTES

The following abbreviations are used: ad, adenovirus; CD, cytosine deaminase; CMV, cytomegalovirus; 5-FC, 5-fluorocytosine; 5-FU, 5-fluorouracil; LacZ, galactosidase; LP, L-plastin; MOI, multiplicity of infection.

ACKNOWLEDGEMENTS

The authors recognize support to A. B. Deisseroth from the Bush Leukemia Research Fund and the Anderson Chair for Cancer Treatment and Research at the UT M. D. Anderson Cancer Center, and from the Hull Development Fund of the Yale Comprehensive Cancer Center, and the Ensign Professorship of Medicine of the Department of Internal Medicine at the Yale University School of Medicine.

REFERENCES

Akbulut, H., Zhang, L., Tang, Y., and Deisseroth, A., 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(5), 388-395 (2003).

Anderson, L., Kilstrup, M., and Neuhard, J., 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 (1989).

Austin, E. A. and Huber, B. E., 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 (1993).

Cannistra, S. A., Cancer of the ovary. *N. Engl. J. Med.*, 329(21), 1550-1559 (1995).

Chung, I., Schwartz, P. E., Crystal, R. G., Pizzorno, G., Leavitt, J., and Deisseroth, A. B., Use of L-plastin promoter to develop an adenoviral system that confers transgene expression in ovarian cancer cells but not in normal mesothelial cells. *Cancer Gene Ther.*, 6(2), 99-106 (1999).

Glazer, R. I. and Lloyd, L. S., 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 (1992).

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

Hirschowitz, E. A., Ohwada, A., Pascal, W. R., Russi, T. J., and Crysta, R. G., *In vivo* adenovirus-mediated gene transfer of the Escherichia coli cytosine deaminase gene to human colon carcinoma-derived tumors induces chemosensitivity to 5-fluorocytosine. *Human Gene Ther.*, 6(8), 1055-1063 (1995).

Huber, B. E., Austin, E. A., Good, S. S., Knick, V. C., Tibbels, S., and Richards, C. A., *In vivo* antitumor activity of 5-fluorocytosine on human colorectal carcinoma cells genetically codified to express cytosine deaminase. *Cancer Res.*, 53, 4619-4626 (1993).

Kufe, D. W. and Major, P. P., 5-Fluorouracil incorporation into human breast carcinoma RNA correlates with cytotoxicity. *J. Biol. Chem.,* 256, 9802-9805 (1991).

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

Lin, C.-S., Chen, Z. P., Park, T., Ghosh, K., and Leavitt, J., Characterization of the human L-plastin gene promoter in normal and neoplastic cells. *J. Biol. Chem.,* 268(4), 2793-2801 (1993a).

Lin, C.-S., Park, T., Chen, Z. P., and Leavitt, J. L., Human plastin genes. *J. Biol. Chem.*, 268(4), 2781-2792 (1993b).

Miller, N. and Whelan, J., Progress in transcriptionally targeted and regulatable vectors for genetic therapy. *Human Gene Ther.*, 8, 803-815 (1997).

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

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., The use of the L-plastin promoter for adenoviral-mediated, tumor-specific gene expression in ovarian and bladder cancer cell lines. *Cancer Res.,* 61(11), 4405-4413 (2001).

Pritchard, D. M., Watson, A. J., Potten, C. S., Jackman, A. L., and Hickman, J. A., 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. U.S.A.,* 94(5), 1795-1799 (1997).

Zhang, W. W., Fang, X., Branch, C. D., Mazur, W., French, B. A., and Roth, J. A., Generation and identification of recombinant adenovirus by liposome-mediated transfection and PCR analysis. *Biotechniques,* 15(5), 868-872 (1993).