

Growth and Gene Expression Profile Analyses of Endometrial Cancer Cells Expressing Exogenous PTEN

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ABSTRACT

The *PTEN* tumor suppressor gene encodes a multifunctional phosphatase that plays an important role in inhibiting the phosphatidylinositol-3-kinase pathway and downstream functions that include activation of Akt/protein kinase B, cell survival, and cell proliferation. Enforced expression of *PTEN* in various cancer cell lines decreases cell proliferation through arrest of the cell cycle, accompanied in some cases by induction of apoptosis. We used cDNA microarrays containing 4009 cDNAs to examine changes in gene-expression profiles when exogenous *PTEN* was induced in *PTEN*-defective cells. The microarrays and subsequent semi-quantitative reverse transcription-PCR analysis revealed transcriptional stimulation of 99 genes and repression of 72 genes. Some of the differentially expressed genes already had been implicated in cell proliferation, differentiation, apoptosis, or cell cycle control, e.g., overexpression of *PTEN*-induced transactivation of *cyclin-dependent inhibitor 1B (p27Kip1)* and *2B (p15INK4B)*, members of the TNF receptor family, tumor necrosis factor-associated genes, and members of the Notch-signaling and Mad families. To our knowledge this is the first report of transactivation of those genes by *PTEN*. The genes differentially expressed in our experiments also included many whose correlation with cancer development had not been recognized before. Our data should contribute to a greater understanding of the broad spectrum of ways in which *PTEN* affects intracellular signaling pathways. Analysis of expression profiles with microarrays appears to be a powerful approach for identifying anticancer genes and/or disease-specific targets for cancer therapy.

INTRODUCTION

The *PTEN/MMAC1*² gene on chromosome 10q23.3 has been identified as a tumor suppressor (1, 2). Deletions and mutations in *PTEN* occur frequently in advanced carcinomas of endometrium, breast, bladder, and endometrioid ovarian tissue and also in glioblastomas and malignant melanomas (1–4). Germ-line mutations of *PTEN* are the cause of Cowden's and Bannayan-Zonana syndromes of cancer predisposition (5, 6).

A recent study demonstrated that *PTEN* acts as a phospholipid phosphatase dephosphorylating the PtdIns (3,4,5)P₃ and PtdIns (3,4)P₂, resulting in PtdIns (4,5)P₂ and PtdIns (4)P, respectively (7). It modulates the PI3K pathway by catalyzing degradation of the PtdIns (3,4,5)P₃ generated by PI3K. This mechanism inhibits downstream functions mediated by the PI3K pathway, such as activation of

Akt/protein kinase B, cell survival, and cell proliferation (8). Overexpression of *PTEN* can decrease cell proliferation and tumorigenicity (9, 10), an observation attributed to the ability of *PTEN* to induce cell cycle arrest and apoptosis (11, 12).

Thus, lack of *PTEN* expression may affect a complex set of transcriptional targets. However, no systematic assessment of *PTEN*-regulated targets in cancer cells has been reported to date. We used a cDNA microarray to investigate transcriptional changes related to *PTEN* signaling or secondary changes caused by the initiation of apoptosis or cell cycle arrest by transfecting wild-type *PTEN* into cancer cells that were deficient in endogenous *PTEN*. Microarray technology, which can permit analysis of the expression of thousands of genes simultaneously, is a proven method for investigating gene-expression profiles in human cancers (13, 14).

MATERIALS AND METHODS

Cell Culture. The 14 endometrial cancer cell lines used in this study are described in Table 1. Ishikawa3-H-12 (15, 16) was obtained from Tsukuba University (Tsukuba, Japan). HEC-1 (17), HEC-6 (18), HEC-50 (19), HEC59 (20), HEC-88 (20), HEC-108 (20), HEC-116 (20), and HEC-151 (21) were obtained from Kitasato University (Sagami-hara, Japan). AN3CA and KLE were obtained from the American Type Culture Collection (Manassas, VA), and Sawano (22), HHUA, and HOUA-I were obtained from the RIKEN Gene Bank (Tsukuba, Japan). Ishikawa3-H-12, Sawano, HEC-1, HEC-6, HEC-50, HEC59, HEC-88, HEC-108, HEC-116, HEC-151, and AN3CA cells were maintained in Eagle's MEM with 10% FBS. HHUA and HOUA-I cells were grown in Ham's F12 medium with 10% FBS. KLE cells were grown in 1:1 mixture of DMEM and Ham's F12 medium with 10% FBS.

DNA Extraction and Mutation Screening. Genomic DNA was extracted from each cell line using standard methods. All nine coding exons of *PTEN* were amplified from these DNA samples, and their nucleotide sequences were determined by direct sequencing using BigDye Terminator RR Mix (Applied Biosystems, Foster City, CA) on an ABI model 3700 DNA sequencer (Applied Biosystems) to reveal any mutations in the *PTEN* gene.

Construction of Recombinant Adenovirus. To construct AdCAPTEN viral vectors, the cDNA of *PTEN* was cloned by RT-PCR using placental mRNA as a template and primers 5'-CTT CAGCCACAGGCTCCCA-3' and 5'-TGGTGTTTTATCCCTCTTGATA-3'. A 1.2-kb fragment of *PTEN* cDNA was cloned into the Swal site of cosmid pAxCawt (TaKaRa; Ref. 23), which contains the CAG promoter (composed of the cytomegalovirus enhancer and chicken β -actin promoter; Ref. 24) and an entire genome of type 5 adenovirus except for the E1 and E3 regions. Recombinant adenoviruses were constructed by *in vitro* homologous recombination in the human embryonic kidney (HEK) cell line 293, using pAxCAPTEN and the adenovirus DNA terminal-protein complex (TaKaRa; Ref. 23). As a control, AdCA viruses were generated from cosmid pAxCawt without a transgene. AdCALacZ viruses encoding the β -gal gene under the control of the CAG promoter were constructed from the control cosmid pAxCALacZ (TaKaRa). Viruses were propagated in the HEK293 cell line and purified by two rounds of CsCl density centrifugation; viral titers were measured in a limiting-dilution bioassay using the HEK293 cells. In this study, we used recombinant adenovirus stocks of fewer than five passages to minimize the possibility of replication-competent adenovirus contamination.

β -Galactosidase Transduction Assay. To assess efficiency of adenovirus-mediated gene transfer, 1×10^5 cells from each culture were plated in

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² The abbreviations used are: *PTEN*, phosphatase and tensin homologue deleted on chromosome 10; *MMAC1*, mutated in multiple advanced cancers 1; PtdIns, phosphatidylinositol; P₃, triphosphate; P₂, biphosphate; PI3K, phosphatidylinositol-3-kinase; FBS, fetal bovine serum; MOI, multiplicity of infection; β -gal, β -galactosidase; MTT, 3, (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; RT-PCR, reverse transcription-PCR; TNF, tumor necrosis factor; PI, propidium iodide.

Table 1 Characteristics of endometrial cancer cell lines (PTEN status and transduction efficiency)

Cell line	PTEN status ^a			Transduction efficiency (%) ^b		Source ^c
	Codon	Mutation	Predicted effect	MOI 20	MOI 100	
HHUA	164	1bp(A) del	Frameshift	48.2	89.7	1
	289	1bp(A) del	Frameshift			
HOUA-1	267	1bp(A) ins	Frameshift	44.4	94.8	1
	289	1bp(A) ins	Frameshift			
Ishikawa 3-H-12	289	1bp(A) del	Frameshift	66.5	100.0	2
	317-318	4bp(ACTT) del	Frameshift			
Sawano	16	2bp(AT) del	Frameshift	27	94.8	2
	130	CGA to GGA	Arg(R) to Gly(G)			
HEC-1		None		79.3	100.0	3
HEC-6	intron 4(+2)	T to C	Splice donor	44	79.5	3
	289	1bp(A) del	Frameshift			
HEC-50		None		43.5	100	3
HEC-59	41	TAC to CAC	Tyr(Y) to His(H)	68.3	100.0	3
	233	CGA to TGA	Stop			
	246	CCG to CTG	Pro(P) to Leu(L)			
	267	1bp(A) del	Frameshift			
HEC-88	130	CGA to GGA	Arg(R) to Gly(G)	95.5	100.0	3
	173	CGC to TGC	Arg(R) to Cys(C)			
	310	GAT to TAT	Asp(D) to Tyr(Y)			
	341	TTT to TGT	Phe(F) to Cys(C)			
HEC-108	6	2bp(AA) del	Frameshift	66.7	100.0	3
	289	1bp(A) del	Frameshift			
HEC-116	Intron 2(-1)	G to A	Splice acceptor	57.6	100.0	3
	173	CGC to TGC	Arg(R) to Cys(C)			
	233	CGA to TGA	Stop			
HEC-151	33	3bp(ATT) del	In-frame deletion	78	100.0	3
	76	2bp(AT) del	Frameshift			
AN3CA	130	1bp(G) del	Frameshift	0.9	5.3	4
KLE		None		66.3	97.3	4

^a PTEN mutations were detected by DNA sequencing.

^b Percentage of β -gal-positive cells 24 h after infection with AdCALacZ. Data represent means of triplicate measurements.

^c Original sources of cell lines: 1, Japanese Collection of Research Bioresources; 2, University of Tsukuba; 3, Kitasato University School of Medicine; 4, American Type Culture Collection.

triplicate in six-well plates and infected 20 h later with AdCALacZ at 20 or 100 MOI for 1 h. Virus-containing solutions were removed at 1 h; cells were washed with PBS and refed with sterile culture medium in the 37°C incubator. After 24 h, the cells were fixed with 2% paraformaldehyde/PBS, washed, and stained in a 1 mg/ml X-Gal (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 K₂[Fe(CN)₆], and 3 mM K₄[Fe(CN)₆] in PBS. β -gal-positive cells in triplicate wells were counted microscopically.

Immunodetection of PTEN Protein. HEC-151 cells in monolayers were infected with AdCAPTEN at a MOI of 100 plaque-forming units/cell. After incubation 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. After 8, 12, 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× Complete Protease Inhibitor Cocktail (Boehringer Mannheim)]. After cells were homogenized and centrifuged at 10,000 × *g* for 30 min, the supernatants were standardized for protein concentrations by the Bradford assay (Bio-Rad). Cellular proteins (20 μ g) 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 antibody for the enhanced chemiluminescence detection system (Amersham Pharmacia Biotech).

Growth Assay. Cell growth was assessed by MTT dye conversion at 570 nm (reference, 630 nm). Seven lines of endometrial cancer cells were seeded at 5 × 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 MOIs of 5, 20, or 100. After 48 h, virus-containing medium was removed and replaced with 1 ml of fresh culture medium. Then 100 μ l of MTT (5 mg/ml in PBS) was added to each well. After 4 h of incubation at 37°C, cells were lysed by the addition of 1 ml of 0.01 N HCl/10% SDS.

Flow Cytometry and TUNEL Assay. Cells were plated at a density of 5 × 10⁵ cells/100-mm dish and infected 20 h later with 100 MOI of AdCAPTEN or AdCA. After 48 h, infected cells were trypsinized, collected in PBS, and fixed in 70% cold ethanol. Subsequent to treatment with RNase, cells were stained with 50 μ g/ml PI 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 any sub-G₁ population, were determined from at least 20,000 ungated cells. Apoptotic cells were detected by fluorescence microscopy (TUNEL assay) using the ApopTag-kit (Oncor) according to the manufacturer's protocol.

Preparation of Microarrays. Microarrays containing 4009 human cDNAs were designed on the basis of DNA sequence data in the Unigene database (National Center for Biotechnology Information; Ref. 25). The microarray used for our experiments included ~700 genes known to play important roles in cell growth or cell cycle; the remainders were chosen for a variety of known or unknown functions, including 52 housekeeping genes. Each target was designed to represent a DNA fragment 0.2–1.0 kbp in size without any repeat sequences. To prepare the target cDNAs, we adopted the RT-PCR method, using as a template a commercial mixture of 12 human poly(A) mRNAs from adult brain, heart, liver, skeletal muscle, spleen, placenta, small intestine, and testis, and from fetal brain, liver, kidney, and lung (Clontech). The cDNAs produced in this manner were purified with the ArrayIt PCR purification kit (Telechem) and adjusted to concentrations of 200 fmol/ μ l. These target cDNAs were mixed with equivalent volumes of Microarray cross-linking solution D (Amersham Pharmacia Biotech) and spotted with a Microarray Spotter Generation III robot on Type7 Microarray Slides (Amersham Pharmacia Biotech). All 4009 target DNAs were arrayed in duplicate and cross-linked to the glass by 50 mJ/cm² of UV irradiation.

Preparation of Probes for the Microarray. HEC-151, HHUA, and Ishikawa3-H-12 cells growing in monolayers were infected with AdCAPTEN or AdCA at a MOI of 50 plaque-forming units/cell and incubated at 37°C for 1 h, with brief agitation every 15 min. After 24 h, the cells were washed twice with PBS, and total RNAs were extracted with TRIzol reagent (Life Technologies, Inc.). Total extracted RNAs were treated with 1 unit of DNase I (Boehringer Mannheim) at 37°C for 1 h in the presence of 1 unit of RNase inhibitor (Toyobo) to remove any contaminating genomic DNA. The DNase I-treated samples were purified twice with spun columns containing oligodeoxy thymidylic acid-cellulose (mRNA Purification Kit; Amersham Pharmacia Biotech). Aliquots consisting of two-thirds of the total mRNA isolated from PTEN- or empty virus-transfected HEC-151 cells were purified and used to generate

fluorescently labeled probes; the remaining third was stored for semiquantitative PCR validation studies (see below).

The mixed mRNAs (2.5 μ g) were incubated with 3 μ g of random hexamers (in a total volume of 26 μ l) at 70°C for 10 min and chilled on ice. Then we added 10 μ l of 5 \times first-strand buffer, 5 μ l of 0.1 M DTT, 1.5 μ l of RNase inhibitor, 1 μ l of 25 mM dGTP, 1 μ l of 25 mM dATP, 1 μ l of 25 mM dTTP, 2 μ l of 1 mM dCTP, 2 μ l of 1 mM Cy3- (for mRNA from AdCAPTEN-infected cells) or Cy5- (for mRNA from control AdCA-infected cells) dCTP (Amersham Pharmacia Biotech.), and 2.5 μ l of Superscript II. After incubation at 37°C for 2 h and denaturation of enzymes in boiling water, template mRNAs were degraded at 37°C for 10 min with 2 μ l of 2.5 N NaOH, then neutralized by 2 μ l of 2.5 N HCl and 10 μ l of 1 M Tris-HCl (pH 6.8). The Cy-labeled probes were purified with a Qiagen PCR purification kit, and their volumes were adjusted to 5 μ l. The labeled probes were boiled and then incubated at 70°C for 40 min with 1 μ l of 1 mg/ml oligo(dA)₈₀. Next, 10 μ l of Microarray Hybridization Solution Buffer version 2 (Amersham Pharmacia Biotech) and 20 μ l of formamide were added.

Hybridization and Scanning of Microarrays. After loading the probes, we covered the slides with coverslips and sealed them with adhesive then incubated the microarrays at 60°C for 14 h in humid boxes. Hybridized slides were washed for 10 min with 1 \times SSC-0.2%SDS at 55°C, twice for 10 min with 0.1 \times SSC-0.2%SDS at 55°C, twice for 1 min with 0.1 \times SSC at room temperature, and finally for 1 min with water at room temperature. Washed slides were air-dried and scanned with a Microarray Scanner Generation III (Amersham Pharmacia Biotech).

Quantitation of Data. Signal intensities of Cy3 and Cy5 were obtained from the microarray images by data-extraction software (Array Vision; Imaging Research Inc.). To reduce background signals from dust particles, we performed median treatment (kernel size, 5 \times 5). The intensity of each spot was corrected by subtracting the immediate surrounding background. Average intensities from duplicate spots were calculated; then we normalized the results for differential efficiencies of labeling and detection on the basis of signals from 52 preselected internal-control genes that are stable in most experiments (red:green intensity ratios close to 1.0). The Cy3: Cy5 ratio for each sample was calculated by averaging intensities of spots. A cutoff value for each expression level was automatically calculated according to the background fluctuation. The fluctuation can be estimated as the variance of log ratio of Cy3: Cy5 minus the variance of log ratio of Cy3: Cy5 of highly expressed genes (upper 30%; where the background fluctuation part is so small that it could be ignored). We used genes of the expression level (greater than \sim 600,000 fluorescent units on a scale of 0–40,000,000 fluorescent units) where the background fluctuation is less than a critical value (1.0), because the lowly expressed genes are embedded in the background fluctuation (see Fig. 5).

Semiquantitative RT-PCR. HEC-151 cells were infected with AdCAPTEN or AdCA at a MOI of 50 plaque-forming units/cell for 1 h and harvested after 24 h. Total RNAs were isolated using Trizol reagent (Life Technologies, Inc.) and treated with DNase I. Equal amounts of RNAs were reverse-transcribed and amplified by the PCR for 20–35 cycles with gene-specific primers at 94°C for denaturing, 55°C for annealing, and 72°C for extension. As an internal control, amplification of β 2-microglobulin mRNA was carried out by RT-PCR using specific primers (sense, 5'-CACCCCACTGA AAAA-

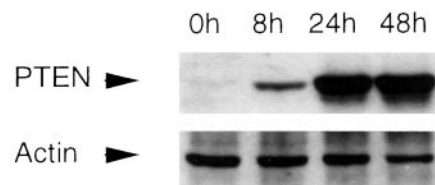


Fig. 2. Expression of the PTEN protein in HEC-151 cells after infection by AdCAPTEN. Cells were lysed 8, 24, and 48 h after virus infection and cellular proteins were immunoblotted with anti-PTEN or anti-actin antibodies.

GATGA-3'; antisense, 5'-TACCTGTGGAGCAACCTGC-3'). Amplified products were analyzed by electrophoresis on 1.5% agarose gels, and their signal intensities were measured with a BAS 1000 bioimaging analyzer (Fujifilm).

RESULTS

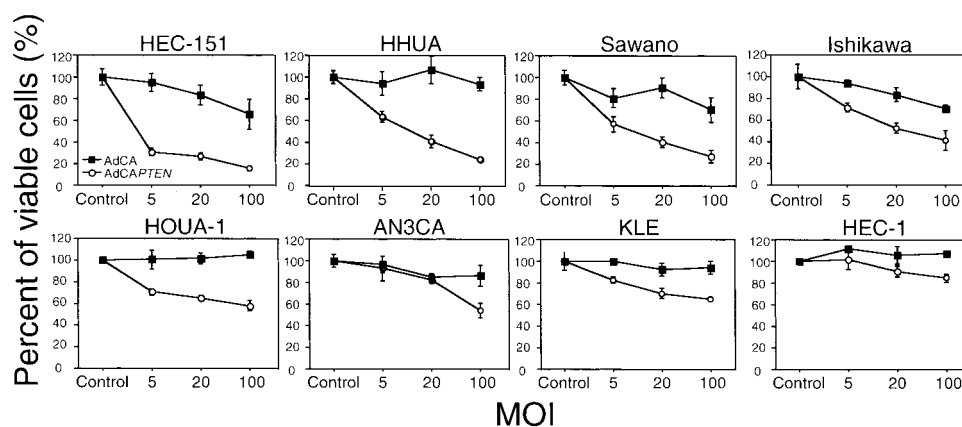
Adenoviral-mediated Gene Transfer and Expression of PTEN.

We examined the status of *PTEN* genes in 14 endometrial cancer cell lines. As summarized in Table 1, mutations of the *PTEN* gene were detected in 11 of the 14 lines, including Ishikawa3-H-12, whose mutation had been reported previously (26). We then evaluated the efficiency of gene transfer by X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside) solution-staining of cells infected with AdCALacZ at different MOIs; Table 1 indicates the percentage of β -gal-positive cells in each cell line. HEC-88, HEC-1, and HEC-151 showed the highest number of β -gal-positive cells, even at MOI 20 (95.5, 79.3, and 78.0%, respectively), whereas AN3CA showed very low proportions (5.3%) of β -gal-positive cells even at MOI 100.

Subsequently we infected AdCAPTEN and examined its effect on cell growth. As Fig. 1 shows, induction of PTEN expression into PTEN-mutant HEC-151, HHUA, Sawano, Ishikawa3-H-12, and HOUA-I cells suppressed proliferation significantly (indicated by MTT dye conversion) as compared with cells transfected with empty virus (AdCA). AN3CA, which had shown a very low efficiency of adenoviral infection, was not affected at MOI 5 or 20 although it contains mutant PTEN. Two cell lines that do express wild-type PTEN, KLE and HEC-1, showed slight decreases in cell growth after transfection of AdCAPTEN. To confirm that exogenous PTEN protein was in fact induced in these cells by adenovirus-mediated gene transfer, we performed immunoblotting analysis in HEC-151 cells using anti-PTEN antibody. Overexpression of PTEN protein was clearly detected as early as 8 h after infection (Fig. 2), although no endogenous PTEN protein was detectable at h 0.

Induction of Cell Cycle Arrest and Cell Death by PTEN. To determine whether the PTEN-mediated growth suppression in cancer

Fig. 1. Inhibition of growth of human endometrial cancer cells by expression of exogenous PTEN. Cells were cultured in six-well plates (5 \times 10⁴ cells/well) and infected with an adenovirus containing a wild-type *PTEN* gene (O, AdCAPTEN) or lacking *PTEN* (■, AdCA) at 5, 20, or 100 MOI. Cell numbers and viability were assessed by MTT dye conversion 48 h after infection. Each data point represents the mean of values from three separate experiments; bars, SD.



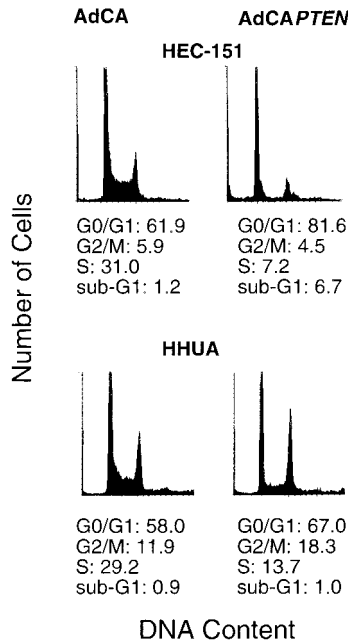


Fig. 3. Effect of PTEN expression on distribution of cell cycle phases in HEC151 and HHUA endometrial cancer cells infected with AdCAPTEN or AdCA at 100 MOI. All floating and attached cells were collected and fixed after 48 h, and DNA content was determined by PI staining. Phase distributions and sub-G₁ populations are indicated as percentages.

cells was attributable to arrest of the cell cycle, we analyzed the distribution of growth phases in HEC-151 cells by flow-cytometry. Induction of PTEN expression resulted in a significant increase in the numbers of cells at G₁, a decrease in the numbers at S-phase, and an increase in the sub-G₁ population (Fig. 3). HHUA cells, which also had shown significant growth suppression, decreased in numbers at S phase and underwent growth arrest at G₁ and G₂, but those changes were not accompanied by an increase in the sub-G₁ population.

By TUNEL assay, we observed that the population of apoptotic cells among AdCAPTEN-infected HEC-151 cells was significantly greater (17%) than that in AdCA-infected cells (1%). No significant difference was observed in HHUA (2% and 1%, respectively; Fig. 4). These data suggest that the mechanisms of growth suppression mediated through PTEN in HEC-151 cells were G₁ arrest and apoptosis,

whereas HHUA cells responded only with G₁ and G₂ arrest—at least, until the same time point.

Analysis by cDNA Microarray. To investigate further the differences in gene expression during cell-cycle arrest and apoptosis caused by the expression of PTEN in HEC-151 cells, we performed a profiling analysis using cDNA microarrays containing 4009 genes. The probe derived from AdCAPTEN-treated cells was labeled with Cy3-dCTP, and probe prepared from AdCA-treated cells was labeled with Cy5-dCTP. The two probes were hybridized simultaneously to duplicate microarrays, and the two fluorescent images were scanned. Cy3 (red) and Cy5 (green) signal intensities were obtained from all of the microarray images. Average intensity from duplicate spots was calculated, then normalization was performed to correct for differential efficiencies of labeling and detection on the basis of signals from 52 preselected internal-control genes that are stable in most experiments (Fig. 5).

We selected cDNAs that revealed green:red signal ratios >2.0 or <0.5 and performed RT-PCR experiments. When the intensity of each band was measured by the BAS 1000 and the ratios AdCAPTEN-infected cells:AdCA-infected cells were calculated, 87.6% of red signals (99 of 113 genes) were confirmed to be up-regulated by RT-PCR experiments and 87.8% of green spots (72 of 82 genes) were also confirmed to be down-regulated. The 171 genes whose expres-

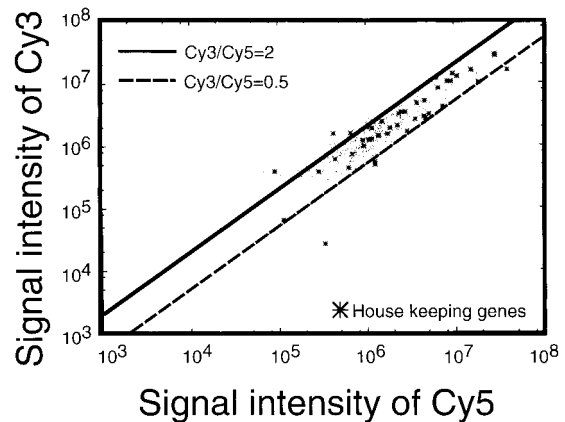


Fig. 5. Representative scatter plots of cDNA microarray analysis. AdCA-infected HEC-151 cells (Cy5-labeled) and AdCAPTEN-infected HEC-151 cells (Cy3-labeled) were labeled and hybridized to cDNA microarray. Upper line, Cy3 = 2 × Cy5; lower line, Cy3 = 0.5 × Cy5.

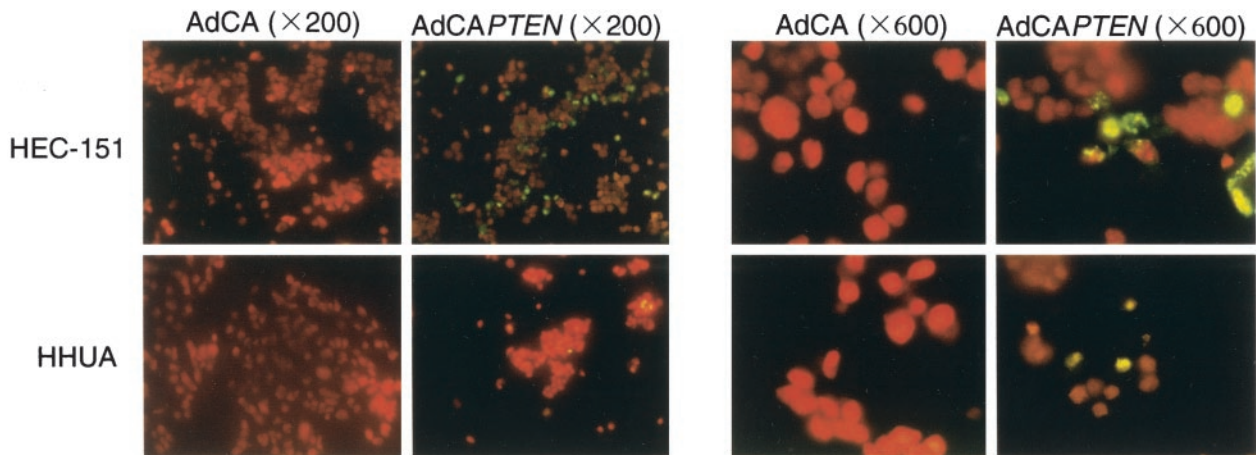


Fig. 4. Detection of induced apoptosis in HEC-151 cells. DNA fragmentation was detected by TUNEL assay (green), and PI was used to counterstain nuclei. Apoptotic cells appear green or yellow; normal cells show only PI staining.

Table 2 Genes of up-regulated by PTEN transfer

Clone ID	Unigene no.	Description	Putative function	Fold change ^a		
				HEC-151	HHUA	Ishikawa
A0493	Hs.238990	<i>CDK inhibitor 1B (p27,Kip1, CDKN1B)</i>	Cell cycle arrest	>100	3.6	6.8
A0485	Hs.72901	<i>CDK inhibitor 2B (p15,INK4B,CDKN2B)</i>	Cell cycle arrest	>100	2.9	4.4
A1775	Hs.183874	<i>Cullin 4A (CUL4A)</i>	Cell cycle arrest	23.3	1.2	(-) ^b
A1778	Hs.77054	<i>B-cell translocation gene 1 (BTG1)</i>	Cell cycle arrest (tob family)	>100	2.0	6.5
A2469	Hs.118442	<i>Cyclin C (CCNC)</i>	Cell cycle arrest (target genes of VD)	3.1	0.8	(-)
A1800	Hs.77313	<i>Cyclin-dependent kinase 10 (CDK10)</i>	Cell cycle	3.3	2.2	8.7
A4817	Hs.20938	<i>RNA-binding protein (SCR3)</i>	Fundamental function (translation)	2.8	2.7	1.4
A2278	Hs.49007	<i>Poly(A) polymerase (PAP)</i>	Fundamental function (RNA processing)	>100	1.3	1.8
A2519	Hs.3530	<i>TLS-associated serine-arginine protein (TASR)</i>	Fundamental function (RNA processing)	8.8	0.9	1.2
A1421	Hs.79658	<i>Casein kinase 1 epsilon (CSNK1E)</i>	Fundamental function (DNA replication and repair)	2.6	2.2	2.1
A4659	Hs.8997	<i>Heat shock 70kD protein 1 (HSPA1A)</i>	Fundamental function (inhibition of protein degradation)	3.7	8.0	1.4
A4602	Hs.4147	<i>Translocating chain-associating membrane protein (TRAM)</i>	Fundamental function (protein translocation)	3.7	1.4	0.9
A4329	Hs.92962	<i>Sec23 homolog A (SEC23A)</i>	Fundamental function (protein transport)	5.9	1.2	2.0
A3979	Hs.139336	<i>ATP-binding cassette member 4 (ABCC4)</i>	Fundamental function (anion transporter)	87.3	1.8	0.9
A1348	AF065164 ^c	<i>Hyperpolarization-activated channel 2 (HCN2)</i>	Fundamental function (channel)	45.8	5.2	3.3
A4927	Hs.194625	<i>Dynein light intermediate chain 2 (DNCL12)</i>	Fundamental function (motor protein)	>100	1.8	1.6
A1703	Hs.154546	<i>Syntaxin 5A (STX5A)</i>	Fundamental function (vesicle transport)	2.8	1.2	1.0
A1174	Hs.112378	<i>LIM and senescent cell antigen-like domains 1 (LIMS1)</i>	Immune response (cell antigen)	55.5	1.6	1.7
A4683	Hs.177559	<i>Interferon γ receptor 2 (IFNGR2)</i>	Immune response	3.2	0.9	1.7
A2244	Hs.73895	<i>TNF-receptor superfamily, member 9 (TNFRSF9)</i>	Signal transduction (TNF and TNF receptor family)	2.1	2.9	(-)
A1281	Hs.129844	<i>Decoy receptor 2 (TNFRSF10D)</i>	Signal transduction (TNF and TNF receptor family)	3.0	1.5	1.1
A1095	Hs.81791	<i>TNF receptor superfamily member 11b (TNFRSF11B)</i>	Signal transduction (TNF and TNF receptor family)	>100	1.5	(-)
A0137	Hs.89862	<i>TNFRSF1A-associated via death domain (TRADD)</i>	Signal transduction (TNF-associated protein)	4.2	2.1	1.9
A6286	Hs.8375	<i>TNF-receptor-associated factor 4 (TRAF4)</i>	Signal transduction (TNF receptor-associated factor)	2.3	1.3	1.5
A1717	Hs.76090	<i>TNF α induced protein 1 (TNFAIP1)</i>	Signal transduction (TNF-induced protein)	3.4	1.5	2.0
A1329	Hs.124024	<i>Deltex (DTX1)</i>	Signal transduction (notch-signalling pathway)	10.0	32.5	>100
A3662	Hs.91143	<i>Jagged1 (Alagille syndrome) (JAG1)</i>	Signal transduction (notch-signalling pathway)	38.4	1.3	0.8
A9582	Hs.173063	<i>Transducin like enhancer of split 2 (TLE2)</i>	Signal transduction (notch-signalling pathway)	2.5	7.8	5.9
A2748	Hs.3260	<i>Presenilin 1 (PSEN1)</i>	Signal transduction (notch-signalling pathway)	2.7	1.2	1.5
A4947	Hs.46348	<i>Bradykinin receptor B1 (BDKRB1)</i>	Signal transduction (G protein-coupled receptor)	2.8	3.1	0.7
A5864	Hs.183800	<i>Ran GTPase-activating protein 1 (RANGAP1)</i>	Signal transduction (small G)	6.7	1.8	1.8
A4887	Hs.37604	<i>Rho GTPase-activating protein 5 (ARHGAP5)</i>	Signal transduction (small G)	27.7	1.9	1.2
A0436	Hs.102402	<i>Mad4 homolog (MAD4)</i>	Signal transduction (Mad family)	2.0	2.7	12.9
A5022	Hs.153863	<i>MAD homolog 6 (Smad6) (MADH6)</i>	Signal transduction (Mad family)	3.8	2.1	1.7
A0578	Hs.171695	<i>Dual specificity phosphatase 1 (DUSP1)</i>	Signal transduction (MAP kinase inhibition)	3.6	2.4	(-)
A1686	Hs.75842	<i>Protein kinase (DYRK1A)</i>	Signal transduction (kinase)	2.7	1.2	1.4
A0964	Hs.75339	<i>Inositol polyphosphate phosphatase-like 1 (INPPL1)</i>	Signal transduction (phosphatase)	2.1	2.7	2.1
A1984	Hs.155079	<i>Protein phosphatase 2A regulatory subunit B (PPP2R5A)</i>	Signal transduction (phosphatase)	2.9	1.2	1.4
A1270	Hs.123085	<i>Chromobox homolog 4 (CBX4)</i>	Signal transduction (repressor of protooncogene)	3.5	1.9	(-)
A2849	Hs.80962	<i>Neurotensin (NTS)</i>	Signal transduction (neurotransmitter)	24.6	2.7	1.2
A1673	Hs.75789	<i>Drg1 protein (NDRG1)</i>	Signal transduction (potential cancer suppressor gene)	3.5	1.3	(-)
A1046	Hs.621	<i>Galectin 3 (LGALS3)</i>	Signal transduction	8.6	1.1	1.1
A4601	Hs.14601	<i>Haematopoietic lineage cell-specific protein (HCLS1)</i>	Signal transduction	10.2	2.1	1.2
A4733	Hs.7879	<i>Interferon-related developmental regulator 1 (IFRD1)</i>	Signal transduction	3.0	0.3	0.7
A3714	Hs.112049	<i>SET-binding factor 1 (SBF1)</i>	Signal transduction	2.6	1.7	0.9
A0341	Hs.2780	<i>jun D (JUND)</i>	Transcription factor (negative regulation of cell proliferation)	2.5	2.7	2.5
A2872	Hs.131953	<i>Basic leucine zipper transcription factor (MAFK)</i>	Transcription factor	19.6	1.7	2.5
A0857	Hs.1004	<i>Bromodomain-containing protein (BR140)</i>	Transcription factor	3.2	2.0	(-)
A0551	Hs.54473	<i>Cardiac-specific homeo box (CSX)</i>	Transcription factor	4.1	1.7	2.5
A4266	Hs.82071	<i>Cbp/p300-interacting transactivator (CITED2)</i>	Transcription factor	15.6	1.3	5.8
A0011	Hs.4055	<i>Core promoter element-binding protein (COPEB)</i>	Transcription factor	6.5	2.5	2.2
A0438	Hs.1395	<i>Early growth response 2 (EGR2)</i>	Transcription factor	2.5	(-)	(-)
A1367	Hs.84728	<i>GC-box-binding protein (BTEB2)</i>	Transcription factor	7.1	1.7	(-)
A4622	Hs.184122	<i>General transcription factor II, I (GTF2I)</i>	Transcription factor	2.8	2.8	3.1
A5048	Hs.34853	<i>Inhibitor of DNA-binding 4 (ID4)</i>	Transcription factor	19.1	(-)	11.2
A1923	Hs.78788	<i>Leucine zipper like transcriptional regulator 1 (LZTR1)</i>	Transcription factor	2.9	1.9	1.7
A0121	Hs.167923	<i>MADS box transcription enhancer factor 2 (MEF2D)</i>	Transcription factor	5.3	2.0	(-)
A2006	Hs.79334	<i>Nuclear factor, interleukin 3-regulated (NFIL3)</i>	Transcription factor	4.8	0.5	1.5
A3745	Hs.144630	<i>Nuclear receptor subfamily 2 (NR2F1)</i>	Transcription factor	4.4	1.2	1.6
A2703	Hs.2982	<i>Sp4 transcription factor (SP4)</i>	Transcription factor	10.1	2.4	2.6
A1618	Hs.83484	<i>SRY (sex-determining region Y) box 4 (SOX4)</i>	Transcription factor	4.2	5.6	2.7
A4050	Hs.82037	<i>TBP-associated factor (TAF2J)</i>	Transcription factor	23.6	(-)	1.3
A2973	Hs.123057	<i>TBP-associated factor (TAF2K)</i>	Transcription factor	2.7	2.0	1.4
A1873	Hs.169832	<i>Zinc finger protein 42 (ZNF42)</i>	Transcription factor	3.7	3.0	2.8
B5101	Hs.9028	<i>Histone deacetylase 5 (NY-CO-9)</i>	Transcriptional regulator (chromatin remodelling)	2.7	32.0	10.2
A3992	Hs.239894	<i>Leucine-rich repeat interacting protein 1 (LRRFIP1)</i>	Transcription repressor	2.7	1.1	0.5
A4009	Hs.78183	<i>Aldo-keto reductase family 1 (AKRIC3)</i>	Metabolic enzyme	4.5	1.6	0.4
A1212	Hs.198365	<i>2,3-bisphosphoglycerate mutase (BPGM)</i>	Metabolic enzyme	6.4	1.2	1.6
A1494	Hs.121576	<i>Aspartate β-hydroxylase (ASPH)</i>	Metabolic enzyme	3.4	(-)	(-)
A2568	U62389 ^c	<i>Isocitrate dehydrogenase</i>	Metabolic enzyme	>100	0.6	(-)
A2121	Hs.771	<i>Liver glycogen phosphorylase (PYGL)</i>	Metabolic enzyme	4.1	2.8	3.6
A1154	Hs.82568	<i>Sterol 27-hydroxylase (CYP27A1)</i>	Metabolic enzyme	19.7	(-)	(-)
A3744	Hs.155455	<i>Liver phosphofructokinase (PFKL)</i>	Metabolic enzyme	2.7	1.3	1.5
A4848	Hs.185973	<i>Membrane fatty acid desaturase (MLD)</i>	Metabolic enzyme	6.0	2.2	1.5
A1610	Hs.172153	<i>Plasma glutathione peroxidase 3 (GPX3)</i>	Catalytic enzyme (detoxification of hydrogen peroxide)	12.3	(-)	3.5
A2378	Hs.77502	<i>Methionine adenosyltransferase II α (MAT2A)</i>	Catalytic enzyme	3.9	2.1	1.7

Table 2 *Continued*

Clone ID	Unigene no.	Description	Putative function	Fold change ^a		
				HEC-151	HHUA	Ishikawa
A0420	Hs.28491	<i>Spermidine/spermine N1-acetyltransferase (SAT)</i>	Catabolic enzyme	4.6	1.6	5.8
A4820	Hs.9482	<i>Target of myb1 homolog (TOM1)</i>	Other (degradation of growth factor receptor)	4.7	2.0	(-)
A1317	Hs.129711	<i>Hepatitis A virus cellular receptor 1 (HAVCR-1)</i>	Other (virus receptor)	5.5	2.1	(-)
A0402	Hs.251664	<i>Insulin-like growth factor II (IGF2)</i>	Cell proliferation (growth factor)	6.8	2.5	0.7
A0168	Hs.199067	<i>Epidermal growth factor receptor (ERBB3)</i>	Cell proliferation (growth factor receptor)	4.4	3.3	3.1
A0092	Hs.81688	<i>Hepatocyte growth factor receptor (MET)</i>	Cell proliferation (growth factor receptor)	>100	1.3	>100
A2366	Hs.78225	<i>Annexin A1 (ANXA1)</i>	Cell proliferation (signal transducer of growth factor)	>100	2.4	1.5
A5158	Hs.147189	<i>HYA22 protein (HYA22)</i>	Unknown (potential cancer suppressor gene)	2.7	4.0	0.9
A1194	NM_000445 ^c	<i>Plectin 1</i>	Unknown (intermediates filament-binding protein)	6.3	4.9	3.0
A3692	U82939 ^c	<i>p53-binding protein (p53BP3)</i>	Unknown (associated with p53)	4.8	2.2	1.1
A0306	Hs.87246	<i>Bcl-2-binding component 3 (BBC3)</i>	Unknown (associated with Bcl-2)	2.5	1.2	(-)
A0431	Hs.210604	<i>Mismatch repair gene PMS1 homologue (PMS5, PMS2L3)</i>	Unknown (mismatch repair gene homologue)	2.3	2.5	1.1
A1425	Hs.83086	<i>GT212</i>	Unknown	3.9	2.0	(-)
A4811	Hs.21753	<i>JM5 protein (JM5)</i>	Unknown	4.5	2.1	7.4
A4243	Hs.118786	<i>Metallothionein 2A (MT2A)</i>	Unknown	3.3	0.9	1.0
A2440	HSU96628 ^c	<i>Nuclear antigen H731-like protein</i>	Unknown	2.3	1.5	(-)
A1232	Hs.85273	<i>Retinoblastoma-binding protein 6 (RBBP6)</i>	Unknown	2.4	1.2	(-)
A2556	Y14820 ^c	<i>SURF-4 protein</i>	Unknown	3.4	1.6	1.6
A3696	Hs.232070	<i>Telomerase-associated protein 1 (TEP1)</i>	Unknown	2.5	3.0	1.3
A4626	Hs.4316	<i>Trinucleotide repeat containing 12 (TNRC12)</i>	Unknown	>100	1.3	(-)
A0284	Hs.76329	<i>Tumor differentially expressed 1 (TDE1)</i>	Unknown	2.8	1.5	3.3

^a Fold change represents a ratio of signal intensity (Cy3/Cy5).

^b Signal intensity is smaller than cut-off value.

^c GenBank accession no.

sion was significantly altered by introduction of wild-type PTEN into PTEN-defective HEC-151 cells are summarized in Tables 2 and 3. For example, cyclin-dependent kinase inhibitor 1B (*p27, CDKN1B*) and cyclin-dependent kinase inhibitor 1B (*p15, CDKN2B*) were identified as up-regulated by PTEN on the microarray slides, with the highest ratios of Cy3/Cy5 signal intensity. Semi-quantitative RT-PCR experiments confirmed the elevated expression (Fig. 6).

For these 171 genes, ratios of the signal intensity (Cy3: Cy5), obtained from microarray analyses using additional two cancer cell lines HHUA and Ishikawa3-H-12, are also shown in Tables 2 and 3. 52.5% (52/99) of PTEN-induced genes in HEC-151 cells were also up-regulated (Cy3: Cy5 signal ratio >2.0) in either HHUA or Ishikawa3-H-12 lines. Similarly, 40.3% (29 of 72) of the repressed genes in HEC-151 cells were also down-regulated (Cy3: Cy5 ratio <0.5) in either HHUA or Ishikawa3-H-12. These results will be useful in making additional confirmation of important genes listed in Table 2. Remaining genes seemed to respond in a HEC-151 cell-specific manner. This might be attributable to pronounced apoptotic susceptibility of HEC-151 cells in response to PTEN *versus* other cells.

DISCUSSION

Hybridization of mRNA-derived probes to cDNA microarrays allows us to perform systematic analysis of expression profiles for thousands of genes simultaneously. This technique has been used successfully to investigate changes in expression profiles after human cells have undergone carcinogenesis (13). In this paper, we have provided primary information on transcriptional changes related to the biophysiological activity of PTEN, an enzyme that mediates important signal-transduction pathways. By combining analysis of gene-expression profiles in PTEN-deficient carcinoma cells with adenovirus-mediated gene transfer, we identified as many as 171 genes whose expression was definitely up- or down-regulated by incorporation of exogenous PTEN.

Up-Regulated Genes. The genes we found to be differentially expressed cover a broad range of functional activities: (a) cell-cycle regulation; (b) apoptotic stimulation; (c) differentiation; (d) transcrip-

tion; and (e) signal transduction. For example, *CDKN1B* (known as *p27Kip1*; clone A0493) and *CDKN2B* (known as *p15INK4B*; clone A0485) were strongly up-regulated in our experiments (Fig. 6). This result was consistent with evidence reported previously that PTEN increases the level of p27Kip1 protein and inhibits the entry of cells into S phase (27, 28). p15INK4B also inhibits cell proliferation by inducing arrest of the cell cycle at G₁ (29).

B-cell translocation gene 1 (BTG1; clone A1778) and Cullin 4A (*CUL4A*; clone A1775) also regulate the cell cycle. BTG1 encodes a member of the antiproliferative Tob/BTG1 family of molecules; its expression is maximum in the G₀-G₁ phases of the cell cycle and is down-regulated when cells progress out of G₁. Transfection experiments have indicated that BTG1 negatively regulates cell proliferation (30). *CUL4A* belongs to the Cullin family, one of whose members, *CUL-1*, appears to be required for G₁ transitions to either G₀ or the apoptotic pathway (31).

Among the multitude of signal-transduction molecules identified on our microarray, the most notable changes were observed in the TNF-receptor family and the TNF-associated group, including TNFRSF9 (clone A2244), TNFRSF11B (clone A1095), TRADD (clone A0137), TRAF4 (clone A6286), TNFAIP1 (clone A1717), and others. TNF receptors contain an "intracellular death" domain that signals TNF-induced responses such as stimulation of apoptosis and activation of nuclear factor κB (32). Wick *et al.* (33) showed that PTEN expression in glioma cells leads to sensitization to CD95-induced apoptosis. Our data in part illustrate their observation. Moreover, our data may provide a clue as to why an impaired response to Fas has been observed in PTEN^{+/-} mice (34).

We also found up-regulation of members of the Notch-signaling pathway, Deltex (*DTX1*; clone A1329), Jagged1 (*JAG1*; clone A3662), presenilin (*PSEN1*; clone A2748) and enhancer of split 2 (*TLE2*; clone A9582). Notch signaling regulates decisions concerning cell fates, differentiation potential, rate of proliferation, and apoptotic cell death (35). Inhibition of Ras-dependent transcription by Notch and Deltex has been reported (36).

As another example, the *Mad* gene encodes a protein that associates

Table 3 Genes of down-regulated by PTEN transfer

Clone ID	Unigene no.	Description	Putative function	Fold change ^a		
				HEC-151	HHUA	Ishikawa
A0157	Hs.23960	<i>Cyclin B1 (CCNB1)</i>	Cell proliferation	0.23	0.59	0.52
A0354	Hs.92374	<i>Cyclin G1 interacting protein (cg11)</i>	Cell proliferation	0.20	0.74	(-) ^b
A0480	Hs.77550	<i>CDC28 protein kinase 1 (CKS1)</i>	Cell proliferation	0.12	0.37	0.60
A0499	Hs.83758	<i>CDC28 protein kinase 2 (CKS2)</i>	Cell proliferation	0.03	0.44	0.57
A0245	Hs.82906	<i>Cell division cycle 20 (CDC20)</i>	Cell proliferation	0.06	0.51	0.52
A0249	Hs.227789	<i>Mitogen-activated protein kinase-activated protein kinase 3 (MAPKAPK3)</i>	Cell proliferation	0.24	0.19	0.38
A0333	Hs.179718	<i>V-myb avian myeloblastosis viral oncogene homolog-like 2 (B-myb, MYBL2)</i>	Cell proliferation	0.27	0.46	0.76
A2369	Hs.77597	<i>Polo (Drosophila)-like kinase (PLK)</i>	Cell proliferation	0.30	0.51	0.30
A2506	Hs.15243	<i>Nucleolar protein 1 (P120 antigen, NOL1)</i>	Cell proliferation	0.33	1.00	0.00
A2452	Hs.108080	<i>Cysteine and glycine-rich protein 1 (CSRP1)</i>	Cell proliferation	0.39	0.93	0.60
A5651	Hs.5181	<i>Proliferation-associated 2G4 (PA2G4)</i>	Cell proliferation	0.25	0.47	0.49
A0018	Hs.48915	<i>Serine/threonine kinase 15 (STK15)</i>	Cell proliferation (centrosome-associated kinase)	0.49	0.65	0.23
A4854	Hs.180655	<i>Serine/threonine kinase 12 (AIM-1, STK12)</i>	Cell proliferation (cell division, chromosome segregation)	0.15	0.57	0.63
A1216	Hs.84746	<i>Chromosome condensation 1 (RCC1, CHC1)</i>	Cell proliferation (cell division)	0.37	0.65	0.90
A2154	Hs.2934	<i>Ribonucleotide reductase M1 polypeptide (RRI, RRM1)</i>	Cell proliferation (cell division)	0.45	0.43	1.15
A0106	Hs.165950	<i>Fibroblast growth factor 4 (FGFR4)</i>	Cell proliferation (growth factor)	0.19	1.79	2.25
A0682	Hs.104	<i>Hepatocyte growth factor (HGF) activator (HGFAC)</i>	Cell proliferation (growth factor activator)	0.21	(-)	(-)
A0080	Hs.78202	<i>SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily a member 4 (SMARCA)</i>	Cell proliferation and transcription	0.31	1.09	0.85
A1970	Hs.79078	<i>MAD2-like 1 (MAD2L1)</i>	Fundamental function (cell cycle checkpoint)	0.00	0.46	0.57
A4383	Hs.25292	<i>Ribonuclease H1 large subunit (RNASEH1)</i>	Fundamental function (DNA replication and repair)	0.34	0.55	0.52
A5087	Hs.74598	<i>Polymerase δ 2 regulatory subunit (POLD2)</i>	Fundamental function (DNA replication and repair)	0.26	0.52	0.49
A4259	Hs.105465	<i>Small nuclear ribonucleoprotein polypeptide F (SNRPF)</i>	Fundamental function (RNA processing)	0.30	0.70	0.75
A2268	Hs.63525	<i>Poly(rC)-binding protein 2 (PCBP2)</i>	Fundamental function (post-translationally modification by phosphorylation)	0.36	0.48	0.53
A1825	Hs.77522	<i>Major histocompatibility complex class II DM α (HLA-DMA)</i>	Immune response	0.19	0.00	(-)
A0378	Hs.394	<i>Adrenomedullin (ADM)</i>	Signal transduction	0.06	0.12	0.24
A1824	Hs.77515	<i>Inositol 1,4,5-triphosphate receptor type 3 (ITPR3)</i>	Signal transduction	0.25	1.19	1.61
A5155	Hs.75545	<i>Interleukin 4 receptor (IL4R)</i>	Signal transduction	0.18	0.68	(-)
A1933	Hs.130227	<i>Tumor necrosis factor receptor superfamily member 14 (TNFRSF14)</i>	Signal transduction (TNF and TNF receptor family)	0.38	1.71	(-)
A3058	Hs.239	<i>Forkhead box M1 (M phase phosphoprotein 2) (FKHL16, FOXM1)</i>	Signal transduction (regulation of transcription, Forkhead gene family)	0.00	0.44	0.65
A4228	Hs.251238	<i>DR1-associated protein 1 (DRAP1)</i>	Signal transduction (repressor of transcription)	0.39	0.67	0.85
A0492	Hs.89657	<i>TBP-associated factor (TAF2H)</i>	Transcription factor	0.34	0.74	0.68
A2776	Hs.76362	<i>General transcription factor IIA 1 (GTF2A1)</i>	Transcription factor	0.42	0.54	0.00
A3049	Hs.166	<i>Sterol regulatory element binding transcription factor 1 (SREBP-1, SREBF1)</i>	Transcription factor	0.37	1.21	0.77
A5119	AF077740 ^c	<i>2-Amino-3-ketobutyrate-CoA ligase</i>	Metabolic enzyme	0.11	0.84	0.74
A2936	Hs.83393	<i>Cystatin E/M (CST6)</i>	Metabolic enzyme inhibitor	0.25	0.00	0.84
A0904	Hs.75105	<i>Emopamil-binding protein (EBP)</i>	Metabolic enzyme	0.00	0.50	0.38
A3036	Hs.154890	<i>Fatty-acid-coenzyme A ligase (FACL2)</i>	Metabolic enzyme	0.25	1.10	1.19
A0688	Hs.135	<i>γ-glutamyltransferase 1 (GGT1)</i>	Metabolic enzyme	0.34	0.88	(-)
A3929	Hs.105435	<i>GDP-mannose 4,6-dehydratase (GMDS)</i>	Metabolic enzyme	0.29	0.85	0.19
A0800	Hs.772	<i>Glycogen synthase 1 (GYS1)</i>	Metabolic enzyme	0.41	0.51	0.74
A4981	Hs.51043	<i>Hexosaminidase B (HEXB)</i>	Metabolic enzyme	0.40	0.55	1.08
A4699	Hs.3828	<i>Mevalonate decarboxylase (MVD)</i>	Metabolic enzyme	0.07	0.93	0.24
A3795	Hs.118721	<i>Neuraminidase 1 (NEU1)</i>	Metabolic enzyme	0.44	0.90	0.32
A2164	Hs.1869	<i>Phosphoglucomutase 1 (PGM1)</i>	Metabolic enzyme	0.10	0.41	0.66
A0899	Hs.75093	<i>Procollagen-lysine 2-oxoglutarate 5-dioxygenase (PLOD)</i>	Metabolic enzyme	0.35	0.59	0.63
A3469	Hs.111024	<i>Solute carrier family 25 member 1 (SLC25A1)</i>	Metabolic enzyme	0.30	0.76	0.97
A0833	Hs.878	<i>Sorbitol dehydrogenase (SORD)</i>	Metabolic enzyme	0.14	0.44	0.73
A0439	Hs.388	<i>Nudix-type motif 1 (8-oxo-dGTPase, NUDT1)</i>	Catalytic enzyme (prevents mutation)	0.47	0.88	0.82
A2832	Hs.80986	<i>ATP synthase H⁺ transporting mitochondrial F0 complex subunit c isoform 1 (ATP5G1)</i>	Production of biochemical energy	0.17	0.56	0.38
A2930	Hs.85539	<i>ATP synthase H⁺ transporting mitochondrial F0 complex subunit e (ATP5I)</i>	Production of biochemical energy	0.18	0.56	0.55
A4132	Hs.153028	<i>Cytochrome b-561 (CYB561)</i>	Production of biochemical energy	0.02	0.60	0.09
A2770	Hs.181028	<i>Cytochrome c oxidase subunit Va subunit (COX5A)</i>	Production of biochemical energy	0.35	0.56	0.76
A2931	Hs.81097	<i>Cytochrome c oxidase subunit VIII (COX8)</i>	Production of biochemical energy	0.28	0.63	0.61
A4177	Hs.109760	<i>NADH dehydrogenase 1 β subcomplex 3 (NDUFB3)</i>	Production of biochemical energy	0.40	0.69	0.75
A2943	Hs.84549	<i>NADH dehydrogenase 1 subcomplex unknown 1 (NDUFC1)</i>	Production of biochemical energy	0.34	0.44	0.71
A5677	Hs.5273	<i>NADH dehydrogenase Fe-S protein 3 (NDUFS3)</i>	Production of biochemical energy	0.29	0.81	0.67
A4248	Hs.109059	<i>Ribosomal protein mitochondrial L12 (RPML12)</i>	Production of biochemical energy	0.46	0.63	0.54
A4693	Hs.18141	<i>Ladinin 1 (LAD1)</i>	Cell structure (component of basement membrane)	0.23	0.83	0.74
A0124	Hs.78452	<i>Solute carrier family 20 member 1 (SLC20A1)</i>	Other (phosphate transporter)	0.35	1.00	0.75
A4686	Hs.169900	<i>Poly(A)-binding protein, cytoplasmic 4 (PABPC4)</i>	Other (pseudogene)	0.43	0.73	0.41
A2518	Hs.106283	<i>Insulin-like growth factor-binding protein 6 (IGFBP6)</i>	Growth inhibitor	0.11	1.09	(-)
A4234	Hs.14623	<i>Interferon γ-inducible protein 30 (IFI30)</i>	Unknown (interferon-induced protein)	0.23	1.06	0.00

Table 3 Continued

Clone ID	Unigene no.	Description	Putative function	Fold change ^a		
				HEC-151	HHUA	Ishikawa
A1054	Hs.833	<i>Interferon-stimulated protein (ISG15)</i>	Unknown (interferon-stimulated protein)	0.33	0.64	0.82
A4184	Hs.24391	<i>BET3 (BET3)</i>	Unknown	0.32	1.04	0.86
A4677	Hs.194662	<i>Calponin 3 acidic (CNN3)</i>	Unknown	0.44	0.91	1.16
A4797	AF069301 ^c	<i>DBI (diazepam binding inhibitor)-related protein</i>	Unknown	0.18	0.60	0.66
A4356	Hs.182265	<i>Keratin 19 (KRT19)</i>	Unknown	0.36	1.22	1.16
A5172	Hs.153591	<i>Not56-like protein (NOT56L)</i>	Unknown	0.37	0.76	0.00
A4301	Hs.101033	<i>Putative GTP-binding protein</i>	Unknown	0.24	1.29	0.76

^a Fold change represents a ratio of signal intensity (Cy3/Cy5).

^b Signal intensity is smaller than cut-off value.

^c GenBank accession no.

with Max, the basic helix-loop-helix-leucine zipper protein. Mad:Max heterodimers repress transcription and suppress Myc-dependent transformation; and MAD4 (clone A0436), which is expressed in differentiating cells, inhibits c-Myc-dependent transformation (37).

Table 2 also identifies up-regulation of several protein kinases and phosphatases. For example *DUSP1* (clone A0578), a dual-specificity phosphatase for tyrosine and threonine, specifically inactivates mitogen-activated protein kinase and suppresses its activation by ras (38). Another gene on this list, differentiation-related gene 1 (*NDRG1*; clone A1673) was recently cloned by differential display as a candidate suppressor of metastases in colon cancer (39); this gene appears to be expressed in normal colon and in primary colon cancers but not in metastatic counterparts. Overexpression of Drg-1 in metastatic colon cancer cells *in vitro* has reduced invasion through Matrigel; *in vivo*, it has suppressed liver metastasis in nude mice.

A third example, histone deacetylase 5 (*NY-CO-9*; clone B5101), may control cell proliferation and differentiation by regulating gene expression as a chromatin remodeling factor (40).

The genes of unknown function that are listed in Table 2 would be of special interest to us. This group contains three genes: p53-binding protein 3 (*p53BP3*; clone A3692), Bcl-2-binding protein (*BBC3*; clone A0306), and *HYA22* (clone A5158) that we isolated earlier from the 700-kb region that was homozygously deleted in a human lung-cancer cell line (41). Tables 2 and 3 may also include genes up-regulated or down-regulated because of a vast overexpression of protein. RNA-

binding protein, poly(A) polymerase, and heat shock proteins would belong to such category.

Down-Regulation. Numerous genes involved in cell-cycle progression and proliferation, along with many that encode metabolic enzymes essential for maintenance of cell survival, were repressed by *PTEN* gene transfer. For example, expression of *CDC28 kinases 1* and *2* (clones A0480 and A0499, respectively), required for cell-cycle transition, were highly repressed in our experiments. *STK15/BTAK* (clone A0018; Fig. 6), which encodes a centrosome-associated kinase, is amplified and overexpressed in multiple human tumors, where it induces centrosome aneuploidy and transformation (42).

In summary, we believe the results reported here support the view that analysis with cDNA microarrays is a useful approach for investigating type II tumor suppressor genes whose altered expression is linked to tumor progression (43) and also for identifying genes whose products could be potential disease-specific targets for cancer therapy.

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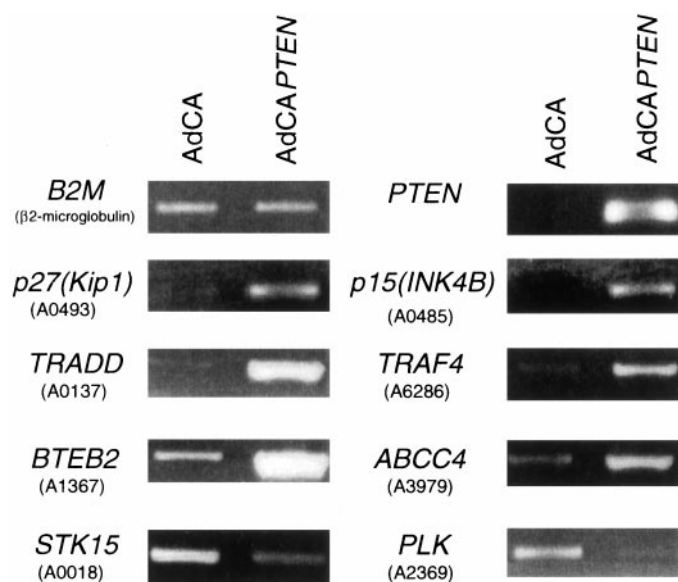


Fig. 6. RT-PCR analysis of mRNAs from AdCA- and AdCAPTEN-infected HEC-151 cells. The integrity of each RNA template was controlled through amplification of β 2-microglobulin.

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