

Growth Suppression of Human Ovarian Cancer Cells by Adenovirus-mediated Transfer of the *PTEN* Gene

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Abstract

A tumor suppressor gene on chromosome 10q23, *PTEN*, encodes a phosphatidylinositol phosphatase that antagonizes activation of the phosphatidylinositol 3'-kinase-mediated pathway involved in cell growth. A gene encoding the catalytic subunit of phosphatidylinositol 3'-kinase (*PIK3CA*) is frequently activated in ovarian cancers; therefore, overexpression of the *PTEN* product through gene transfer might be an effective strategy for treating ovarian cancers. To test the potential for this type of gene therapy, we constructed a recombinant adenovirus encoding wild-type *PTEN* and examined its effects on nine cell lines derived from human ovarian carcinomas. Transduction of the *PTEN* gene significantly inhibited growth of six of these cell lines compared with infection with virus alone, and the degree of inhibition correlated with the efficiency of gene transfer as determined by β -galactosidase assay. Results of flow cytometry suggested that the observed effects were mediated by two mechanisms, apoptosis and/or arrest in the G₁ phase of the cell cycle, and that high adenoviral transduction efficiency of cells was associated with induction of apoptosis. We also found that the level of transcription of *Integrin α v* in ovarian cancer cells correlated with the efficiency of transduction ($P = 0.014$) and with the degree of growth inhibition after *PTEN* gene transfer ($P = 0.009$). These findings carry significant implications for adenovirus vector-based *PTEN* gene therapies for ovarian cancers.

Introduction

Among gynecological malignancies, ovarian cancer is the leading cause of death. Despite introduction of new chemotherapeutic agents into treatment protocols, to date no overall improvement has been achieved in long-term survival. Hence, developing alternative strategies is a matter of urgency. The *PIK3CA* gene on chromosome 3q26, which encodes the catalytic subunit of PI3K,² is frequently increased in copy number in ovarian cancers (1); PI3K mediates a major growth-control pathway, acting both to stimulate cell growth and to block apoptosis (2–4). This pathway is antagonized by a tumor suppressor gene (*PTEN*) on chromosome 10q23, which encodes a phosphatidylinositol phosphatase. The *PTEN* product opposes activation of phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate, second messengers downstream of PI3K (5–7). Although mutations of the *PTEN* gene were reported to be rare in ovarian cancers (8, 9), gene transfer may be an effective therapy for this type of tumors, if a high dose of the *PTEN* product is able to block the activated PI3K-mediated cell growth pathway. Transfection of a *PTEN* expression plasmid into glioma cells indeed can suppress

growth by arresting cells in the G₁ phase (10). Moreover, adenovirus-mediated *PTEN* gene transfer into glioma cells is able to suppress tumorigenicity (11) and induces apoptosis initiated by disruption of the interactions of the cells with the extracellular matrix (12).

To test the potential for *PTEN* gene therapy of ovarian cancers, we introduced wild-type *PTEN* genes into each of nine cell lines derived from human ovarian carcinomas and correlated the effect of exogenous *PTEN* on transcription of *PTEN*, *PIK3CA*, and receptors for the adenovirus vector. We found that the level of transcription of *Integrin α v* in these cell lines correlated with the efficiency of transduction and also with the extent of growth inhibition by overexpressed *PTEN*. Flow cytometry suggested that the growth-inhibitory activity of *PTEN* was mediated by two mechanisms, apoptosis and/or cell cycle arrest in the G₁ phase, and that high adenoviral transduction efficiency of cells associated with induction of apoptosis. Our results carry significant implications for development of adenovirus vector-based gene therapies for this disease.

Materials and Methods

Ovarian Cancer Cell Lines. Cell lines Caov-3, ES-2, MDAH 2774, NIH: OVCAR-3, OV-1063, SK-OV-3, and SW 626 were obtained from the American Type Culture Collection (Manassas, VA). MCAS and TYK-nu cell lines were obtained from the Japanese Collection of Research Bioresources (Tokyo, Japan). NIH:OVCAR-3 and OV-1063 cells were maintained in RPMI 1640 with 10% FBS. ES-2 and SK-OV-3 cells were grown in McCoy's 5A medium with 10% FBS. MDAH 2774 and SW 626 cells were maintained in Leibovitz's L-15 medium with 10% FBS. MCAS and TYK-nu cells were grown in Eagle's MEM with 10% FBS, and Caov-3 cells were maintained in DMEM with 10% FBS.

RT-PCR Analysis. Using a semiquantitative RT-PCR method, we assessed transcription levels of *PTEN*, *PIK3CA*, *HCAR*, *Integrin α v*, *Integrin β 3*, and *Integrin β 5* relative to β 2-microglobulin in all nine ovarian cancer cell lines. Total RNAs were isolated using Trizol reagent (Life Technologies, Inc.), treated with DNase I, and reverse-transcribed using Superscript II reverse transcriptase (Life Technologies, Inc.). Each RT-PCR reaction consisted of 25 or 30 cycles of 30 s at 94°C, 30 s at 55°C (for *PTEN*, *PIK3CA*, and β 2-microglobulin) or 60°C (for *Integrin α v*, β 3, and β 5) and 1 min at 72°C. Amplification of β 2-microglobulin revealed similar signal strengths in all samples, as a control for the integrity of each RNA template. PCR products were electrophoresed in 2% agarose gels, blotted onto nylon membranes, and hybridized with [γ -³²P]ATP-labeled internal primers. Signal intensities were measured with a bioimaging analyzer (BAS 1000; Fujifilm) and autoradiography. The RNA preparations were checked for contamination by genomic DNA by comparing PCR reactions for all sets of primers with or without reverse transcriptase. Primers used for amplification and internal probes for hybridization were as follows:

PTEN: forward (F), 5'-CTT CAG CCA CAG GCT CCC A-3'; reverse (R), 5'-TGG TGT TTT ATC CCT CTT GAT A-3'; and internal (I), 5'-GAC CAA TGG CTA AGT GAA GAT-3'

PIK3CA: F, 5'-GTA TGT CTA TCC GCC ACA TGT-3'; R, 5'-CAG TCA TGG TTG ATT TTC AGA G-3'; and I, 5'-TCA CCA GAA TTG CCA AAG CAC-3'

HCAR: F, 5'-ACA ACT GTC AGA TAT TGG CAC-3'; R, 5'-GAT GAA

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² The abbreviations used are: PI3K, phosphatidylinositol 3'-kinase; FBS, fetal bovine serum; RT-PCR, reverse transcription-PCR; MOI, multiplicity of infection.

TGA TTA CTG CCG ATG-3'; and I, 5'-GAA GTT CAT CAC GAT ATC AG-3'

Integrin α : F, 5'-GAC TCC TGC TAC CTC TGT G-3'; R, 5'-GCT CTC GCT CCT GTT TCA TC-3'; and I, 5'-TTC AAC CTA GAC GTG GAC AG-3'

Integrin β 3: F, 5'-AGG ATT ACC CTG TGG ACA TC-3'; R, 5'-TCA CTA CCA ACA TGA CAC TG-3'; and I, 5'-GTG AAG AAG CAG AGT GTG TC-3'

Integrin β 5: F, 5'-GGA CGT CAT TCA GAT GAC AC-3'; R, 5'-TGG TTG GAT GCA GTG TAC TC-3'; and I, 5'-GGA CGT CAT TCA GAT GAC AC-3'

β 2-microglobulin: F, 5'-CAC CCC CAC TGA AAA AGA TGA-3'; R, 5'-TAC CTG TGG AGC AAC CTG C-3'; and I, 5'-ATC TTC AAC CTC CAT GAT G-3'

Construction of Recombinant Adenovirus and Conditions of Infection.

To construct AdCAPTEN viruses, the cDNA of *PTEN* was cloned by RT-PCR using placental mRNA as a template and the following primers: 5'-CTT CAGCCACAGGCTCCCA-3' and 5'-TGGTGTTTTATCCTCTTGATA-3'. A 1.2-kb blunt-ended fragment of *PTEN* cDNA was inserted into the *Swa*I site of the cosmid pAxCawt (TaKaRa; Ref. 13) that contained the CAG promoter (composed of the cytomegalovirus enhancer and chicken β -actin promoter; Ref. 14) and an entire genome of type 5 adenovirus except for the E1 and E3 regions. This procedure generated pAxCAPTEN. Recombinant adenoviruses were constructed by *in vitro* homologous recombination in the human embryonic kidney cell line 293 using pAxCAPTEN and the adenovirus DNA terminal-protein complex (TaKaRa; Ref. 13). As a control, AdCA viruses were generated from the cosmid pAxCawt without a transgene. AdCALacZ viruses encoding the β -galactosidase gene under the control of the CAG promoter were constructed from the control cosmid pAxCaiLacZ (TaKaRa). Viruses were propagated in the 293 cell line and purified by two rounds of CsCl density centrifugation. Viral titers were measured in a limiting-dilution bioassay using the 293 cells. Cancer cells were infected by viral solutions to cell monolayers and incubated at 37°C for 1 h with brief agitation every 15 min. Culture medium was added, and the infected cells were returned to the 37°C incubator.

Immunodetection of PTEN Protein. SK-OV-3 cells were infected with AdCAPTEN at a MOI of 100 plaque-forming units/cell. After 0, 6, 12, 18, and 24 h, the cells were washed twice with PBS and harvested in lysis buffer [150 mM NaCl, 1% Triton X-100, 50 mM Tris-HCl (pH 7.4), 1 mM DTT, and 1 \times Complete Protease Inhibitor Cocktail (Boehringer Mannheim)]. After cells were homogenized and centrifuged at 10,000 \times g for 30 min, the supernatants were standardized for protein concentration by the Bradford assay (Bio-Rad). Proteins were separated by 8% SDS-PAGE and immunoblotted with goat anti-PTEN and goat anti-actin polyclonal antibodies (Santa Cruz Biotechnology). Horseradish peroxidase-conjugated donkey anti-goat IgG (Santa Cruz Biotechnology) served as the secondary reagent for the ECL Detection System (Amersham).

Cellular Proliferation Assay. Each line of ovarian cancer cells was seeded at 1 \times 10⁴ cells/well in six-well culture plates for 20 h before viral infection. The cells were infected in triplicate with AdCAPTEN or AdCA at a MOI of 5, 20, or 100. Triplicate dishes of each treatment were counted by the trypan blue exclusion method using a hemocytometer on the 5th day after infection.

β -Galactosidase Transduction Assay. To assess efficiency of the adenovirus-mediated gene transfer, 1 \times 10⁵ cells were plated in triplicate in six-well plates and infected 20 h later with AdCALacZ at 100 MOI for 1 h. Virus-containing solutions were removed at 1 h; cells were washed with PBS, refed with culture medium, and returned to the 37°C incubator. After 24 h, the cells were fixed with 2% paraformaldehyde/PBS, washed, and stained in a 1 mg/ml

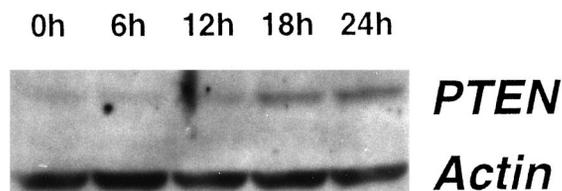


Fig. 1. Chronicity of expression of PTEN protein in transfected ovarian cancer cell line SK-OV-3. Proteins were extracted from the cells at the time points indicated. The levels of PTEN expression were compared with expression of actin.

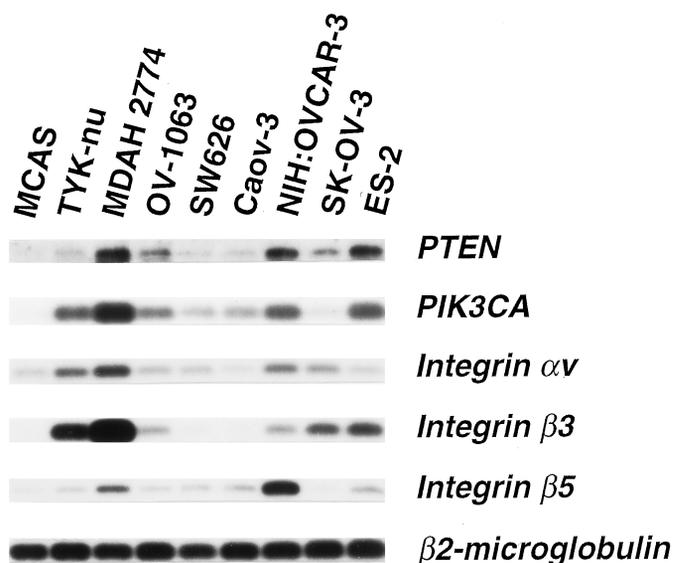


Fig. 2. Semiquantitative RT-PCR analysis of the transcription levels of *PTEN*, *PIK3CA*, and *Integrin* subunits α , β 3, and β 5 in all nine cell lines. The integrity of each RNA template was controlled through amplification of β 2-microglobulin.

5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside solution consisting of 0.1 M sodium phosphate (pH 7.3), 1.3 mM MgCl₂, 3 mM K₃[Fe(CN)₆], and 3 mM K₄[Fe(CN)₆] in PBS. β -galactosidase-positive cells in triplicate wells were counted microscopically.

Flow Cytometry. Cells were plated at a density of 5 \times 10⁵ cells/100-mm dish and infected 20 h later with 100 MOI of AdCAPTEN or AdCA. Forty-eight h after infection, cells were trypsinized, collected in PBS, and fixed in 70% cold ethanol. After RNase treatment, cells were stained with 50 μ g/ml propidium iodide in PBS. Flow cytometry was performed on a Becton Dickinson FACScan and analyzed by ModFit software (Verity Software House, Inc., Topsham, ME). The percentages of nuclei in G₀-G₁, S, and G₂-M phases of the cell cycle and sub-G₁ population were determined from at least 20,000 ungated cells.

Results and Discussion

Expression of recombinant *PTEN*-containing adenovirus (AdCAPTEN) was examined in the ovarian cancer cell line SK-OV-3 by Western blotting of cell lysates (Fig. 1). Overexpression of *PTEN* protein was detected as early as 18 h after infection. To evaluate the effect of the transgene on proliferation of ovarian cancer cells, we selected an additional eight cell lines derived from human ovarian carcinomas. We examined genetic alterations of the *PTEN* gene in each line by direct sequencing of the coding region and found that none contained mutations in this gene. We also examined the transcription level of *PTEN* and *PIK3CA* in each line, using semiquantitative RT-PCR (Fig. 2). Interestingly, cell lines showing a high level of endogenous *PTEN* transcription showed a high level of *PIK3CA*, and those showing a very low level of *PTEN* expression revealed a relatively low level of *PIK3CA* expression except one cell line, TYK-nu.

To examine effects of *PTEN* transfection using the recombinant adenovirus on the cellular proliferation, 1 \times 10⁴ cells of each ovarian cancer lines were infected in triplicate with AdCAPTEN or AdCA at a MOI of 5, 20, or 100, and the cell numbers were counted on the 5th day after infection. As shown in Fig. 3, six of the nine cell lines, MDAH 2774, TYK-nu, SW 626, NIH:OVCAR-3, OV-1063, and SK-OV-3, showed significant decreases in cell number by infection with AdCAPTEN compared with cells infected with control virus alone; the numbers of these cells were decreased to 13–38% of the control cells. The remaining three cell lines, Caov-3, ES-2, and

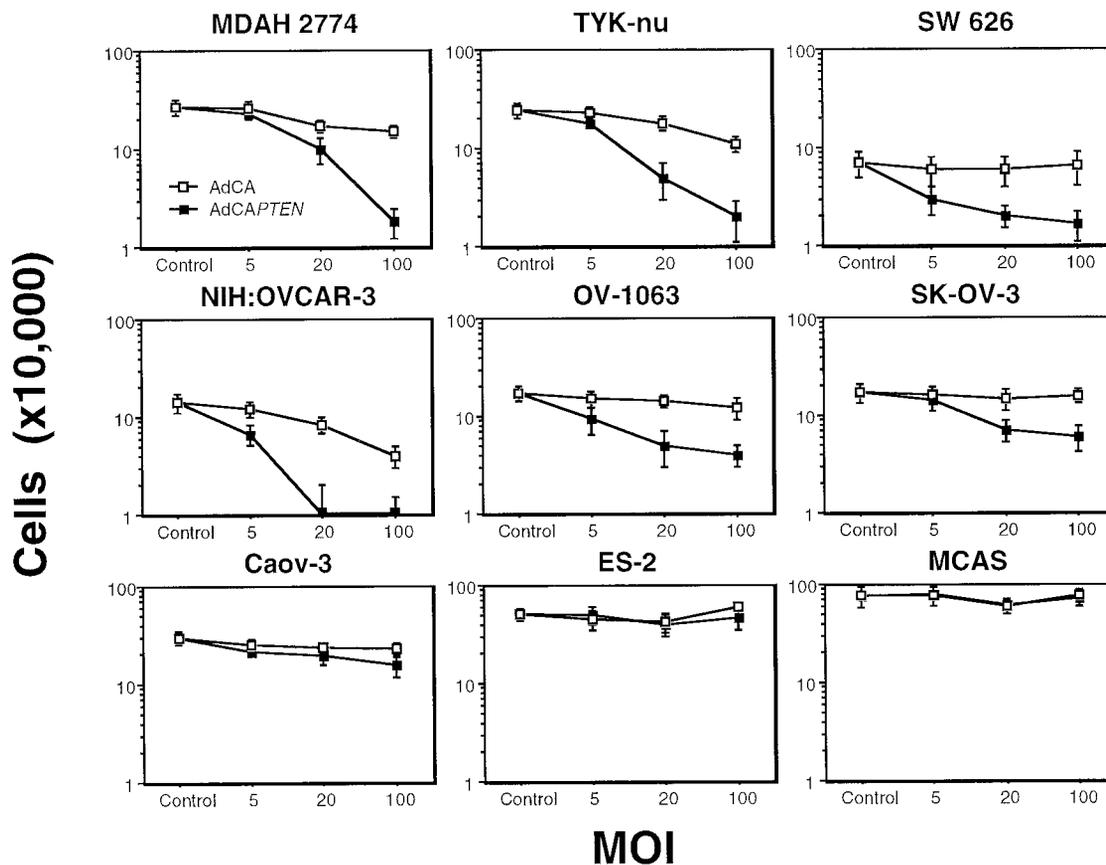


Fig. 3. Inhibition of human ovarian cancer cells by AdCAPTEN. Nine cell lines were infected with a PTEN-expressing viral construct (AdCAPTEN) or control virus (AdCA). Mean cell numbers on the 5th day after infection are plotted; bars, SD (three replicates).

MCAS, had little or no effect by transfection of AdCAPTEN. These findings indicate that genotypically wild-type PTEN cells can be growth inhibited by a high dose of exogenous PTEN. Similar results were reported by Li *et al.* (15), where adenoviral PTEN gene transfer suppressed the growth of breast cancer cells, regardless of the mutational status of endogenous PTEN.

To investigate a relationship between the efficiency of the gene transfers by the virus vector and the growth-suppressive effects, we performed β -galactosidase transduction assays using AdCALacZ, which carries the β -galactosidase gene of *Escherichia coli*. Six of the nine ovarian cancer cell lines that revealed significant growth suppression in the cellular proliferation assay by transfection of AdCAPTEN showed transduction efficiencies as high as 70–100%

when transfected at a MOI of 100 (Table 1). In contrast, the two cell lines, ES-2 and MCAS, that had no growth-suppressive effect showed 12 and 9% efficiencies of β -galactosidase transduction, respectively.

To further examine the molecular mechanisms of growth suppression, we performed flow cytometric analysis and found that a marked increase in the sub-G₁ population in three of the six cell lines that showed significant growth suppression, MDAH 2774, TYK-nu, and SW 626 (Fig. 4). Among the other three growth-inhibited cell lines, the two lines NIH:OVCAR-3 and SK-OV-3 showed a moderate increase of sub-G₁ population and a significant increase in the proportion of G₀-G₁ phase population. OV-1063 showed a moderate increase in the sub-G₁ population. The cell line Caov-3, which showed a little growth suppression and moderate (44%) gene trans-

Table 1 PTEN gene transfer to ovarian cancer cells and quantified transcription levels of PTEN, PIK3CA, and adenovirus receptors

Cell line	Growth inhibition ^{a,b} (%)	Transduction efficiency ^{b,c} (%)	PTEN ^d	PIK3CA ^d	Integrin subunit ^d		
					αv ^b	$\beta 3$	$\beta 5$
MDAH 2774	87	100	1.8	5.1	2.4	23.4	1.0
TYK-nu	82	93	0.2	1.5	1.0	3.5	0.1
SW 626	75	100	0.1	1.2	0.7	0.4	0.4
NIH:OVCAR-3	75	83	1.0	1.9	1.0	1.0	1.4
OV-1063	67	70	0.5	1.2	0.5	0.8	0.3
SK-OV-3	62	71	0.3	0.6	0.7	1.9	0.0
Caov-3	32	44	0.1	1.0	0.3	0.2	0.1
ES-2	23	12	1.2	2.3	0.4	2.5	0.4
MCAS	15	9	0.2	0.3	0.3	0.0	0.0

^a The growth-inhibitory effects of AdCAPTEN infection were calculated as $(x - y)/x$ (x , mean cell number 5 days after AdCA infection at 100 MOI; y , mean cell number 5 days after AdCAPTEN infection at 100 MOI).

^b The correlations between transcription levels of Integrin αv and adenoviral transduction efficiencies or growth-inhibitory effects were both statistically significant (Integrin αv versus growth inhibition, $P = 0.009$, $\rho = 0.924$; Integrin αv versus transduction efficiency, $P = 0.014$, $\rho = 0.869$; Spearman's rank correlation).

^c Mean percentages of 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside-positive cells were measured microscopically 24 h after AdCALacZ infection at 100 MOI.

^d The transcription levels of PTEN, PIK3CA, Integrin αv , Integrin $\beta 3$, and Integrin $\beta 5$ were examined using semiquantitative RT-PCR. The intensity of each band was calculated as a ratio against the intensity of the corresponding β_2 -microglobulin band by densitometric analysis.

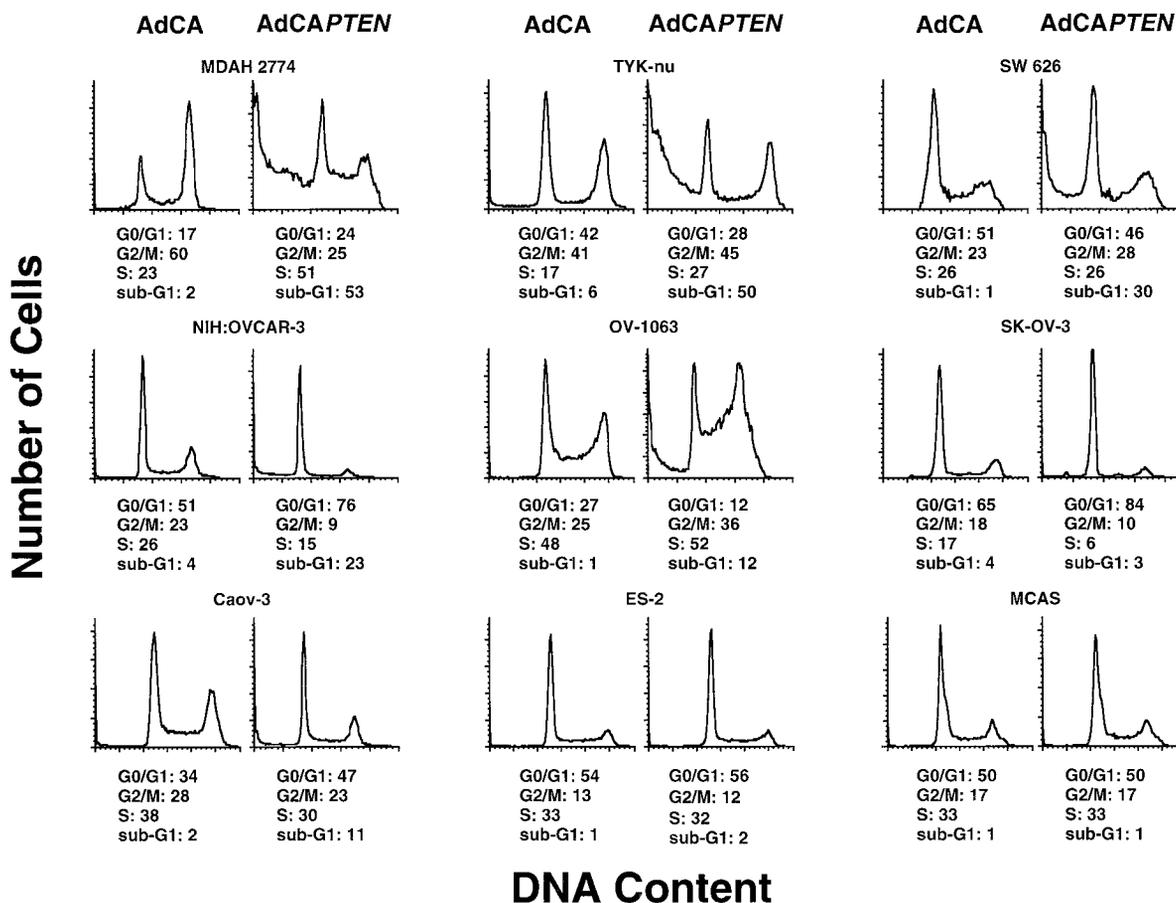


Fig. 4. Flow cytometric analysis of AdCA or AdCAPTEN-infected ovarian cancer cells. A representative profile from a single experiment is shown for each cell line analyzed, along with the percentages of the cell cycle phase distribution and sub-G₁ population.

duction efficiency, revealed a moderate increase of sub-G₁ population and a moderate increase in the proportion of G₀-G₁ phase population. The two cell lines that had no growth-suppressive effect showed no alterations by flow cytometric analysis (Fig. 4). These findings suggested that the growth-inhibitory activity of exogenous PTEN is mediated by two mechanisms, apoptosis and/or arrest at the G₁ phase of the cell cycle, and that high adenoviral transduction efficiency of cells was associated with induction of apoptosis by *PTEN* gene transfer.

Expression of fiber receptors (HCAR in particular) and of α v integrins β 3 and β 5 is thought to be required for efficient entry of adenovirus into cells and subsequent gene transfer (16–21). Therefore, we examined transcription of HCAR and of integrin subunits α v, β 3, and β 5 by RT-PCR analysis (Fig. 2). No transcript of HCAR was detected, even after 30 cycles of PCR amplification, a result consistent with Northern blot analyses reported by Tomko *et al.* (21). According to those investigators, no signal was detected in the ovary, whereas the brain revealed the highest level of HCAR transcription among the tissues tested. Susceptibility of glioma cells to adenovirus-mediated gene transfer has been correlated with expression of HCAR but not α v integrins (22). Although we detected no HCAR expression in the ovarian cancer cell lines examined, we obtained transduction efficiencies comparable with those reported in glioma cells (23). Hence, we suspect that ovarian cells express molecules having functions similar to HCAR. Furthermore, our RT-PCR analysis found that transcription of *Integrin α v* in ovarian cancer cells correlated significantly with the efficiency of adenoviral gene transfer ($P = 0.014$; $P = 0.869$) and also with the degree of growth inhibition by exogenous PTEN

($P = 0.009$ and $P = 0.924$; Spearman's rank correlation; Table 1). These findings suggest that different limiting factors affect adenoviral gene transfer, and that they may be tissue specific. Vanderkwaak *et al.* (24) reported that incorporation of an integrin-binding RGD motif to the adenovirus fiber knob enhanced the gene transfer efficiency by modified adenovirus vector in the context of ovarian cancer.

We have demonstrated here that recombinant adenovirus expressing wild-type PTEN can significantly inhibit the growth of ovarian cancer cells through two mechanisms, apoptosis and/or cell cycle arrest at the G₁ phase. Moreover, we have shown that transcription of *Integrin α v* in these cells correlates with the efficiency of gene transfer and with the growth-inhibitory effect of transduced *PTEN*. Our data suggest that adenovirus-mediated transfer of the *PTEN* gene may be a potential approach for treatment of patients with ovarian cancer.

References

- Shayesteh, L., Lu, Y., Kuo, W. L., Baldocchi, R., Godfrey, T., Collins, C., Pinkel, D., Powell, B., Mills, G. B., and Gray, J. W. PIK3CA is implicated as an oncogene in ovarian cancer. *Nat. Genet.*, *21*: 99–102, 1999.
- Downward, J. Ras signaling and apoptosis. *Curr. Opin. Genet. Dev.*, *8*: 49–54, 1998.
- Klippel, A., Escobedo, M. A., Wachowicz, M. S., Apell, G., Brown, T. W., Giedlin, M. A., Kavanaugh, W. M., and Williams, L. T. Activation of phosphatidylinositol 3-kinase is sufficient for cell cycle entry and promotes cellular changes characteristic of oncogenic transformation. *Mol. Cell. Biol.*, *18*: 5699–5711, 1998.
- Kennedy, S. G., Wagner, A. J., Conzen, S. D., Jordan, J., Bellacosa, A., Tsichlis, P. N., and Hay, N. The PI 3-kinase/Akt signaling pathway delivers an anti-apoptotic signal. *Genes Dev.*, *11*: 701–713, 1997.
- Maehama, T., and Dixon, J. E. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *J. Biol. Chem.*, *273*: 13375–13378, 1998.

6. Myers, M. P., Pass, I., Batty, I. H., Van der Kaay, J., Stolarov, J. P., Hemmings, B. A., Wigler, M. H., Downes, C. P., and Tonks, N. K. The lipid phosphatase activity of PTEN is critical for its tumor suppressor function. *Proc. Natl. Acad. Sci. USA*, *95*: 13513–13518, 1998.
7. Stambolic, V., Suzuki, A., de la Pompa, J. L., Brothers, G. M., Mirtsos, C., Sasaki, T., Ruland, J., Penninger, J. M., Siderovski, D. P., and Mak, T. W. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell*, *95*: 29–39, 1998.
8. Maxwell, G. L., Risinger, J. I., Tong, B., Shaw, H., Barrett, J. C., Berchuck, A., and Futreal, P. A. Mutation of the *PTEN* tumor suppressor gene is not a feature of ovarian cancers. *Gynecol. Oncol.*, *70*: 13–16, 1998.
9. Yokomizo, A., Tindall, D. J., Hartmann, L., Jenkins, R. B., Smith, D. I., and Liu, W. Mutation analysis of the putative tumor suppressor PTEN/MMAC1 in human ovarian cancer. *Int. J. Oncol.*, *13*: 101–105, 1998.
10. Fumari, F. B., Huang, H. J., and Cavenee, W. K. The phosphoinositol phosphatase activity of PTEN mediates a serum-sensitive G₁ growth arrest in glioma cells. *Cancer Res.*, *58*: 5002–5008, 1998.
11. Cheney, I. W., Johnson, D. E., Vaillancourt, M. T., Avanzini, J., Morimoto, A., Demers, G. W., Wills, K. N., Shabram, P. W., Bolen, J. B., Tavtigian, S. V., and Bookstein, R. Suppression of tumorigenicity of glioblastoma cells by adenovirus-mediated *MMAC1/PTEN* gene transfer. *Cancer Res.*, *58*: 2331–2334, 1998.
12. Davies, M. A., Lu, Y., Sano, T., Fang, X., Tang, P., LaPushin, R., Koul, D., Bookstein, R., Stokoe, D., Yung, W. K., Mills, G. B., and Steck, P. A. Adenoviral transgene expression of MMAC/PTEN in human glioma cells inhibits Akt activation and induces anoikis. *Cancer Res.*, *58*: 5285–5290, 1998.
13. Miyake, S., Makimura, M., Kanegae, Y., Harada, S., Sato, Y., Takamori, K., Tokuda, C., and Saito, I. Efficient generation of recombinant adenoviruses using adenovirus DNA-terminal protein complex and a cosmid bearing the full-length virus genome. *Proc. Natl. Acad. Sci. USA*, *93*: 1320–1324, 1996.
14. Niwa, H., Yamamura, K., and Miyazaki, J. Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene (Amst.)*, *108*: 193–199, 1991.
15. Li, J., Simpson, L., Takahashi, M., Miliareisis, C., Myers, M. P., Tonks, N., and Parsons, R. The PTEN/MMAC1 tumor suppressor induces cell death that is rescued by the AKT/protein kinase B oncogene. *Cancer Res.*, *58*: 5667–5672, 1998.
16. Wickham, T. J., Mathias, P., Cheresch, D. A., and Nemerow, G. R. Integrins $\alpha\beta 3$ and $\alpha\beta 5$ promote adenovirus internalization but not virus attachment. *Cell*, *73*: 309–319, 1993.
17. Henry, L. J., Xia, D., Wilke, M. E., Deisenhofer, J., and Gerard, R. D. Characterization of the knob domain of the adenovirus type 5 fiber protein expressed in *Escherichia coli*. *J. Virol.*, *68*: 5239–5246, 1994.
18. Wickham, T. J., Roelvink, P. W., Brough, D. E., and Kovesdi, I. Adenovirus targeted to heparan-containing receptors increases its gene delivery efficiency to multiple cell types. *Nat. Biotechnol.*, *14*: 1570–1573, 1996.
19. Hong, S. S., Karayan, L., Tournier, J., Curiel, D. T., and Boulanger, P. A. Adenovirus type 5 fiber knob binds to MHC class I $\alpha 2$ domain at the surface of human epithelial and B lymphoblastoid cells. *EMBO J.*, *16*: 2294–2306, 1997.
20. 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. Isolation of a common receptor for coxsackie B viruses and adenoviruses 2 and 5. *Science (Washington DC)*, *275*: 1320–1323, 1997.
21. Tomko, R. P., Xu, R., and Philipson, L. HCAR and MCAR: the human and mouse cellular receptors for subgroup C adenoviruses and group B coxsackieviruses. *Proc. Natl. Acad. Sci. USA*, *94*: 3352–3356, 1997.
22. Miller, C. R., Buchsbaum, D. J., Reynolds, P. N., Douglas, J. T., Gillespie, G. Y., Mayo, M. S., Raben, D., and Curiel, D. T. Differential susceptibility of primary and established human glioma cells to adenovirus infection: targeting via the epidermal growth factor receptor achieves fiber receptor-independent gene transfer. *Cancer Res.*, *58*: 5738–5748, 1998.
23. Gomez-Manzano, C., Fueyo, J., Kyritsis, A. P., Steck, P. A., Roth, J. A., McDonnell, T. J., Steck, K. D., Levin, V. A., and Yung, W. K. Adenovirus-mediated transfer of the *p53* gene produces rapid and generalized death of human glioma cells via apoptosis. *Cancer Res.*, *56*: 694–699, 1996.
24. Vanderkwaak, T. J., Wang, M., Gomez-Navarro, J., Rancourt, C., Dmitriev, I., Krasnykh, V., Barnes, M., Siegal, G. P., Alvarez, R., and Curiel, D. T. An advanced generation of adenoviral vectors selectively enhances gene transfer for ovarian cancer gene therapy approaches. *Gynecol. Oncol.*, *74*: 227–234, 1999.

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