

Ovarian Epithelial Cell Lineage-specific Gene Expression Using the Promoter of a Retrovirus-like Element¹

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Abstract

We have isolated 462 bp of sequence termed ovarian-specific promoter 1 (OSP-1) that is part of a retrovirus-like element specifically expressed in the rat ovary. We have evaluated the ability of OSP-1 to activate gene expression in normal and neoplastic cell lines derived from the ovaries of rats and women. We have found that there was marked specificity in the ability of OSP-1 to drive reporter gene expression in an ovarian epithelial cell lineage manner. The expression of herpes simplex virus thymidine kinase (HSV-TK) under OSP-1 control was sufficiently ovarian cancer cell line specific to render ganciclovir ~50-fold more toxic in the A2780 human ovarian cancer cell line compared with clones of the HCT-116 and HT-29 colon cancer cell lines. Furthermore, ganciclovir had marked antitumor efficacy *in vivo* in severe combined immunodeficient mice bearing A2780^{OSP-1-HSV-TK} as a s.c. xenograft. We suggest that these data support the use of OSP-1 as a tool to provide specificity to the gene therapy of ovarian cancer and to drive ovarian-specific oncogene expression for the creation of transgenic mouse models of ovarian cancer.

Introduction

Ovarian carcinoma is the leading cause of death from gynecological malignancies in women of Western industrialized countries and is most frequently diagnosed at an advanced stage (1). Current standard treatment for advanced stage ovarian cancer consists of surgical debulking in combination with cytotoxic chemotherapy using a platinum drug and paclitaxel (2). Despite the encouraging clinical complete response rates (40–60%) achieved with combination therapy, the majority of patients eventually relapse and become refractory to further treatment (3). In most studies, long-term survival for patients with advanced stage disease rarely exceeds 30%. On the basis of these facts, it is obvious that alternative therapeutic modalities are needed, and gene therapy has been proposed as one possibility (4). It is also noteworthy that no representative spontaneous mammalian model reflecting human epithelial ovarian cancer exists. This void may further contribute to the lack of highly efficacious therapies for the disease because it limits the relevance of the preclinical drug screening possible.

Recently, advancements have been made in identifying certain tissue-specific promoters of utility for the creation of transgenic models of some solid tumor types and to yield tumor-specific transgene expression for the purpose of gene therapy. Perhaps the most common approach for the gene therapy of cancer is the transduction

of a gene whose product is capable of converting an inactive drug into a cytotoxic metabolite. Two genes are being widely used for this purpose. These are the *TK*⁴ gene from HSV and the cytosine deaminase gene. The HSV-TK enzyme converts the nontoxic prodrug GCV to monophosphate GCV, which is ultimately converted to toxic triphosphates by endogenous kinases, whereas cytosine deaminase converts 5-fluorocytosine to the cytotoxic 5-fluorouracil.

Although many studies have been undertaken with adenovirus vector-mediated HSV-TK/GCV-based prodrug therapy for ovarian cancer (4), the issue of specificity of expression of the transgene has not been investigated. This is in contrast to the evaluation of tissue-specific promoters for the gene therapy of cervical carcinoma, glioma, melanoma, and thyroid cancers (5–10). In this report, we describe the documentation that a potential OSP confers specificity of reporter gene expression in cells of ovarian epithelial cell lineage including human ovarian cancer cells. This promoter was derived from the cDNA sequence of a retrovirus-like element that is specifically expressed in the rat ovary (11). Specifically, it is based on the well-accepted assumption that the 5' and 3' LTRs of retroviruses are identical in sequence and that their transcriptional activation domain resides in the U3 portion of the 5' LTR. Hence, although transcription is initiated downstream of the U3 portion of the 5' LTR, the sequence of the U3 element can be deduced from the transcribed 3' LTR. On the basis of the ability of this DNA sequence to control expression of reporter genes and through activation of HSV-TK to convert GCV to its cytotoxic form in an ovarian epithelial cell lineage-specific manner, we propose that this promoter should be considered for use in the clinical gene therapy of ovarian cancer and deserves consideration for use to create transgenic models of ovarian cancer.

Materials and Methods

Cell Culture. The human ovarian cancer cell lines (A2780, OVCAR2, OVCAR3, OVCAR4, OVCAR5, OVCAR7, OVCAR8, OVCAR10, SKOV3, PEO1, PEO4, A1847, and UPN251) used in this study were isolated from ovarian cancer patients who were either untreated or treated with platinum-based chemotherapy and were described in our previous publications (12, 13). The non-ovarian cell lines HeLa, HT-29, HCT-116, NIH3T3, Hec-1A, and Hec-1B were obtained from American Type Culture Collection (Rockville, MD). The parental hepatoma cell line BEL-7404 was a gift from Dr. M. M. Gottesman (National Institute of Health, Bethesda, MD). The normal rat ovarian surface epithelial cells were obtained from the ovaries of adult female Fisher rats by selective trypsinization (11, 14). The human ovarian and non-ovarian cell lines were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (Life Technologies, Inc., Grand Island, NY), 100 units/ml streptomycin, 100 units/ml penicillin, 0.3 mg/ml L-glutamine, and 0.25 unit/ml porcine insulin. The normal rat ovarian surface epithelial cells, rat ovary Nutu19 cell line, and the murine cell line NIH3T3 were cultured in DMEM

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⁴ The abbreviations used are: TK, thymidine kinase; HSV, herpes simplex virus; CAT, chloramphenicol acetyltransferase; OST, ovarian-specific transcription; OSP, ovarian-specific promoter; GCV, ganciclovir; LTR, long terminal repeat; β gal, β -galactosidase; C/EBP β , CCAAT/enhancer binding protein β .

supplemented with L-glutamine, insulin, and 4% fetal bovine serum. Cell lines were maintained in exponential growth in a humidified incubator at 37°C in a atmosphere of 5% CO₂ and 95% air.

Construction of OSP-1-driven Vectors. OSP-1 was obtained by the PCR using the forward primer 5'-CAGAGGTACCTAAAACAAGTTG-3' and the reverse primer 5'-AGACTGGCGCGCTAGCAGAGCC-3' from the template DNA of the OST 30-1 clone (11). The 462-bp PCR fragment was cloned in the TA cloning vector pCRII (Invitrogen). To create OSP-1 CAT reporter plasmid (pCATOSP-1), the *XhoI/HindIII* fragment of OSP-1 was subcloned into the promoterless pCAT3-Basic vector. To construct the OSP-1-luciferase reporter plasmid, the *EcoRI* fragment of OSP-1 was initially ligated to *SmaI* adaptors and then subcloned into *SmaI* site of the promoterless pgl3-Basic luciferase (Promega) vector. The tandem copies of OSP-1 reporter vectors (two copies of pgl2-OSP-1; three copies of pgl3-OSP-1) were made by self-religation of *EcoRI* fragments of OSP-1, followed by subcloning in pgl3 vector. The positive control SV40-driven luciferase plasmid, pgl-SV40, was obtained from Promega. The backbone expression vector with OSP-1 (referred to as PcOSP-1 plasmid) was made by removing the CMV promoter with *BglIII/BamHI* digestion, followed by religation and subcloning of the OSP-1 fragment at the *EcoRI* site in pcDNA3 (Invitrogen) vector. The HSV-TK gene-containing plasmid pAB109 was supplied by Dr. David Curriel's laboratory (University of Alabama, Birmingham, AL). To create the OSP-1-driven HSV-TK plasmid, called OSP-1-TK, the HSV-TK fragment was excised by *BglIII/PvuII* and subcloned into the PcOSP-1 vector at the *EcoRV* site. All of the plasmid constructs were verified for proper orientation by sequence analysis.

Transient Transfections and Reporter Assays. Cells growing at 50–60% confluence in six-well tissue culture plates were used for transient transfection assays. A total of 5 µg of plasmid [4.5 µg of reporter plasmids pCATOSP-1 or pglOSP-1 plasmid and 0.5 µg of internal control plasmid CMV-β galactosidase (pCMV-βgal)] were mixed with an equal volume of serum-free RPMI 1640-diluted LT-1 reagent (Mirus). The LT1-DNA complexes were incubated at a 0.6 ml of RPMI 1640 volume on cultures for 4 h at 37°C. After 4 h incubation, cells were rinsed with PBS and cultured with complete medium. After 2 days, cells were washed once with PBS and lysed with 0.5 ml of 1× cell lysis buffer (Promega). The cell lysate was used for reporter assays. The CAT enzyme activity was analyzed on the basis of acetylation of ¹⁴C-labeled chloramphenicol separated by TLC. The acetylated and nonacetylated chloramphenicol bands were cut out on the TLC plates and quantitated by determination of radioactivity using beta scintillation. The percentage of acetylation was calculated as cpm of acetylated chloramphenicol divided by total cpm of acetylated and nonacetylated chloramphenicol and multiplied by 100. The luciferase activity was measured with the luciferase assay system from Promega. To normalize the transfection efficiency, CMV-βgal plasmid pcCMV-βgal, which was included in the transfection, was assayed with *o*-nitrophenyl-β-D-galactopyranoside substrate based on a βgal enzyme assay system (Promega).

Stable Transfection of Tumor Cells. The *ScaI* linearized PcOSP-1-TK plasmid was introduced into the A2780 ovarian and HT-29 and HCT-116 colon carcinoma cell lines by electroporation. To accomplish this, the cells were trypsinized and washed twice with PBS by centrifugation. The cell pellets were resuspended in ice-cold PBS, and the cell concentrations were adjusted to 1 × 10⁶ cells/ml. Vector DNA (20 µg) was added, and cells were maintained on ice for 15 min. The electroporation was carried out at 975 µF, 250 V using the Gene Pulser II system (Bio-Rad Laboratories, Hercules, CA). The transduced neomycin-resistant clones were selected by growth of the cells in 0.5 mg/ml G418 (Life Technologies, Inc.)-containing medium. The cell clones were isolated using cloning cylinders and were expanded for further studies. The total RNA extracted from the G418-resistant clones was electrophoresed on formaldehyde-containing gel and transferred to nylon membranes as described previously (11). The HSV-TK fragment was used as a probe in Northern analysis to confirm expression. In addition to the clonal lines isolated, a portion of the G418-resistant clones from each transfection was pooled to create pooled, transduced cell lines.

In Vitro GCV Cytotoxicity Assay. To determine the cytotoxicity of GCV, 1000 or 3000 cells in a volume of 150 µl were plated in complete culture medium in flat-bottomed, 96-well microtiter trays overnight at 37°C and 5% CO₂ in a humidified incubator. After 24 h incubation, varying amounts of GCV (Cytovene-IV; Roche Laboratories Inc., Nutley, NJ) were added to yield a final concentration range of 0.03–30 µM. On day 3, sensitivity to GCV was

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          SRY
1  CAGAGGTACC TAAACAAGT TGAGTGAAG ATTCCACTCA AGGACAAAAA

          C/EBP beta          IK1
51  GAAAATGGGG GAATGAAAGA CCCTCTCCCA GAAAGGGAAT TGTACAAGCC

101  CAAGGCCCTC AACTATAGAA GTAAAAAATA AGTTTTTAGA TATGTGGGAC

          ER          C/EBP beta
151  AAGCTGACCC ATCATTTAGA CGAACAGAC CAGAAAAGAA AACAAAATGC

201  AGAAACCAGA CTAATTATG GGCAGTCTC CATGGCCAC CTGGCAGGAA

251  AAAGAAAAAG TCCCTGACCC CCCGGAAGCC CTGACCACCA TGTACTTTTT

          AP1
301  GCTAACTCAC TGTTATGATT ATAGCCAAA TAGGTAACAT ACACGTATGC

          CAT box
351  CTCATTTACT CCACCAATCA CATCCCTGTA ACCATGCATC TGCTTCTGTA

          TATA box
401  AGCCTGCTTC TGCTCCCAA AATACTATAA AAAGCCCACC CTGGCTCTGC

451  TAGGCGGCC AGTCT

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Fig. 1. Nucleotide sequence of the OSP-1 promoter. The sequence is shown by convention as 5' to 3'. The transcription initiation (CAT box and TATA box) and the regulatory elements are indicated by *bold letters*, and the corresponding binding proteins are shown by *overlining* the sequences. SRY, sex-determining region Y gene product; IK1, *Ikaros1* (the *Ikaros* gene encodes a family of functionally diverse zinc finger DNA-binding proteins); ER, estrogen receptor; AP1, AP1 binding proteins.

determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide-based colorimetric assay as described previously (15). The reported IC₅₀s are the result of triplicate determinations on at least three separate occasions.

Tumor Implantation and in Vivo Sensitivity to GCV. Female C.B.17/ICR severe combined immunodeficient mice were bred in the Laboratory Animal Facility at Fox Chase Cancer Center. All of the animals were maintained in specific pathogen-free conditions in plastic cages equipped with air filters. The animals received commercial food and water *ad libitum*. The protocol of the animal experiment was approved by the institutional animal care and use committee of Fox Chase Cancer Center. Two clonal populations of A2780^{PcOSP-1-TK} cells or empty vector containing cells growing as monolayers in flasks were harvested with trypsin, washed with complete medium, and resuspended in PBS. The mice (6–8 weeks of age) were injected s.c. into the flank region with 0.2 ml of cell suspensions (about 5 × 10⁶ cells) on day 0. On day 8, the mice were divided randomly as six mice/group into three groups (control, early treated, and late treated). GCV diluted to 2 mg/ml with 0.9% normal saline was injected i.p. to yield a dose of 50 mg/kg. The control group received i.p. injections of normal saline. The s.c. tumors were measured by a caliper every 4 days. The tumor volume was calculated by using the following equation:

$$V = 1/2 ab^2$$

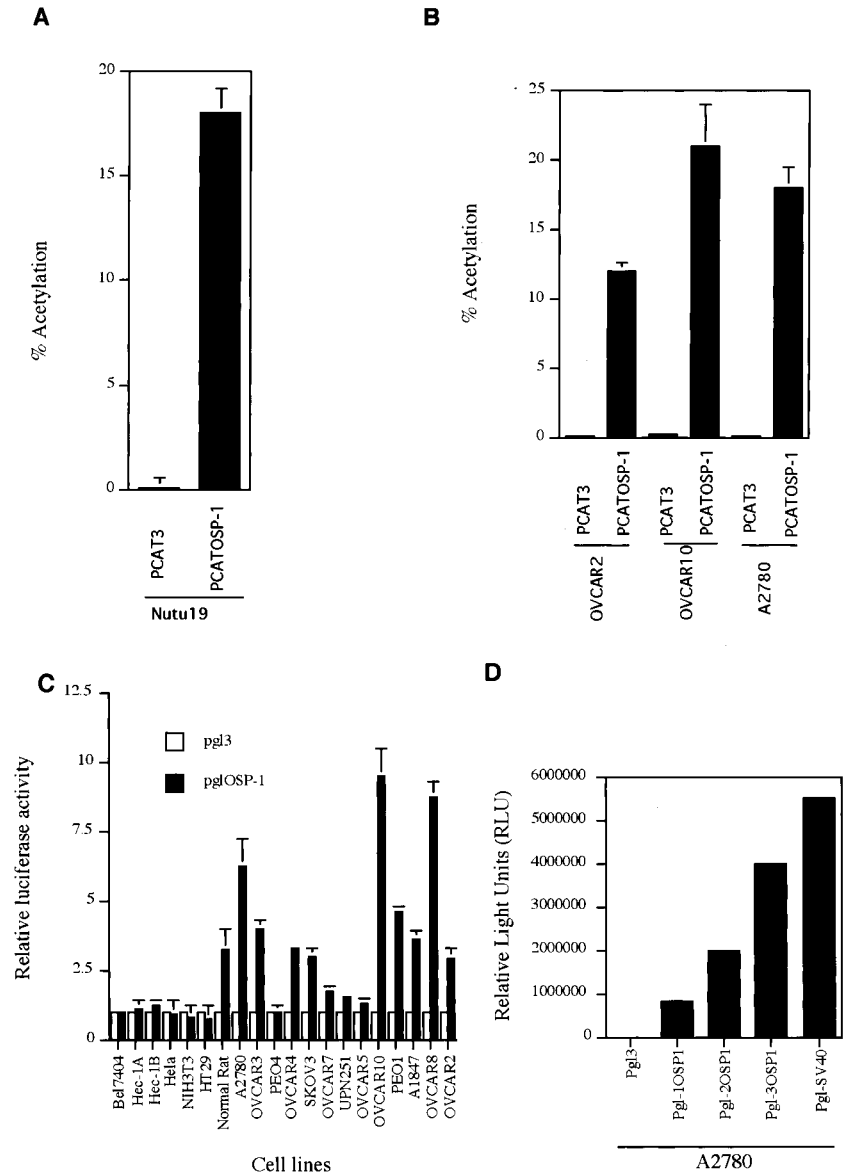
where *a* is the longest diameter and *b* is the shortest diameter of the s.c. tumor. The survival was monitored, and side effects including body weight changes were observed closely.

Statistical Analysis. The Wilcoxon test for paired samples was used to evaluate differences between animal groups.

Results

Isolation of OSP-1 from Rat Ovarian-specific, Retrovirus-like Element Transcripts. We identified previously a repetitive family of DNA sequences in the rat genome, members of which were specifically transcriptionally active in the rat ovary and had the characteris-

Fig. 2. Reporter gene expression using OSP-1 promoter. **A**, rat Nutu19 ovarian surface epithelial cell line was transiently transfected with promoterless CAT vector, pCAT3, and OST-OSP-1-driven CAT vector, pCATOSP1. CAT activity was measured after 48 h of posttransfection, as described in "Materials and Methods." CAT activity was normalized to transfection efficiency using CMV-driven β gal internal control vector. **B**, human ovarian surface epithelial cell lines OVCAR2, OVCAR10, and A2780 were transiently transfected with pCAT3 and pCATOSP1 vectors. CAT activity was measured after 48 h of transfection. The results shown are averages of three transfection experiments for each plasmid; bars, SD. **C**, luciferase reporter gene expression in ovarian (A2780, OVCAR2, OVCAR3, OVCAR4, OVCAR5, OVCAR7, OVCAR8, OVCAR10, SKOV3, PEO1, PEO4, A1847, and UPN251), non-ovarian (Bel7404, Hec1A, Hec1B, HeLa, NIH3T3, and HT29) cell lines and normal rat surface epithelial cells using OSP-1 promoter. Cells from rat and human ovarian surface and non-ovarian sources were transiently transfected with promoterless luciferase vector, pgl3, and OSP-1-driven pglOSP1 vectors. Luciferase activity was measured after 48 h of transfection. Luciferase activity was normalized to transfection efficiency using β gal internal control vector. The data are fold induction over that obtained with the pgl3 vector alone, which was normalized to 1. The results shown are averages of three or four transfection experiments for each plasmid; bars, SD. **D**, luciferase reporter gene expression by tandem copies of OSP-1 in ovarian A2780 cell line. A2780 cell line was transiently transfected with pgl3, pgl1-OSP-1 (single copy of OSP-1), pgl2-OSP-1 (two copies of OSP-1), pgl3-OSP1 (three copies of OSP-1) and positive control pgl-SV40 plasmids. Luciferase activity was measured after 48 h of transfection. Luciferase activity was normalized to transfection efficiency using β gal internal control vector. The results shown are the representative of three independent experiments, which had identical results.



tics of retrovirus-like elements (11). We have termed these DNA elements as OST units. We hypothesized that this OST was the result of the combination of ovarian-specific *cis*-acting elements present in the U3 portion of the 5' LTRs of these elements and the presence of *trans*-activation factors unique to ovarian cells.

To begin to examine this possibility, we first reconstructed the sequence of what would be a prototypical genomic OST unit, based on the well-accepted assumption that the complete 5' and 3' LTRs of retroviral genomic elements are identical or have only slightly diverged. Hence, there should be sufficient information within the sequence of a full-length retrovirus-like transcript to reconstruct its genomic template because the U3 sequence present in the 3' portion of the transcript can theoretically be used as the source for the sequence of the U3 portion of the 5' LTR. To test this possibility, we obtained the U3 portion of the 3' LTR fragment by the PCR and named it OSP-1. The PCR product was designed to have sequences essential for transcription initiation and devoid of termination signals. The sequence was analyzed for transcription factor binding sites using the MatInspector program (16). As shown in Fig. 1, the OSP-1 fragment had retained TATA box elements and known regulatory

elements important in ovarian physiology, such as estrogen response elements and CCAAT enhancer-binding protein β elements.

Transactivation of Reporter Genes by OSP-1. OSP-1 was subcloned into *XhoI/HindIII* sites of pCAT3-Basic vector to obtain a construct called pCAT-OSP-1, where the CAT reporter gene is under the control of OSP-1. To evaluate the OSP-1 promoter functionality, the pCAT-OSP-1 and pCAT3-Basic reporter vectors were transfected into the rat ovarian cancer cell line Nutu-19 by lipofection. The pCMV- β gal plasmid with β gal under control of the CMV promoter was used to normalize for transfection efficiency. The ability of OSP-1 to induce CAT activity is shown in Fig. 2A. Although expression of CAT was not detected in promoterless pCAT3-Basic vector-transfected cells, CAT transcription was highly induced in the pCAT-OSP-1-transfected transformed rat ovarian surface epithelial cell line, Nutu19. These data supported the notion that we had isolated DNA with *cis*-regulatory activity. Because the element was functional in transformed rat ovarian surface epithelial cells, we speculated that it might cross species bounds and function in human ovarian cells. To investigate this possibility, we transiently transfected the reporter vector pCAT-OSP-1 and promoterless vector pCAT3 into A2780,

OVCAR2, and OVCAR10. The internal control β gal-normalized cell extracts were assayed for CAT activity. As shown in Fig. 2B, OSP-1 activated CAT expression in these human ovarian cancer cell lines.

To evaluate OSP-1 specificity and uniformity of activity, several additional ovarian and non-ovarian cell lines were examined using constructs with OSP-1-driving luciferase. This was accomplished by cloning OSP-1 into the *Sma*I site of the pgl3 vector, now designated pglOSP-1, thus placing the sensitive luciferase reporter gene under the control of OSP-1. The ovarian reporter vector pglOSP-1 or its promoterless counterpart pgl3 was transiently transfected along with internal control pCMV- β gal plasmid into the following human ovarian cancer cell lines: OVCAR2, OVCAR3, OVCAR4, OVCAR5, OVCAR7, OVCAR8, OVCAR10, PEO1, PEO4, UPN251, SKOV3, A2780, and A1847. To examine cell type specificity, we also transfected the reporter plasmids into non-ovarian cell lines, including HeLa, NIH3T3, HT29, and Bel7404, and endometrial cancer cell lines Hec-1A and Hec-1B. We have also included normal rat ovarian surface epithelial cells to determine the OSP-1 activity in nonneoplastic cells. Before measuring luciferase activity, the cell extracts were normalized for β gal activity. As seen in Fig. 2C, OSP-1 had modest or no activity in non-ovarian cell lines, whereas it had substantial promoter activity in 9 of 13 of the ovarian cancer cell lines and in

Table 1 Effect of GCV concentration on OSP-1-TK transfected cells

Cell line ^a	GCV in μ M (IC ₅₀) ^b
A2780 ^{OSP-1-TK pooled}	9.6 \pm 0.41
A2780 ^{OSP-1-TK1}	0.41 \pm 0.02
A2780 ^{OSP-1-TK2}	0.5 \pm 0.03
HT29 ^{OSP-1-TK pooled}	>30
HT29 ^{OSP-1-TK1}	24 \pm 3.1
HT29 ^{OSP-1-TK2}	25.5 \pm 2.9
HCT116 ^{OSP-1-TK pooled}	>30
HCT116 ^{OSP-1-TK1}	26 \pm 1.6
HCT116 ^{OSP-1-TK2}	23.5 \pm 2.6

^a HSV-TK gene-transfected cell lines were assayed for GCV cytotoxicity. Pooled cell lines and individual clones were used to determine GCV sensitivity.

^b Cytotoxicity was determined by 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay. The IC₅₀ is the concentration that inhibited growth by 50%. The IC₅₀ is expressed as the mean of two experiments. Within experiments, quadruplicate wells were done at each concentration. GCV was used at a concentration of 0.1–30 μ M. Control cells (nontransfected) exhibited cytotoxicity, causing ~10% of dead cells at 30 μ M concentration of GCV.

normal rat ovarian surface epithelial cells. We also transfected tandem copies of OSP-1-containing luciferase reporter plasmids and the positive control Pgl-SV40 into the A2780 ovarian cell line. As seen in Fig. 2D, two and three tandem copies of OSP-1 showed increased promoter activity when compared with a single copy of the OSP-1 promoter.

Specificity of HSV-TK-mediated Prodrug Activation by OSP-1.

Having established that OSP-1 could induce the transcription of reporter genes in ovarian cancer cell lines, we wished to determine whether the degree of induction was sufficient for therapeutic purposes. Therefore, we examined its ability to sensitize ovarian cancer cell lines to GCV by expressing the HSV-TK gene under its control. The widely used ovarian cancer cell line A2780 and colon carcinoma cell lines HT-29 and HCT116 cell lines were stably transfected with the plasmid PcOSP-1-TK, where HSV-TK gene expression was anticipated to be regulated by OSP-1 (Fig. 3A). Twenty randomly selected G418-resistant clones from each cell line were pooled and assessed for their sensitivity to GCV. The *in vitro* GCV toxicity was markedly increased in the A2780^{PcOSP-1-TK} pooled cell line when compared with HT-29^{PcOSP-1-TK} and HCT116^{PcOSP-1-TK} pooled cell lines (Table 1). Specifically, the IC₅₀ was found to be 9.6 μ M for the A2780^{PcOSP-1-TK} cell line, whereas GCV conferred only moderate cytotoxicity at high concentrations (30 μ M) on the HSV-TK transduced non-ovarian cell lines. GCV at high concentrations (30 μ M) is toxic to cells in the absence of functional HSV-TK. To determine whether the higher level of HSV-TK gene expression could enhance the sensitization to GCV, we selected the highest HSV-TK-expressing individual clones from each transfection by Northern blot analysis (Fig. 3B) for GCV treatment. The IC₅₀s for A2780^{PcOSP-1-TK} clones 1 and 2 were 0.4 and 0.5 μ M, respectively, whereas HSV-TK highest expressing HT-29^{PcOSP-1-TK} and HCT116^{PcOSP-1-TK} still were largely insensitive to GCV, with IC₅₀s at >23 μ M (Table 1).

In Vivo Antitumor Effect on A2780^{PcOSP-1-TK} Xenografts: In Vivo Cytotoxicity. We next examined GCV activity in xenograft models using A2780^{PcOSP-1-TK1}, A2780^{PcOSP-1-TK2}, and A2780^{Pc-TK}. The GCV treatment was given at various time points, including a GCV early treatment (low tumor burden) and late treatment regimen (high tumor burden). The GCV early treated mice received GCV (150 mg/kg/day) for 5 days beginning 8 days after tumor implantation. This GCV treatment was halted for 10 days and resumed at 50 mg/kg/day for another 7 days. For the GCV late treated mice, GCV treatment was started 23 days after tumor implantation, when small s.c. nodules were palpated in each mouse. This late treatment was carried out at a dosage of 50 mg/kg/day for 2 weeks. We found that in the GCV early treated mice, no tumor developed, even after 3 months of observation (Fig. 3C), indicating that in all probability the animals were cured. In

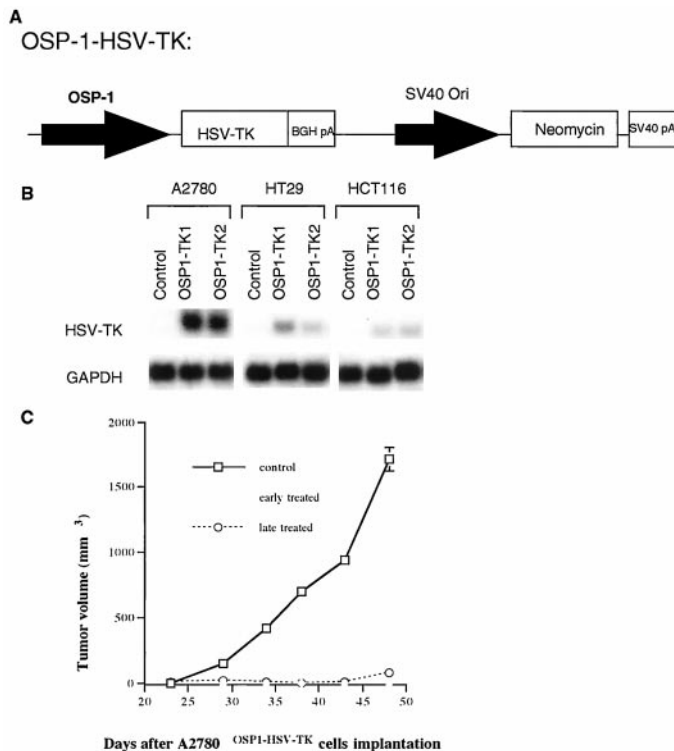


Fig. 3. A, OSP-1 promoter-driven HSV-TK expression vector. HSV-TK-containing expression vector, PcOSP-1-TK, was made by subcloning the HSV-TK gene into PcOSP-1 plasmid. The PcOSP-1 vector was created by replacing the CMV promoter with the OSP-1 promoter in the pCDNA3 vector. The PcOSP-1-TK plasmid contains neomycin resistance expression cassette under the control of the SV40 promoter. B, Northern blot analysis of HSV-TK gene expression in A2780, HT-29, and HCT-116 cell lines. Cell lines were stably transfected with PcOSP-1-TK or control plasmid pCDNA3 as described in "Materials and Methods." Ten μ g of total RNA from each sample were used for Northern analysis. The Northern blots were probed with ³²P-labeled HSV-TK and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) fragments. In Northern blot hybridization, identical quantities of ³²P-labeled probes were used, and the blots were exposed to an autoradiogram for similar time periods. C, GCV-mediated *in vivo* antitumor effect on A2780^{OSP-1-TK1} xenografts. A2780^{OSP-1-TK1} cells were injected s.c. on day 0 in three groups (control, early treated, and late treated) of mice. The mice were treated with GCV according to the treatment regimen. Tumor size was measured, and the volume was calculated using $a \times b^2 \times 1/2$. Each point represents the mean value of six animals; bars, SD. Essentially identical results were obtained in an independent experiment with another clonal cell line, A2780^{OSP-1-TK2}.

comparison, the tumor volume of the control vehicle-treated group became quite large, reaching nearly 2000 mm³ in volume (10% body weight) within 50 days. In the late treated group, tumor volume remained unchanged during the first 3 weeks of GCV treatment, but later the size gradually increased to a maximum of 2000 mm³ on day 80. Statistical analysis showed significant differences among these three groups ($P < 0.01$).

Discussion

In this study, we have described a potential OSP, OSP-1, that was isolated from a member of a family of retrovirus-like elements (OST units) specifically expressed in the rat ovary. We showed that OSP-1 indeed has transcriptional regulatory activity. This activity crosses species bounds and appears to be confined to epithelial cells of ovarian origin. Furthermore, the ability of OSP-1 to up-regulate gene expression is sufficiently strong that it may have utility in expressing therapeutic genes, such as those that activate prodrugs, *e.g.*, ganciclovir or 5-fluorocytosine.

OST units are widely distributed in the rat genome, and we have shown previously that they were transcriptionally active in the rat ovary but not in a wide range of other normal rat tissues, such as brain, heart, kidney, lung, spleen, uterus, fallopian tubes, and thymus (11). This ovary-specific expression pattern of the OST units prompted us to pursue the analysis and characterization of their means of activation. To isolate the putative regulatory elements in the U3 portion of the 5' LTR of OST units, the genomic template was reconstructed from a full-length transcript derived from one of these units. The database analysis of the putative promoter revealed the presence of many potential *cis*-regulatory units that could well be linked to ovarian physiology. Among these there were three estrogen response elements and five C/EBP β sites. The C/EBP β regulatory elements had been linked to the induction of gonadotropin (follicle-stimulating hormone) responsive genes (17). Besides known ovarian physiology relevant regulatory sequences, other known transcription factor binding elements were also found. We hypothesize that the correct combination of transacting factors to activate these OST units only occurs in the rat ovary.

The reporter gene analysis showed the transcriptional activity of OSP-1 promoter in 9 of 13 human ovarian cancer cell lines. The modest or absence of activity in PEO4, OVCAR7, UPN251, and OVCAR5 cell lines may account for the very nature of ovarian cancer biology, which is highly variable. Perhaps the transcription factors needed for OSP-1 promoter function are altered or absent in the correct combination in these cell lines.

It is well known that certain viral promoters, such as CMV and SV40, can constitutively activate genes in diverse cell types. However, certain viruses have organ-tropism, and they and their related retrovirus-like elements may show developmentally related and/or tissue-specific expression (18). OST units are the first such retrovirus-like elements to be found to be expressed in an ovary-specific manner. Although the present *in vitro* results are convincing that OSP-1 can drive gene expression in an ovarian epithelial cell-specific manner, our current efforts are designed to express *lacZ* gene under the control of the OSP-1 promoter in transgenic mice to more clearly ascertain the specificity of the OSP-1 promoter.

In conclusion, we isolated a DNA sequence that shows *cis*-regulatory activity in cells of ovarian lineage. Its activity is sufficiently strong to make it a candidate for the use in gene therapy of ovarian cancer, including regulation of prodrug-activating enzymes or to produce conditionally replicative viral vectors. Furthermore, the value of OSP-1 may not only be limited to cancer gene therapy, but it could also be of utility as a tool to create transgenic animals prone to develop ovarian cancer.

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