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Molecular Characterization of a New Ovarian Cancer Cell Line, YDOV-151, Established from Mucinous Cystadenocarcinoma

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Ovarian cancer is a leading cause of death among gynecological malignancies. Established cancer cell lines are useful tools for clinical and basic researches. We have therefore established a new human ovarian cancer cell line, YDOV-151, derived from the mucinous cystadenocarcinoma and characterized it by the microarray analyses. A mucinous origin of the YDOV-151 was evident from light microscopy, and its epithelial-like character was confirmed with electron microscopy. No pathogenic mutations were found in the BRCA1 and BRCA2 genes. The subcutaneous transplantation of YDOV-151 cells into nude mice successfully induced the tumor mass after 3 weeks. cDNA microarray analysis revealed 1,926 genes (> 2-fold differences, $P < 0.05$) that distinguished the YDOV-151 from human ovarian surface epithelial (HOSE) cells. To identify candidate biomarkers, we selected five genes (SFN, RGC32, CDCA7, LAMP3, and SLCO4A1), each of which was up-regulated (> 7-fold) in YDOV-151 and had an available antibody assay for further validation. In SYBR Green real-time PCR, the relative expression levels of RGC32 (651-fold), LAMP3 (1,930-fold), and SLCO4A1 (20,598-fold) were significantly higher in YDOV-151 than in HOSEs ($P < 0.001$). RGC32 may be involved in cell cycle regulation, LAMP3 may promote metastasis, and SLCO4A1 is a member of anion-transporting polypeptides. The newly established ovarian cancer cell line, YDOV-151, would be a useful model for elucidating the biology and the pathogenesis of mucinous cystadenocarcinoma. In addition, the identification and validation of up-regulated genes may provide a genetic approach for identifying biomarkers in ovarian cancer. ———— ovarian cancer; cell line; characterization; microarray; biomarker.

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Ovarian cancer continues to be the leading cause of death among gynecological malignancies in the Western world (Jemal et al. 2007). Because of the insidious onset of this disease and the lack of reliable screening tests, over 70% of patients with ovarian cancer are already at an advanced stage at initial diagnosis (Cannistra 2004). As a result, ovarian cancer causes more deaths than any other cancer of the female reproductive system. Although many patients with advanced disease respond initially to standard combination therapy of surgery and chemotherapy, nearly 90% will develop recurrence and necessarily yield to the disease (Cooper and DePriest 2007). When discovered at an early stage, either by chance or due to symptoms, the prognosis is usually excellent, with the 5-year survival exceeding 90%.

Microarray technology enables us to analyze and measure thousands of tumor-specific gene expression profiles. To identify more effective biomarkers to detect early-stage cancer and monitor biological responses to therapy, genomics-based approaches have been used in medical research (Raetz and Moos 2004). cDNA microarray technology has been especially useful to identify genes involved in ovarian carcinogenesis (Hough et al. 2000). In addition, gene expression fingerprints representing large numbers of genes may allow precise and accurate grouping of human tumors and may identify patients unlikely to be cured by conven-

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tional therapy.

Ovarian cancers vary in histological appearance, cell biology, and tumor marker expression. Therefore, it is important to derive tumor cell lines from various ovarian cancers to serve as models to investigate the molecular and cellular pathways, and to identify the new tumor markers. Although a number of ovarian cancer-derived cell lines have been established and widely characterized (Ishiwata et al. 1984; Alama et al. 1996; Kim et al. 1997), most of them are serous type tumors. The newly established cell lines of mucinous tumors may serve as additional models to extend our knowledge of the natural history and treatment of ovarian cancer.

In this study, we established a new ovarian cancer cell line, designated YDOV-151, from mucinous cystadenocarcinoma. We subsequently assessed the cell morphology, growth rate in culture, tumor marker expression, the presence of mutations in BRCA1, BRCA2, and p53, tumorigenecity, drug sensitivity, and gene expression profile in this cell line.

Materials and Methods

Patient information

This study was carried out in accordance with the ethical standards of the Helsinki Declaration and was approved by the Institute of Review Boards (IRBs) of Gangnam Severance Hospital. The YDOV-151 cell line was established in February 2006 from malignant ascites of a 20-year-old women presenting with recurrent ovarian cancer. The patient was diagnosed with the International Federation of Gynecology and Obstetrics (FIGO) stage Ic ovarian cancer in January 2005 after surgical staging with unilateral salpingo-oophorectomy, pelvic and para-aortic lymph node dissection, and omentectomy. The histopathologic diagnosis indicated poorly differentiated mucinous cystadenocarcinoma of right ovary with capsular rupture. In the immunohistochemical analysis, the tumor cells were positive for cytokeratin (CK) 7 and negative for CK 20, which is compatible with the features of ovarian tumors (Baker and Oliva 2005).

Postoperatively, the patient received six courses of combination chemotherapy with paclitaxel and cisplatin. However, residual disease was found at second look operation and she received additional three courses of chemotherapy. In September 2005, the patient still had a residual disease on peritoneal wall, which was treated with four courses of topotecan and carboplatin. In January 2006, the patient received docetaxel and carboplatin due to disease progression. The patient developed abdominal ascites requiring paracentesis and expired in March 2006, 14 months after the original diagnosis.

Establishing YDOV-151 cell line and culture conditions

All biosamples were obtained with informed consent from the participant. Freshly obtained ascitic fluid was pelleted, which washed, and resuspended in culture media. The cells in media were then seeded for 3 hr onto a 60 mm^2 culture dish (Becton Dickinson and Company, Franklin Lakes, NJ) to rapidly remove attaching fibroblasts. Next, the medium with unattached cells was transferred to another dish. All cultures were maintained at 37°C in a humidified 5% CO_2 ambient air atmosphere and grown in a mixture of Medium 199 and MCDB 105 (1:1) (Sigma, St Louis, MO) supplemented with 10% fetal bovine serum (FBS) (Gemini Bio-Products, Calabasas, CA). A polymerase chain reaction (PCR)-based method was performed to test for possible contamination by mycoplasma in the newly established cell line according to the manufacturer's instructions (*i*-MycoPCR mycoplasma detection kit; iNtRON Biotechnology Inc., Seongnam, Korea). In brief, template DNA from 100 cells in suspension and *i*-MycoPCR Master mix solution was added to *i*-Master PCR tubes at a total volume of 20 μL. After dissolving the blue pellet by pipetting, the PCR was performed.

In vitro morphology

For morphologic studies, cells were observed daily by phase-contrast microscopy (Olympus, Tokyo, Japan) and histopathologically compared with the original tumors. For transmission electron microscopy, the obtained specimens were prefixed with 2% glutaraldehyde and paraformaldehyde, buffered with 0.1 M phosphate buffered saline (PBS), and postfixed with 1.33% OsO_4. They were then washed with alcohol that was gradually substituted with propylene oxide, embedded in an Epon mixture, cut into ultra-thin sections, and double-stained with uranyl acetate and lead citrate. Ultra-thin sections were observed and photographed with a Philips CM 10 transmission electron microscope (TEM; Philips Scientifics, Eindhoven, The Netherlands).

Growth characteristics

Population doubling times and growth curves were determined using a Cell Titer 96® Aqueous one Solution cell proliferation Assay kit (Promega, Madison, WI). Briefly, 3×10^3 viable cells were seeded onto 96-well plates in 100 μL culture media lacking FBS. Two different treatment groups were created with no or 10% FBS. Growth analysis was performed every day for the first 4 days and every other day for 10 days thereafter. For the assay, we added 20 μL of MTS (a novel tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS[(a)]) solution to every well of a 96-well plate and cultured the cells for 4 hours in a 37°C incubator, at which time the optical density (OD) value at 490 nm was measured using an ELISA reader (EL311; Bio-Tek Instruments, New York, NJ).

Tumor markers assay

The concentrations of cancer antigen 125 (CA125), CA19-9, and carcinoembryonic antigen (CEA) in the conditioned supernatant of ovarian cancer cell cultures were determined by chemiluminescent enzyme immunoassay using Architect i2000SR system (Abbott Diagnostics, Abbott Park, IL). Cells (1.5×10^6) were seeded into 25 cm^2 flasks and the conditioned supernatant was harvested four days later and stored at –80°C until assayed.

Tumorigenecity assay

To estimate the YDOV-151 tumor-forming activity, we subcutaneously transplanted 5×10^6 cells with greater than 95% viability by trypan blue exclusion into 5-week-old athymic female nude mice (BALB/c nu/nu, $n = 3$). The injected mice were monitored every 2 days to determine the time at which tumors emerged. The amount of swelling at each inoculation site was also measured. Injected mice were sacrificed 8 weeks after inoculation. For histological examination, tumor mass, liver, lung, kidney, ovary, heart, omentum, lymph node, and spleen were fixed in phosphate-buffered 10% formalin, and processed routinely to obtain paraffin sections. Four-micrometer-thick sections were stained with hematoxylin and eosin (HE) and

compared with the original slides. All studies were done in accordance with the American Association for Accreditation of Laboratory Animal Care guidelines and adhered to all national and international standards.

Human leukocyte antigen (HLA) genotyping

Genomic DNA was obtained using a standard salting-out procedure (Miller et al. 1988) and polymerase chain reaction (PCR) amplified using locus-specific primers for HLA-A, -B, and -Cw of exon 2 and 3 and subsequently subjected to direct sequencing for PCR-sequencing-based typing (PCR-SBT), and for HLA-DRB1, PCR-sequence specific oligonucleotide probe (PCR-SSOP) hybridization was used after PCR amplification with allele-specific primers for exon 2.

Mutational analysis of the BRCA1 and BRCA2 genes

Mutational screening of the BRCA1 and BRCA2 genes was carried out using PCR-denaturing high-performance liquid chromatography (DHPLC). Specifically, DNA was collected from tumor cells using the QIAamp DNA Blood Mini kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. PCR of BRCA1/2 exons were performed in a 25-μL volume containing 2 μL template DNA (about 50-100 ng/L), 10 pmol of each primer, 200 μM dNTPs, standard PCR buffer (1.5 mM $MgCl_2$), and 5 U Taq DNA polymerase (Takara Bio Inc., Kyoto, Japan). Thirty three and forty eight primers were used to amplify exons of BRCA1 and BRCA2, respectively (Friedman et al. 1994; Wagner et al. 1999). Thermal cycling was performed with a GeneAmp 2700 thermocycler (Applied Biosystems Inc., Foster City, CA). DHPLC analysis was performed using the WAVE Maker System (Transgenomic Inc., San Jose, CA) as reported previously (Gross et al. 2000). For heteroduplex and multiplex detection, crude PCR products were subjected to an additional 5 min 95°C denaturing step followed by gradual reannealing from 95°C to 25°C over 50 min. DHPLC gradients and temperatures were determined using WAVE Maker System software. Next, the purified BRCA1 and BRCA2 PCR products were analyzed by solution extraction and bidirectional sequencing using the BigDyeDeoxy terminator cycle sequencing kit (Applied Biosystems Inc., Foster City, CA) according to the manufacturer's instructions. Sequencing reactions were separated on an ABI 3100 Genetic Analyzer (Applied Biosystems Inc., Foster City, CA).

Mutational analysis of p53 exon 4

A single-stranded conformation polymorphism (SSCP) analysis was used to analyze cancer cells for mutations within exon 4 of the p53 gene. DNA was prepared using standard proteinase plus detergent digestion followed by phenol: chloroform extraction and dialysis. The primers used encompassed a 259-bp region containing codon 72 within exon 4 of the p53 gene and a 107-bp region in intron 6. Amplifications were performed in a 25-μL volume using a hybrid thermocycler. Amplified DNA was sequenced using the AmpliCycle sequencing kit (Perkin-Elmer, Branchburg, NJ) in the presence of appropriate radiolabeled primer. Sequencing reactions products were resolved by electrophoresis on a 7% denaturing polyacrylamide gel and imaged by autoradiography. All mutations detected were verified in a repeat sequencing experiment that began with DNA extracted from the dissected cells.

cDNA microarray analysis

Total RNA was extracted from YDOV-151 and four human ovarian surface epithelial (HOSE) cells (HOSE 198, 209, 211, 213) using Trizol (Invitrogen Life Technologies, Carlsbad, CA), and purified using RNeasy columns (Qiagen, Valencia, CA) according to the manufacturers' suggested protocol. Total RNA was amplified and purified using the Ambion Illumina RNA amplification kit according to the manufacturer's instructions (Ambion, Austin, TX) to yield biotinylated cRNA. Following purification, the cRNA was quantified using an ND-1000 Spectrophotometer (NanoDrop, Wilmington, DE).

A total of 750 ng of labeled cRNA samples were hybridized to each Sentrix Human Ref-6-V2 Expression Bead Chip according to the manufacturer's instructions (Illumina Inc., San Diego, CA). Array signal detection was carried out using Amersham fluorolink streptavidin-Cy3 (GE Healthcare Bio-Sciences, Little Chalfont, UK) according to the Bead Chip protocol. Arrays were scanned with an Illumina Bead Array Reader according to the manufacturer's instructions. Array data processing and analysis was performed using Illumina Bead Studio software.

A total of 23,920 probes were used in the final analysis. Data were extracted using software provided by the manufacturer (Bead Studio v. 1.0.0.5) and normalized by Quantile normalization. One-way analysis of variance (ANOVA) and local-pooled-error (LPE) testing was applied to determine differentially expressed genes across the three experimental groups. Following analysis, 1926 genes were selected with the conditions; (1) P-value of ANOVA < 0.05, (2) |Fold (B/A)| > 2 and P-value < 0.05, (3) |Fold (C/A)| > 2 and P-value < 0.05. We used Avadis Prophetic version 3.3 (Strand Genomics, Bangalore, India) for statistical analyses.

SYBR Green real-time polymerase chain reaction

The SNU-840 cell line was purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea) and SKOV3, TOV 112D, OVCA 429, and RMUG-s cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA). These cell lines were maintained in DMEM/F12 supplemented with 10% FBS in the presence of 5% CO2 at 37°C in a humidified incubator. Total RNA was extracted from 11 ovarian cancer cell lines (YDOV-13, 105, 139, 151, 157, 161, SKOV3, TOV 112D, OVCA 429, SNU-840, RMUG-s) and 8 HOSEs (HOSE 10, 15 186, 198, 201, 213, 216, 225) using the RNeasy Mini kit (Qiagen, Valencia, CA). Next, a total of 2 μg RNA from each sample was reverse transcribed into cDNA by the SuperScript™ III first-strand synthesis system (Invitrogen, Carlsbad, CA) according to the manufacturer's suggested protocol. The expression levels of selected mRNAs were measured by SYBR Green real-time PCR using an ABI 7300 instrument (Applied Biosystems, Forster, CA). The specific primers were as follows: *SFN* (forward primer 5′-AAA GTG GTC TTG GCC AGA GAG A-3′, reverse primer 5′-AAA GTG GTC TTG GCC AGA GAG A-3′), *RGC32* (forward primer 5′-ACT CAA CCT TCT ACC AGG CCA C-3′, reverse primer 5′-TTC ACT GTC TAA ATT GCC CAG AAA T-3′), *CDCA7* (forward primer 5′-CTC CTA ATT TCT TCT GCC CGA A-3′, reverse primer 5′-ATT ACA TTG CCC ACC AAC CTT T-3′), *LAMP3* (forward primer 5′-CTG CAG GTG AAA ACA ACC GAT-3′, reverse primer 5′-TCA GAC GAG CAC TCA TCC ACA-3′), and *SLCO4A1* (forward primer 5′-CAT TCC TGC ACT AAC GGC AAC-3′, reverse primer 5′-AAC TAC AAT CCA CTG GAT TCC CA-3′). The PCR was performed in 20 μL buffer containing 2 μL cDNA, 5 pmole of each primer, and power SYBR Green PCR master mix (Applied

Biosystems, Forster, CA). The thermal cycling conditions consisted of pre-incubation for 2 min at 50°C, then denaturation for 10 min at 95°C followed by 40 cycles of denaturation for 15 sec at 95°C and annealing/extension for 1 min at 60°C. The comparative Ct method was used to calculate relative quantification of gene expression as described previously (Livak and Schmittgen 2001). The normalization formula was as follows: target amount = $2^{-\Delta\Delta Ct}$, where $\Delta\Delta$Ct = [Ct (Candidate gene) – Ct (Candidate gene GAPDH)] – [Ct (HOSE186) – Ct (HOSE 186 GAPDH)].

Results

Histopathology of the original tumor

Grossly, the right ovary was enlarged and the surface showed a scattered, nodular area with mild erosion. The cut ovary surface revealed a multilocular cystic mass with mucinous contents. A myxoid and sponge-like solid area was scattered across the inner surface. There were no identifiable psammoma bodies. The original tumor showed irregularly proliferating atypical epithelial cells with papillary configuration, representing a mucinous tumor (Fig. 1A, B).

Establishment of cell culture

The proliferating cells were observed 3 days after cell culture was initiated. The cells grew as adherent monolayer after passage and lost contact inhibition, with evidence of cellular piling when cells reached a density greater than $1 \times 10^5/cm^2$. Three weeks after the primary cell seeding, the first passage of the cells was performed. The established cell line was designated YDOV-151. To date, it has undergone 31 continuous passages for more than 36 months. In mycoplasma test, YDOV-151 was verified to be free from Mycoplasma contamination.

General features of YDOV-151 cells

In the established cell line, tumor cells grew as monolayer, but sometimes formed multi-layered sheets or clusters. The cells appeared to be epithelial cell, forming a sheet of polygonal cells with a pavement-like arrangement when viewed by phase-contrast microscopy (Fig. 1C, D). Transmission electron microscopy revealed that the cells had intracellular and intercellular lumina, indented nuclei, abundant secretory granules, and numerous microvilli on the surface (Fig. 1E, F). These features suggest that the cells were epithelial, especially glandular and adenomatous in origin.

Early passage (passage 10) YDOV-151 cell growth characteristics were studied in media containing 0% or 10% FBS media as described in the methods section. The average population doubling time is 19 hr and cell viability is greater than 90% according to Trypan Blue dye exclusion tests. A growth kinetics curve according to FBS concentration is presented in Fig. 2. The preoperative CA125 and CA19-9 values of this patient were 30.6 and 234.4 U/mL, respectively. In the tumor marker assay, the CA125 (10.8 U/mL), CA19-9 (0.11 U/mL), and CEA (0.5 μg/mL) concentrations in the YDOV-151 conditioned medium were not increased. A drug sensitivity test showed that YDOV-151 cells were most sensitive to cisplatin (chemosensitivity index: 144.7) among the twelve drugs tested. Detailed drug sensitivity indices for each drug are presented in Table 1.

Transplantation in nude mice

About 3 weeks after inoculation of YDOV-151 cells into nude mice, subcutaneous tumors appeared at the sites of inoculation and grew slowly during the next 3 weeks, and reached at a maximum size of 6 mm at 6 weeks after inoculation. There was a 33.3% (one of three mice) incidence of tumor in the three mice tested (data not shown).

Genetic analysis of YDOV-151

We performed genotyping of HLA-A, HLA-B, HLA-C and HLA-DRB1 loci of YDOV-151. The two digit level of genotype was A*38/A*38, B*58/B*58, Cw*03(10)/Cw*03(Cw*10), and DRB1*03/DRB1*04. Sequencing of BRCA1 and BRCA2 demonstrated 5 heterozygous point mutations and 4 heterozygous missense mutations in BRCA1 and 5 homozygous point mutations and 3 homozygous missense mutations in BRCA2 (Fig. 3). In analysis of exons 4 of the *p53* gene, wild-type sequence (215 G/G) was detected.

Gene expression profiling

A cDNA microarray was used to analyze and compare the gene expression patterns of YDOV-151 and four HOSEs. Hierarchical clustering analysis revealed that approximately 1,926 genes were differentially expressed (> 2-fold, $P < 0.05$). Of the genes classified with altered expression, 838 were up-regulated and 1,088 were down-regulated in the YDOV-151 cell line. To investigate the association between these differentially expressed genes and functional ovarian cancer pathways, we grouped some of the genes with remarkable fold changes (> 5-fold or 10-fold) according to their function. A partial list of genes that were grouped by different functions is presented in Tables 2 and 3.

SYBR Green real-time PCR analysis of selected gene products

To validate the differentially expressed genes identified by cDNA microarray, we tested the expression of five genes by SYBR Green real-time PCR in 11 ovarian cancer cell lines including YDOV-151 and in eight HOSEs. SFN, RGC32, CDCA7, LAMP3, and SLCO4A1 gene analysis revealed that the mean $2^{-\Delta\Delta Ct}$ value, which represents relative gene expression, ranged from 2.6-fold (SFN) to 2093-fold (SLCO4A1) greater for cancer cell lines than HOSE cells. The relative gene expression of RGC32 (651-fold), LAMP3 (1,930-fold), and SLCO4A1 (20,598-fold) in YDOV-151 was significantly higher than that of eight HOSEs ($P < 0.001$) (Fig. 4). In general, SYBR Green real-time PCR results were consistent with the expression patterns of the

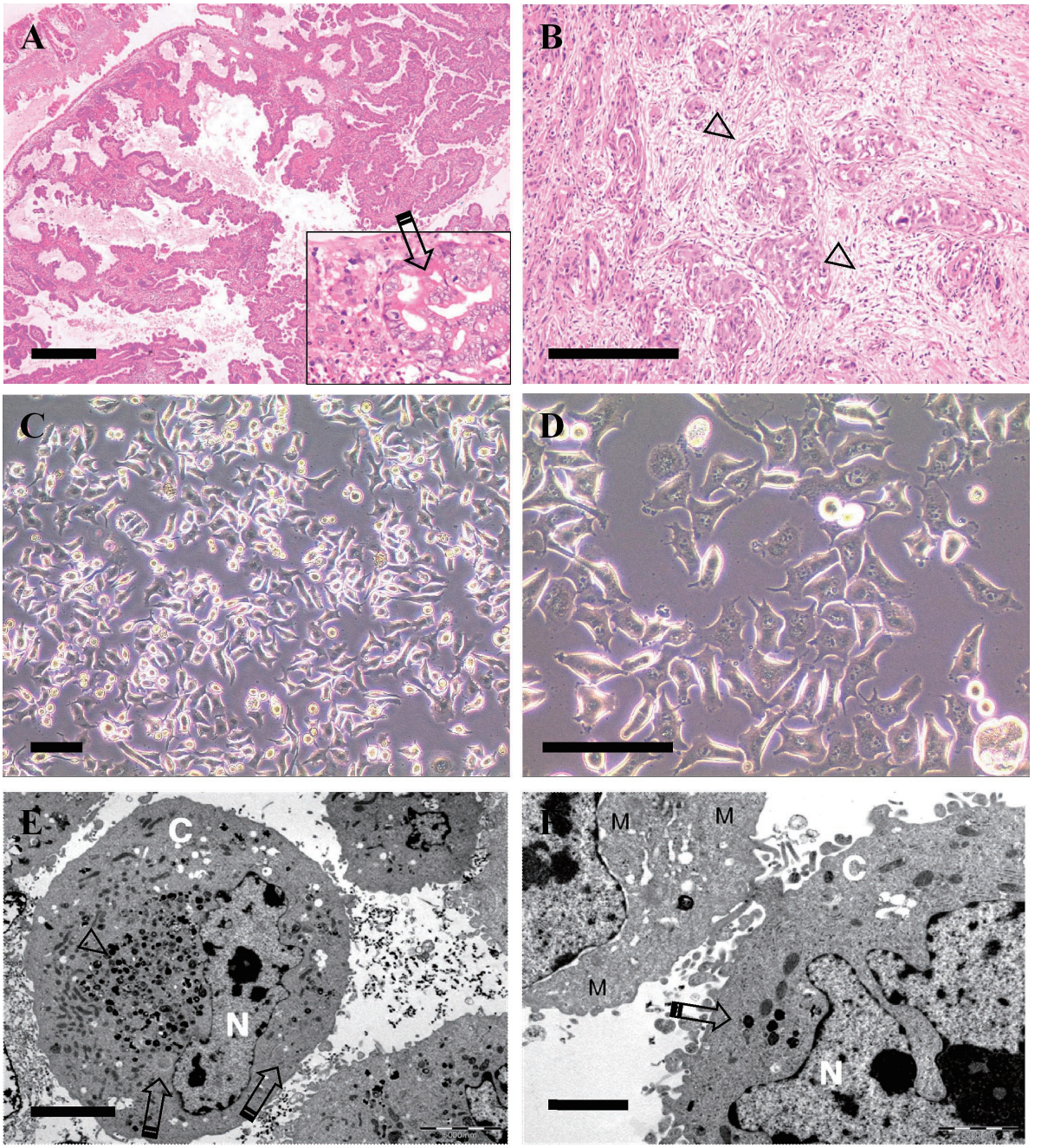

Fig. 1. Morphologic features of the YDOV-151 cell line.
(A, B) H&E-stained paraffin section of the ovarian mucinous cystadenocarcinoma from which the YDOV-151 was derived (Bars; 100 μm). A, Ovarian cyst shows irregularly proliferating atypical epithelial cells with papillary configuration, representing a mucinous tumor. In addition, there are atypical cribriform pattern glands with suspicious early stromal invasive foci (Inlet) (dashed arrow). B, Sections of peritoneal lesion show atypical malignant nests with desmoplastic stroma (arrowhead). (C, D) In phase-contrast microscopy of YDOV-151 (Bars; 100 μm), the monolayer-cultured cells appeared to be epithelial, showing a polygonal shape with elongated processes. (E, F) Ultrastructural aspects of YDOV-151 cells as examined by transmission electron microscopy. C: cytoplasm, N: nucleus. E, The mucin granules (dashed arrow) contain reticular content of medium density. The tumor cells show perinuclear mitochondria (arrowhead) in the cytoplasm. (Bar; 5000 nm) F, Higher magnification shows mucin granules (M) and abundant mitochondria (dashed arrow). (Bar; 2000 nm)

microarray data.

Discussion

We newly established the mucinous ovarian cancer cell line, YDOV-151, and characterized it by the gene expression profile, morphologic features, growth curve, tumor markers, mutational status, tumorigenecity, and drug sensitivity test.

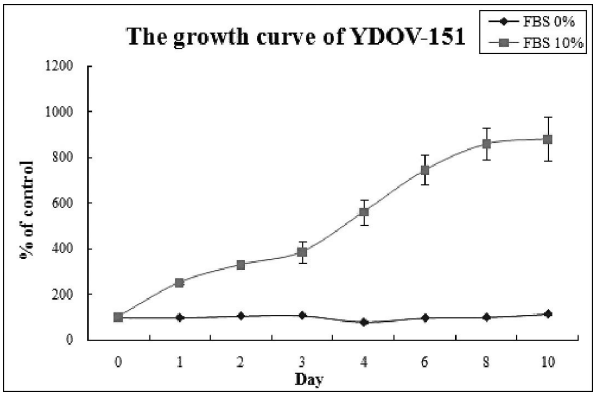

Fig. 2. Growth kinetics of cultured YDOV-151 cells under a 5% CO_2 atmosphere at 37°C.
YDOV-151 cells (3×10^3) were cultured in 96-well plates in media containing 0% (●) or 10% (■) FBS. Cell numbers were estimated at the indicated times as described in materials and methods. Each point represents the mean value from 6-wells of a 96-well plate. The population doubling time was 19 hours.

Table 1. Tumor inhibition rate tested at serial concentration of drugs (TDC) in the YDOV-151.

Drug	Concentration (X TDC)			Index[a] (Rank)[b]
	0.2 X	1.0 X	5.0 X	
Cisplatin	1.5	67.8*	86*	144.7 (1)
Irinotecan	6.9*	37.5*	77*	178.6 (2)
Paclitaxel	0*	0*	99.8*	200.2 (3)
Carboplatin	0	16.1*	80.7*	203.2 (4)
Etoposide	5.8*	22.5*	55.2*	216.5 (5)
Topotecan	13.1*	28*	37*	221.9 (6)
Gemcitabine	15.7*	18*	18.5*	247.8 (7)
5-FU	0	11.8*	33.2*	255.0 (8)
Doxorubicin	6*	10.8*	16.5*	266.7 (9)
Oxaliplatin	0	0	31.3*	268.7 (10)
Bleomycin	0	0	8.7*	291.3 (11)
Docetaxel	0*	0*	5.1*	294.9 (12)

TDC, tested drug concentration.
*P value < 0.05.
[a]Chemosensitivity Index = 300 – SUM of tumor inhibition rate (0.2 X – 5.0 X).
[b]Rank = more desirable in drug tested.

Epithelial ovarian cancer is currently classified into four major histological subtypes: serous, mucinous, endometrioid, and clear, each of which has a morphological and immunohistochemical similarity to normal epithelium. Mucinous ovarian cancer accounts for approximately 10% of epithelial ovarian cancers (McGuire et al. 2002). Molecular differences between mucinous and serous ovarian cancers suggest that these tumors should be regarded as separate entities. Previous studies have identified overexpression of the *KRAS* oncogene and a relative absence of mutations in the tumor suppressor gene *p*53 in mucinous tumors, whereas serous tumors show the converse (Fujita et al. 2003). Both the difficulty in accurate diagnosis of the primary disease and the relative rarity of mucinous tumor has contributed to a lack of knowledge regarding the molecular basis of its development and progression.

Because pure cells in culture can be used for a variety of studies that cannot be carried out on tissue specimens, the study of cancer cell lines has played an important role in our understanding of cancer biology. Moreover, the heterogeneity of ovarian cancer needs the establishment of numerous ovarian cancer cell lines with unique biological properties. Therefore, establishing a new cell line is important for cancer-related research on cell function, chemotherapeutic drug sensitivity, and developing new therapeutic methods. The newly established and genetically characterized mucinous ovarian cancer cell line, YDOV-151, may help enhance our understanding of epithelial ovarian cancer biology.

YDOV-151 cells have a polygonal shape and grow as monolayer. Most tumor cells were morphologically similar to the primary tumor from which the cells were derived. Our estimation of doubling time for YDOV-151 is 19 h, which is shorter than that of other mucinous ovarian carcinoma cell lines whose doubling times range between 28 to 37 h (van Niekerk et al. 1988; Sato et al. 2002). In a tumor marker assay, YDOV-151 appears to weakly express CA125 (10.8 U/mL) and CA19-9 (0.11 U/mL), although the preoperative serum CA125 and CA19-9 levels of the patient were 30.6 U/mL and 234.4 U/mL, respectively. We suspect that this result was a property of the tumor cells themselves rather than a loss of ability to express tumor markers in culture, and may reflect a preoperative elevation of these markers partly attributable to a systemic inflammatory response to a tumor burden. Lastly, YDOV-151 cells are successfully engrafted in nude mice and, therefore, should be useful for ovarian cancer mouse models.

In the present study, the HLA-A, -B, -C, and -DRB1 gene allelic polymorphisms in YDOV-151 were investigated by molecular methods. Cancer development is a multistep process that includes the accumulation of genetic alterations. As a result, tumor cells often express new antigens which are presented in the context of major histocompatibility complex (MHC) molecules (Rosenberg 2001). The HLA system, the human version of the MHC, is a highly polymorphic genetic system and plays an important role in immune response by presenting peptides to T lymphocytes (Parham 1996). HLA gene polymorphism is considered to be a factor useful in identifying susceptibility loci for more than 100 genetic or infectious diseases. Relatively little is known about the molecular pathogenesis of ovarian cancer, and insight may be gained by identifying genetic risk factors such as HLA alterations.

It is believed that approximately 5-12% of ovarian cancers are attributable to an inherited susceptibility, with the BRCA1/2 mutations accounting for the majority of hereditary ovarian cancers (Frank et al. 1998). Estimates of

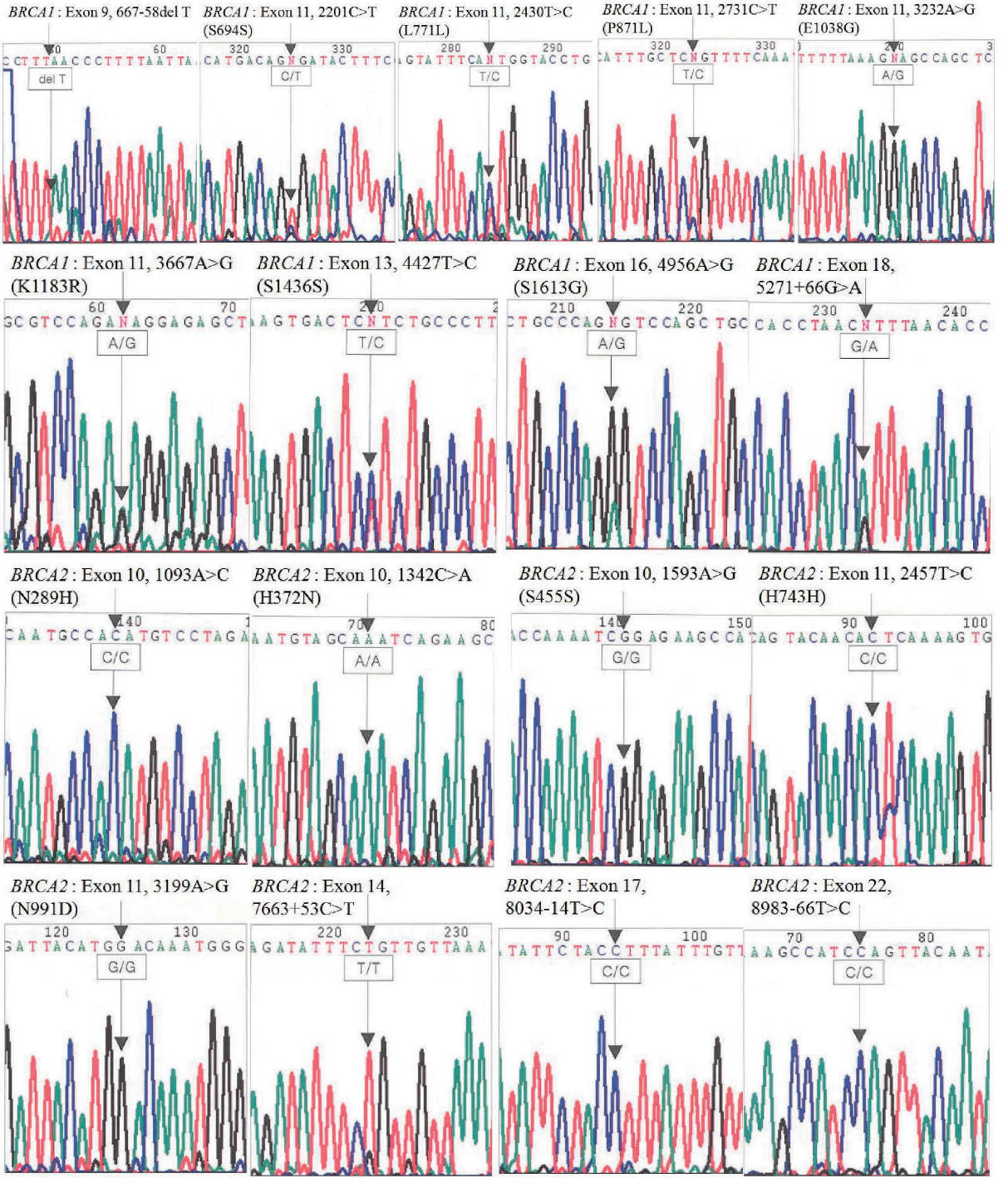

Fig. 3. BRCA1/2 PCR-DHPLC (denaturing high-performance liquid chromatography) sequencing in YDOV-151. Nine BRCA1 polymorphisms (667-58delT on exon 9 [int 8], 2201C > T, 2430T > C, 2731C > T, 3232A > G, and 3667A > G on exon 11, 4427T > C on exon 13, 4956A > G on exon 16, 5271 + 66G > A on exon 18 [int 18]) and 8 BRCA2 homozygous mutations (1093A > C, 1342C > A, and 1593A > G on exon 10, 2457T > C and 3199A > G on exon 11, 7663 + 53C > T on exon 14 [int 14], 8034-14T > C on exon 17 [int 16], 8983-66T > C on exon 22 [int 21]) were detected in this study. The arrow indicates point or missense mutations in the YDOV-151 cells.

the frequency of these mutations in ovarian cancer range between 2-12% for BRCA1 and 2-6% for BRCA2 mutations (Risch et al. 2001; Majdak et al. 2005). BRCA1/2 act as tumor suppressors and play a role in maintaining genomic stability through DNA damage recognition and repair, transcriptional regulation, and cell cycle control (Scully and Livingston 2000). Abnormal DNA repair mechanisms contribute to carcinogenesis and also modify a tumor's

Table 2. Selected groups of up-regulated genes expressed at least 5-fold higher in YDOV-151 compared with four HOSEs.

TargetID	Definition	Symbol	Fold[a]	Accession
Cell adhesion				
ILMN_12740	cadherin 3, type 1	CDH3	31.55	NM_001793
ILMN_23446	epithelial V-like antigen 1	EVA1	10.85	NM_144765
Cell signalling				
ILMN_4070	tumor-associated calcium signal transducer 1	TACSTD1	39.70	NM_002354
ILMN_6835	laminin, alpha 3	LAMA3	35.98	NM_198129
ILMN_11468	RAB25, member RAS oncogene family	RAB25	33.95	NM_020387
ILMN_25104	forkhead box A1	FOXA1	13.28	NM_004496
ILMN_28725	stanniocalcin 2	STC2	8.23	NM_003714
Immunity and defense				
ILMN_8896	S100 calcium binding protein A4	S100A4	8.43	NM_002961
ILMN_22638	neuronal pentraxin II	NPTX2	5.26	NM_002523
ILMN_137905	glutathione peroxidase 3	GPX3	5.12	NM_002084
Iron transport				
ILMN_3183	solute carrier organic anion transporter family, member 4A1	SLCO4A1	91.82	NM_016354
mRNA transcription				
ILMN_4354	iroquois homeobox protein 3	IRX3	68.27	NM_024336
ILMN_27218	transcription elongation factor A (SII), 3	TCEA3	6.73	NM_003196
Biological process unclassified				
ILMN_14549	response gene to complement 32	RGC32	12.05	NM_014059
ILMN_138827	cell division cycle associated 7	CDCA7	9.92	NM_145810
ILMN_21715	lysosomal-associated membrane protein 3	LAMP3	7.38	NM_014398

[a] Fold = gene expression level of YDOV-151/ mean gene expression level of four HOSEs.

Table 3. Selected groups of down-regulated genes expressed at least 10-fold lower in YDOV-151 compared with four HOSEs.

TargetID	Definition	Symbol	Fold[a]	Accession
Cell cycle control				
ILMN_3680	kallikrein 11	KLK11	–130.93	NM_006853
DNA recombination				
ILMN_6405	REC8-like 1	REC8L1	–26.90	NM_005132
Immunity and defense				
ILMN_16098	thrombospondin 2	THBS2	–24.68	NM_003247
ILMN_5682	complement component 3	C3	–56.76	NM_000064
mRNA transcription				
ILMN_2220	LIM domain binding 2	LDB2	–22.06	NM_001290
Protein synthesis/modification				
ILMN_21403	carboxypeptidase A4	CPA4	–17.86	NM_016352
ILMN_20248	dipeptidyl-peptidase 4	DPP4	–28.08	NM_001935
Signal transduction				
ILMN_3875	vascular cell adhesion molecule 1	VCAM1	–14.29	NM_001078
ILMN_5665	gap junction protein, alpha 1, 43kDa	GJA1	–14.47	NM_000165
ILMN_6950	secretory granule, neuroendocrine protein 1	SGNE1	–29.11	NM_003020
ILMN_21430	chromosome 20 open reading frame 75	C20orf75	–68.39	NM_152611
ILMN_30352	heart alpha-kinase	HAK	–78.37	NM_052947

[a] Fold = gene expression level of YDOV-151/ mean gene expression level of four HOSEs.

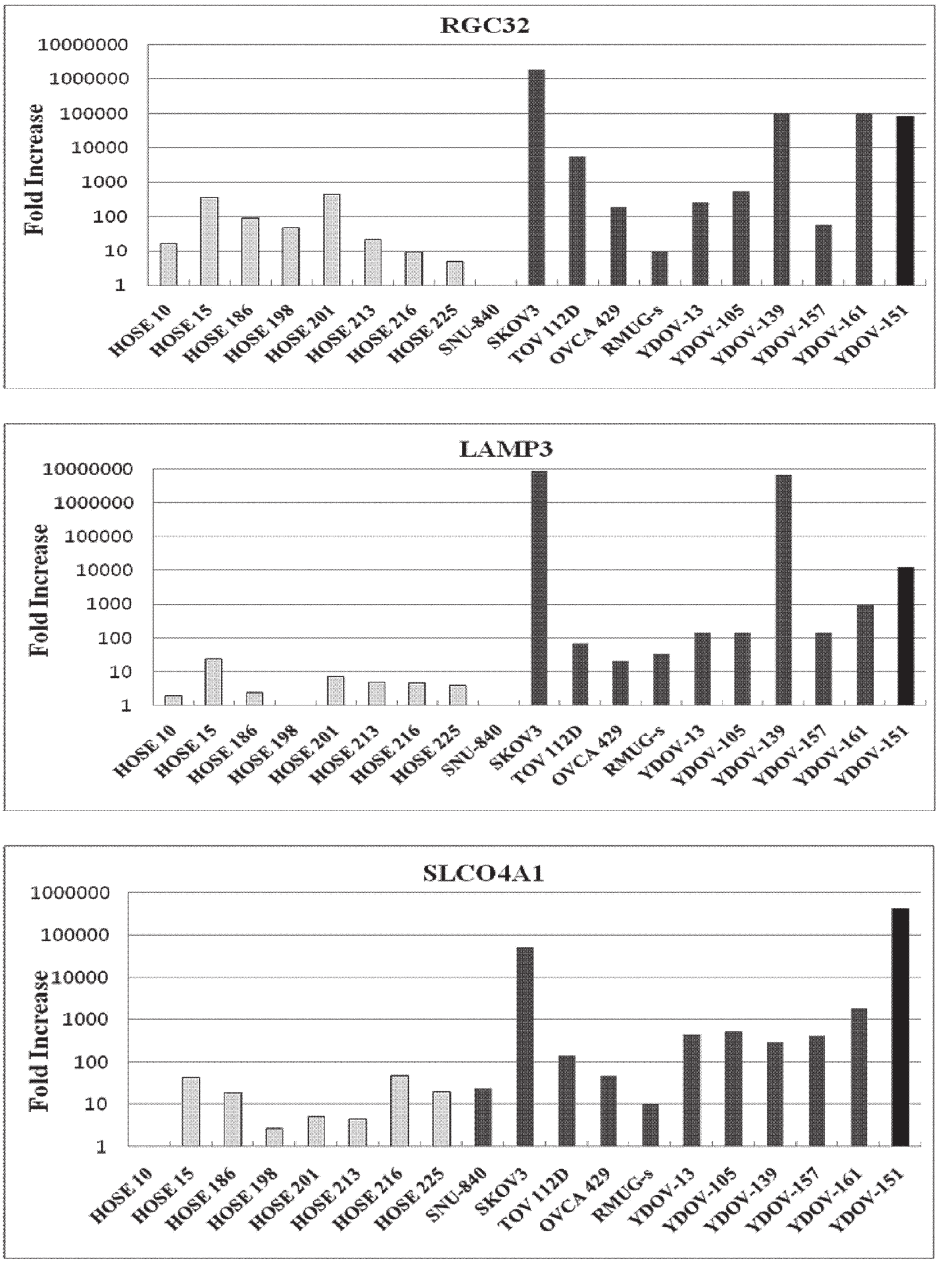

Fig. 4. Validation of differentially regulated genes in YDOV-151 using SYBR Green real-time PCR. Expression analysis of 5 genes revealed that RGC32 (358-fold), LAMP3 (450-fold), and SLCO4A1 (2093-fold) genes were strongly up-regulated in 11 cancer cell lines compared to 8 HOSEs.

response to chemotherapy. BRCA1 genotyping by PCR-DHPLC sequencing method revealed 9 polymorphisms and heterozygosity showed that no allele has been lost in the tumor. In BRCA2 genotyping, 8 homozygous point or missense mutations were detected and homozygous variants could indeed point to loss of one allele. Previous studies reported that certain genetic polymorphisms are associated with increased breast and ovarian cancer risk (Durocher et al. 1996). However, it has also been reported that germline mutations and genetic polymorphisms in BRCA1/2 are not important in ovarian cancer, and polymorphisms in selected ovarian cancer susceptibility genes are not associated with the clinicopathologic characteristics of ovarian cancer in Korean populations (Kim et al. 2005). Thus, the research on the role of genetic polymorphisms in BRCA1/2 is still controversial.

The p53 tumor suppressor gene is the most frequently mutated gene in human cancers (Greenblatt et al. 1994) and plays a critical role in cell cycle and apoptosis regulation. The p53 gene has been shown to be mutated in approximately 15-80% of ovarian cancers (Jacobs et al. 1992; Kohler et al. 1993; Kupryjanczyk et al. 1993). However, p53 mutations, which are considered important in defining paclitaxel sensitivity, are less frequent in mucinous tumors

(Reles et al. 2001). Cell culture experiments have shown that tumor cell sensitivity to various chemotherapeutic agents depends on the efficient induction of apoptosis mediated by functional p53 protein. Loss of p53 can enhance resistance to chemotherapy (Vikhanskaya et al. 1997). The hypothesis that ovarian cancer cells with functional p53 are more sensitive to cisplatin is further supported by the findings of gene therapy. Introducing wild-type p53 protein via adenovirus gene transfer into A2780/CP cisplatin resistant cells significantly sensitized these cells to platinum cytotoxicity, indicating that p53 is involved in cisplatin resistance (Song et al. 1997). In our study, we found wild-type p53 in YDOV-151, which was consistent with drug sensitivity test that the cell line was most sensitive to cisplatin. However, we analyzed the p53 mutation only in the exon 4, which is a limitation of our p53 mutational analysis.

Progress in molecular medicine, particularly the use of microarray technology, has allowed the identification of numerous cancer-related genes. Candidate biomarker genes can further be validated by real-time PCR analysis of mRNA expression and/or immunohistochemical analysis of protein expression. Because PCR is not dependent on antibody availability and sensitivity, it can be used for the initial validation of candidate genes. Taking into consideration that no single marker identified thus far is sensitive- or specific-enough for early ovarian cancer diagnosis, there is a clear need to identify additional ovarian cancer marker. Therefore, we compared the YDOV-151 with four healthy ovarian epithelial cell lines using a cDNA microarray to identify genes upregulated in mucinous ovarian tumor and to identify novel biomarkers for this disease. The YDOV-151 was clearly distinguishable from HOSEs based on their patterns of gene expression. Gene ontology analysis revealed that a number of genes involved in carcinogenesis, including cell adhesion and cell signaling, were upregulated in the YDOV-151. Although KRAS mutations are often associated with mucinous ovarian tumor, we did not find any evidence of upregulated KRAS expression in this microarray analysis. Finally, some of these microarray findings were validated by SYBR Green real-time PCR analyses on 8 HOSEs and 11 ovarian cancer cell lines. For all five genes tested, overexpression in ovarian cancer cells was confirmed.

In conclusion, the present study shows that the newly established mucinous ovarian tumor cell line, YDOV-151, provides an additional model for basic research on ovarian cancer and can be used to identify new biomarkers for the early detection, screening, and evaluation of drug responses in ovarian cancer. Differentially expressed genes consistently found in several microarray studies are likely to be associated with carcinogenesis and good candidate tumor markers, although better evaluation of these markers and an understanding of their relation to the biological and molecular events involved in ovarian cancer carcinogenesis requires a larger patient cohort.

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