Establishment of a New Ovarian Cancer Cell Line CA5171

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Abstract

A new cell line, CA5171, derived from a chemotherapy-naïve, high-grade undifferentiated ovarian carcinoma was established and characterized. The CA5171 cells presented with cobblestone morphology and a doubling time of 24 hours. Gene mutation analysis showed that the cells belonged to the type II ovarian cancer pathway with mutations of PIK3CA, PTEN, and TP53. Single-nucleotide polymorphism array analysis showed no homozygous gene deletion; however, several loci of gene copy number gains were noted in chromosome 1, 2, 5, 9, 10, 12, 15, 16, 20, and X. The in vitro and in vivo experiments showed that the cells were sensitive to paclitaxel and doxorubicin, but resistant to cisplatin. The cells also presented epithelial–mesenchymal transition properties that may have been related to their invasion and migration potential. The CA5171 cells show the potential as a new cell line for studies on epithelial ovarian carcinoma.

Keywords

epithelial ovarian carcinoma, cell line, type II pathway

Introduction

The prognosis of epithelial ovarian carcinoma is generally poor.^{1,2} Current treatments include cytoreduction surgery and adjuvant chemotherapy with a platinum and paclitaxel regimen, which has an overall response rate of 80%, although these patients usually relapse within 2 years and ultimately die of recurrence.³

The pathogenesis of epithelial ovarian carcinoma includes types I and II tumor pathways.⁴ Type I tumors are composed of low-grade serous, low-grade endometrioid, clear cell, mucinous, and Brenner carcinomas that are generally indolent. Type II tumors consist of high-grade serous carcinomas, undifferentiated carcinomas, and carcinosarcomas that are aggressive. The type I tumors are relatively genetically stable and are characterized by specific gene mutations of *KRAS*, *BRAF*, *ERBB2*, *CTNNB1*, *PTEN*, or *PIK3CA*. The type II tumors are genetically highly unstable and frequently have *TP53* mutations. Understanding the pathogenesis is the key for the development of new strategies for ovarian cancer.

Cancer cell lines are useful tools for investigating tumorigenesis and drug resistance. In this study, we reported a new cell line established from solid tumor of a chemotherapy-naïve epithelial ovarian carcinoma patient. The CA5171 cells may provide a new choice of study tool for epithelial ovarian carcinoma.

Materials and Methods

Clinical Information of the Patient

A 57-year-old menopausal woman who had Trousseau syndrome presented with thrombosis of the lower limbs. She did not have any known familial history of cancer; however, a pelvic complex tumor was noted after initial workup. She underwent cytoreduction surgery, and bilateral adnexal solid tumors with multiple tumor implants were noted in the abdominal and pelvic cavities. Fresh tumor tissue was collected during the surgery, and the pathological report showed stage IIIc high-grade undifferentiated ovarian carcinoma. Adjuvant

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platinum-based chemotherapy was given; however, she died two months later due to disease progression.

Establishment of the Cancer Cell Line

The study protocol was reviewed and approved by National Taiwan University Hospital Research Ethics Committee. The CA5171 cell line was established from the tumor tissue of the patient described earlier. The primary culture media for the tumor cells were supplemented with 10% fetal bovine serum, 50 U/mL of penicillin/streptomycin, 2 mmol/L of L-glutamine, 1 mmol/L of sodium pyruvate, 2 mmol/L of nonessential amino acids, and recombinant human insulin, and were grown at 37°C in an atmosphere of 5% carbon dioxide. Briefly, the tumor tissue was dissected into small pieces, and then put in a 100-mm petri dish that contained the culture medium supplemented with collagenase at 37°C. After 4 hours, the suspended cells were collected into 10 mL of culture media, and the remaining pieces of tumor tissue were discarded. The culture medium containing the suspended cells was divided into four 60-mm petri dishes. The tumor cells were divided in a ratio of 1:2 once per week until passage 20, twice per week until passage 70, and thereafter the cells were maintained without recombinant human insulin.

In Vitro Measurements of the Proliferation of the Cells

The CA5171 tumor cells (2×10^5 /well) were incubated with the culture medium in triplicate, in flat-bottomed 6-well plates. The tumor cells were then trypsinized and counted directly in a counting chamber with a light microscope at 24, 48, and 72 hours.

Tumor Cell Invasion Assay

The tumor cell invasion assay was performed using a Boyden chamber with filter inserts (pore size, 8 μ m) coated with Matrigel (40 μ g; Collaborative Biomedical, Becton Dickinson Labware, Bedford, Massachusetts) in 24-well dishes (Nucleopore, Pleasanton, California). Briefly, 2×10^5 tumor cells were seeded in the upper chamber, and the same medium was placed in the lower chamber. The plates were incubated for 24 hours, and then the cells were fixed in methanol and stained with 0.05% crystal violet in phosphate-buffered saline (PBS) for 1 hour at room temperature. The cells on the upper side of the filters were removed by cotton-tipped swabs, and the filters were washed with PBS. The cells on the lower side of the filters were defined as invasive cells and counted in 10 different fields of each filter at a 200-fold magnification.

Cell Migration Assay

The cell migration assay was performed using wound-healing assays. The tumor cells were seeded into 6-well plates overnight. A sterile 200- μ L pipette tip was used to scratch the cells to form a wound, and the cells were then cultured for

Table 1. Primer Sequences of Reverse-Transcription Polymerase Chain Reaction.

Gene		Primers
GAPDH	Sense	5-ACCCAGAAGACTGTGGATGG-3
	Antisense	5-TGCTGTAGCCAAATTCGTTG-3
CK7	Sense	5-CAGGATGTGGTGGAGGACTT-3
	Antisense	5-TGAGGGTCCTGAGGAAGTTG-3
CK20	Sense	5-ACGCCAGAACAACGAATACC-3
	Antisense	5-ACGACCTTGCCATCCACTAC-3
CA125	Sense	5-AGCATCCTGGACGTAACCAC-3
	Antisense	5-CAGGTGGAAGGGTGTCTGT-3
TP53	Sense	5-GTTCCGAGAGCTGAATGAGG-3
	Antisense	5-TCTGAGTCAGGCCCTTCTGT-3
EGFR	Sense	5-CAACACCCTGGTCTGGAAGT-3
	Antisense	5-GCTTCGGAGATGTTGCTTC-3
FLT-1	Sense	5-AATCATTCCGAAGCAAGGTG-3
	Antisense	5-GGAGGTATGGTGCTTCCTGA-3
KDR	Sense	5-CCCACGTTTTTCAGAGTTGGT-3
	Antisense	5-CTACCGGTTTGCCTCCAAT-3
SNAIL1	Sense	5-CCAATGCTCATCTGGGACTC-3
	Antisense	5-TGACATCTGAGTGGGTCTGG-3
SNAIL2	Sense	5-GAGCATACAGCCCCATCACT-3
	Antisense	5-GCAGTGAGGGCAAGAAAAAG-3
ZEB1	Sense	5-GCAGCTGAAGAGGACCAGAG-3
	Antisense	5-TGGTGATGCTGAAAGAGACG-3
ZEB2	Sense	5-TCCTAATATTCCGCCTGTCG-3
	Antisense	5-TCAGCAGTTCATCGGAGTTG-3
TCF3	Sense	5-GCACTGGCCTCGATCTACTC-3
	Antisense	5-GGCCTTCAGCTCCTTCTTCT-3
TWIST1	Sense	5-GTCCAGCTCGCCAGTCTC-3
	Antisense	5-CATCTTGGAGTCCAGCTCGT-3
E-cadherin	Sense	5-TGCCCAGAAAATGAAAAAGG-3
	Antisense	5-GTGTATGTGGCAATGCGTTC-3

Abbreviation: RT-PCR, reverse-transcriptase polymerase chain reaction.

24 hours. The cells that migrated to the wound were defined as migrating cells and visualized with an inverted Olympus phase-contrast microscope (Olympus Taiwan, Taipei, Taiwan). The healing rate was quantified by measuring the number of migrating cells that were counted at 4 different areas in each assay.

Reverse-Transcription Polymerase Chain Reaction

Total RNA of the cells was isolated using TRIzol reagent (Invitrogen, Carlsbad, California) following the manufacturer's instructions. The RNA was first reverse transcribed to complementary DNA using a Moloney murine leukemia virus reverse transcriptase kit (Invitrogen Life Technologies, San Diego, California). To generate CK7, CK20, CA125, TP53, EGFR, FLT-1, KDR, ZEB1, ZEB2, SNAIL1, SNAIL2, TCF3, TWIST1, and E-cadherin, several sets of primers were performed for 30 cycles. GAPDH was used as a housekeeping gene to compare with the aforementioned target genes (Table 1). The polymerase chain reaction (PCR) products were then analyzed in 1% agarose gel with ethidium bromide staining in tris-borate-EDTA solution.

Table 2. Primer Sequences of Gene Mutation Analysis.

Gene	Exon	Primer Sequence	
KRAS	1	Primer 1	5-GTAAACGACGGCCAGTTTGAAACCCAAGGTACATTTTCAG-3
		Primer 2	5-TCTTAAGCGTCGATGGAGGAG-3
KRAS	2	Primer 1	5-GTAAACGACGGCCAGTATGCATGGCATTAGCAAAGAC-3
		Primer 2	5-CGTCATCTTTGGAGCAGGAAC-3
BRAF	15	Primer 1	5-GTAAACGACGGCCAGTTTGTGAATACTGGGAAGTATGAAA-3
		Primer 2	5-TCATCCTAACACATTTCAAGCC-3
TP53	3	Primer 1	5-GTAAACGACGGCCAGTGAGGAATCCCAAAGTTCCAAAC-3
		Primer 2	5-ACGTTCTGGTAAGGACAAGGG-3
TP53	4	Primer 1	5-GTAAACGACGGCCAGTGGGCCAGACCTAAGAGCAATC-3
		Primer 2	5-AAGCTCCTGAGGTGTAGACGC-3
TP53	5	Primer 1	5-AGGCCCTTAGCCTCTGTAAGC-3
		Primer 2	5-GTAAACGACGGCCAGTCTGCTCAGATAGCGATGGTG-3
TP53	6	Primer 1	5-GTAAACGACGGCCAGTAGAAATCGGTAAGAGGTGGGC-3
		Primer 2	5-CATCCTGGCTAACGGTGAAC-3
TP53	7	Primer 1	5-GTAAACGACGGCCAGTTTGGGCAGTGCTAGGAAAGAG-3
		Primer 2	5-GTTGGGAGTAGATGGAGCCTG-3
PIK3CA	1	Primer 1	5-TGCTTTGGGACAACCATACATC-3
		Primer 2	5-CTTGCTTCTTTAAATAGTTCATGCTTT-3
PIK3CA	1	Primer 1	5-CCCCTCCATCAACTTCTTCAA-3
		Primer 2	5-ATTGTATCATACCAATTTCTCGATTG-3
PIK3CA	9	Primer 1	5-TCAGCAGTTACTATTCTGTGACTGG-3
		Primer 2	5-GTAAACGACGGCCAGTTGCTGAGATCAGCCAAATTCA-3
PIK3CA	20	Primer 1	5-GTAAACGACGGCCAGTGACATTTGAGCAAAGACCTGAAG-3
		Primer 2	5-TGGATTGTGCAATTCCTATGC-3
PTEN	1	Primer 1	5-TTCCATCCTGCAGAAGAAGC-3
		Primer 2	5-GTAAACGACGGCCAGTTCCGTCTAGCCAAACACACC-3
PTEN	2	Primer 1	5-GTAAACGACGGCCAGTTCTGTGATGTATAAACCGTGAGTTTC-3
		Primer 2	5-CCCTGAAGTCCATTAGGTACGG-3
PTEN	3	Primer 1	5-GTAAACGACGGCCAGTCATGATTACTACTCTAAACCCATAGAAGG-3
		Primer 2	5-TCAAATATGGGCTAGATGCCA-3
PTEN	4	Primer 1	5-ATAAAGATTCAAGCAATGTTTGTAG-3
		Primer 2	5-GTAAACGACGGCCAGTGACCAACTGCCTCAAATAGTAGG-3
PTEN	5	Primer 1	5-TGCAACATTTCTAAAGTTACCTACTTG-3
		Primer 2	5-GTAAACGACGGCCAGTTTACTTGTCAATTACACCTCAATAAA-3
PTEN	6	Primer 1	5-AATGGCTACGACCCAGTTACC-3
		Primer 2	5-GTAAACGACGGCCAGTTTGGCTTCTTTAGCCCAATG-3
PTEN	7	Primer 1	5-GTAAACGACGGCCAGTTGCAGATACAGAATCCATATTTTCG-3
		Primer 2	5-AATGTCTCACCAATGCCAGAG-3
PTEN	8	Primer 1	5-TGCAACAGATAACTCAGATTGCC-3
		Primer 2	5-GTAAACGACGGCCAGTTGTCAAGCAAGTTCTTCATCAGC-3
PTEN	9	Primer 1	5-GTAAACGACGGCCAGTAAAGATCATGTTTGTACAGTGCTTAA-3
		Primer 2	5-TGACACAATGTCCTATTGCCA-3
CTNNB1	2	Primer 1	5-AAATATTTCAATGGGTATATCACAG-3
		Primer 2	5-GTAAACGACGGCCAGTTCCACAGTTCAGCATTTACCTAAG-3

Mutational Analysis

The primers for the relevant exons of genes including *KRAS*, *BRAF*, *TP53*, *PIK3CA*, *PTEN*, and *CTNNB1* are listed in Table 2.⁵ The CA5171 cells were PCR amplified, sequenced, and assessed for potential alterations. The sequences were then analyzed by the Mutation Surveyor program (Soft Genetics LLC, State College, Pennsylvania).

Single-Nucleotide Polymorphism Array Analysis

Single-nucleotide polymorphisms (SNPs) were genotyped using 250K NSPI arrays (Affymetrix, Santa Clara, California)

at the Microarray Core Facility of China Medical School, Taichung, Taiwan. Briefly, genomic DNA was digested using a restriction enzyme followed by PCR amplification. The products were then digested with DNase I to become fragmented products with sizes ranging from 250 to 2000 bp. The fragmented PCR products were labeled with biotin and hybridized to the arrays. The resulting images were scanned using a Gene Chip Scanner 3000 (Affymetrix). Analysis of SNP array data was performed using Partek Genomic Suite Software (Partek Inc, St Louis, Missouri). All procedures and parameters followed a previous study,⁶ except that the minimum requirement of consecutive genomic markers was set as 5 in this study. We

used an arbitrary cutoff of >3 copies to define amplification, a cutoff of <0.5 copies to define homozygous deletion, and a copy number between 0.5 and 1.5 to define hemizygous deletions.

Responses to Cytotoxic Drugs

The tumor cells (1×10^3 /well) were seeded in triplicate in flat-bottomed, 96-well microtiter plates and incubated for 24, 48, or 72 hours with the culture media and cytotoxic drugs to be tested. Briefly, the cells were harvested and treated with paclitaxel (0.005, 0.05, 0.5, or 5 $\mu\text{mol/L}$), doxorubicin (0.005, 0.05, 0.5, or 5 $\mu\text{mol/L}$), and cisplatin (0.05, 0.5, 5, or 50 $\mu\text{mol/L}$). The cells treated with PBS were used as the negative controls. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (30 μL ; 5 mg/mL) was added and the mixture was incubated for 5 hours, after which 100 μL of dimethyl sulfoxide was added to dissolve formazan crystals under vigorous shaking for 30 minutes. The absorption at an optical density of 570 nm was detected by an enzyme-linked immunosorbent assay reader.

In Vivo Tumor Growth Experiments

Six- to eight-week-old female nude mice (3 per group) were challenged with 2×10^6 , 5×10^5 , 1×10^5 , or 2×10^4 cells subcutaneously into the right back. The mice were monitored regularly for evidence of tumor growth by inspection and palpation twice a week. The size of each tumor size was measured and recorded serially. Tumor volume was calculated by the following formula: tumor volume = $0.75 \times \pi \times (\text{radius} [\text{mm}])^3$. The tumors were fixed with 4% buffered formaldehyde, and the sections were stained with hematoxylin and eosin.

In Vivo Cytotoxic Drug Experiments

Six- to eight-week-old female NOD/SCID mice (3 per group) were challenged with 2×10^6 cells subcutaneously into the right back. The mice were monitored regularly for evidence of tumor growth by inspection and palpation twice a week. The size of each tumor was measured and recorded serially. From the fifth week after CA5171 cell inoculation, the intratumoral cytotoxic drug was given twice per week for 3 weeks. The dosage of cytotoxic drug was 10 mg/kg for paclitaxel, 5 mg/kg for doxorubicin, and 20 mg/kg for cisplatin. The control group received only PBS. The change of tumor volume was the difference of the tumor volume in the fifth week after tumor cell inoculation (just before the treatment of cytotoxic drugs) and the tumor volume in the sixth, seventh, and eighth week after tumor cell inoculation (1, 2, and 3 weeks after the treatment of cytotoxic drug, respectively) in each mouse. The percentage of the change in the tumor volume is the percentage of division of the change of tumor volume by the volume of 5 weeks after the tumor cell inoculation. These were calculated in the following formulas:

$$\text{Tumor volume}(V) = 0.75 \times \pi \times (\text{radius} [\text{mm}]^3).$$

$$\begin{aligned} \text{The change of tumor volume } (\Delta V) &= V_{\text{after drug treatment}} \\ &- V_{\text{before drug treatment (fifth week after tumor cell inoculation)}} \end{aligned}$$

$$\begin{aligned} \text{The percentage of the change in the tumor volume } (\%) &= [\Delta V / V_{\text{before drug treatment (fifth week after tumor cell inoculation)}}] \\ &\times 100\% \end{aligned}$$

Statistical Analysis

Data were analyzed using SPSS version 15.0 (SPSS Inc, Chicago, Illinois). The Student *t* test was used for continuous variables and Fisher exact test was used for categorical variables. All data were expressed as mean $\pm$ standard error from 3 different experiments.

Results

The Basic Characteristics

The cells exhibited a cobblestone morphology characteristic of epithelial cells (Figure 1A). The doubling time was about 24 hours (Figure 1B). The invasion assays showed that 300.0 ± 6.9 invasive cells were noted after 48 hours, 583.6 ± 8.6 after 72 hours, and 775 ± 7.9 after 96 hours (Figure 1C). The migration assays showed that 89.0 ± 3.8 migrated cells were noted after 6 hours, and 217.6 ± 7.2 after 12 hours (Figure 1D). The cells expressed *CK7*, *CA125*, *TP53*, *EGFR*, *VEGFR* (*FLT-1* and *KDR*), *SNAIL1*, *ZEB1*, *TCF3*, and *TWIST1*, but not *CK20*, *SNAIL2*, and *E-cadherin* (Figure 2).

Mutations of PIK3CA, PTEN, and TP53

The gene mutations in *PIK3CA*, *PTEN*, and *TP53* and wild type in *KRAS*, *BRAF*, and *CTNNB1* were found in the cells. The guanine in DNA nucleotide 1624 of exon 9 of *PIK3CA* mutated to adenine, making the coding amino acid change from glutamic acid to lysine. The cytosine in DNA nucleotide 80 of exon 1 of *PTEN* mutated to guanine, changing the tyrosine to cysteine. The cytosine in DNA nucleotide 701 of exon 7 of *TP53* mutated to guanine, changing the tyrosine to cysteine.

The DNA Copy Number Alterations

The SNP array showed DNA copy number alterations in the cells (Figure 3, Supplementary Figures 1-12). No homozygous gene deletion was found but several loci of gene amplification were noted in chromosomes 1, 2, 5, 9, 10, 12, 15, 16, 20, and X (Table 3). The 12q24.33 region was one of 2 main regions with a 3-fold gain in copy number which harbored the loci of *ANKLE2*, *FBRSL1*, *GALNT9*, *LOC100130238*, *LOC645277*, *P2RX2*, *PGAM5*, *POLE*, *PXMP2*, and *NOC4L*. Another main region was ch15q11.1 to ch15q11.2, harboring the *BCL8*, *CXADRP2*, *GOLGA6L6*, *GOLGA8C*, *HERC2P3*,

Figure 1. The general characteristics of the CA5171 cells. A, The morphology of the CA5171 cells under microscopy. B, The cell counting assay by microscopic examination was performed to evaluate the ability of proliferation. The doubling time of the CA5171 cells was about 24 hours. C, The Boyden chamber assay was performed to evaluate the invasion ability. D, The modified wound-healing assay was performed to evaluate the migration ability.

Figure 2. The reverse-transcription polymerase chain reaction for 3 clones of the CA5171 cells. The CA5171 cells expressed CK7, CA125, TP53, EGFR, VEGFR (FLT-I and KDR), SNAIL 1, ZEB 1, TCF 3, and TWIST 1, but not CK20, SNAIL 2 or E-cadherin.

Figure 3. The single-nucleotide polymorphism arrays of all the chromosomes in the CA5171 cells. The red dots indicate a copy number of more than 2.5, and the blue dots indicate a copy number of less than 1.5 (also see Supplementary Figures I-12). (The color version of this figure is available in the online version at <http://rs.sagepub.com/>.)

LOC646214, LOC727924, NF1P1, OR4M2, OR4N4, POTE8, OR4N3P, and REREP3.

The Response to Cytotoxic Drugs

The cell viability did not change much when treating with serial concentrations of paclitaxel for 24 hours (Figure 4A). When treating with paclitaxel for 48 hours, the median lethal dose (LD_{50}) was about 0.005 $\mu\text{mol/L}$. The LD_{50} was less than 0.005 $\mu\text{mol/L}$ when treating with paclitaxel for 72 hours. The LD_{50} of doxorubicin for 24 or 48 hours was between 0.5 and 5 $\mu\text{mol/L}$ (Figure 4B). The LD_{50} was between 0.05 and 0.5 $\mu\text{mol/L}$ when treating with doxorubicin for 72 hours. There were no decreases in cell viability at any concentration or duration of cisplatin (Figure 4C). These results indicated that the cells were sensitive to paclitaxel and doxorubicin, but resistant to cisplatin in vitro.

The Ability of In Vivo Tumorigenesis

The mice inoculated with the cells developed tumors gradually (Figure 5A). The tumors were detected 11 weeks after inoculation of 2×10^4 cells and 5 weeks after inoculation of 2×10^6 cells (Figure 5B). Hematoxylin and eosin staining revealed high nucleus to cell ratio with prominent nucleolus and frequent mitosis activity. These cells were arranged in sheets with frequent necrosis. The pictures were compatible with undifferentiated carcinoma.

The Response to Cytotoxic Drugs in Tumor-Bearing Mice

The in vivo mice model of cytotoxic drugs was shown in Figure 5D. The tumor volume in the control group increased from $43.82\% \pm 1.47\%$, $71.33\% \pm 6.16\%$ to $122.19\% \pm 12.39\%$, respectively, in the 1, 2, and 3 weeks after treated with PBS. In the paclitaxel group, the tumor volume decreased from $12.05\% \pm 3.01\%$, $25.52\% \pm 4.68\%$ to $34.33\% \pm 5.10\%$, respectively, in the 1, 2, and 3 weeks after treatment. In the doxorubicin group, the tumor volume also decreased from $10.09\% \pm 6.55\%$, $21.62\% \pm 7.11\%$, to $29.78\% \pm 6.84\%$, respectively, in the 1, 2, and 3 weeks after treatment. However, in cisplatin group, the tumor volume increased from $35.11\% \pm 3.48\%$, $71.17\% \pm 5.62\%$, to $100.12\% \pm 5.99\%$, respectively, in the 1, 2, and 3 weeks after treatment.

Our results indicated the cells were sensitive to paclitaxel and doxorubicin, but resistant to cisplatin in vivo, which was compatible with the in vitro experiments.

Discussion

CA5171 is a new human epithelial ovarian cancer cell line originating from a chemotherapy-naïve woman with advanced-staged undifferentiated ovarian carcinoma. Most of the established ovarian cancer cell lines are derived from ascites and less commonly, from solid tumors.⁷⁻¹⁶ Cell lines derived from chemotherapy-naïve patients have even rarely been reported. It is important because the cells derived from tumor tissues of patients receiving chemotherapy may have genetic alterations

Table 3. Gene Copy Number Gains in the CA5171 Cell Line.

Gene Symbol	Transcript ID	Chromosome	Location	Mean
<i>LRRN2</i>	NM_006338	chr1	1q32.1	4.41601
<i>PAK7</i>	NM_020341	chr20	20p12.2	3.72525
<i>NRP1</i>	NM_003873	chr10	10p11.22	3.49875
<i>PDE4D</i>	NM_001104631	chr5	5q11.2	3.49856
<i>ANKLE2</i>	NM_015114	chr12	12q24.33	3.45816
<i>FBRSL1</i>	NM_001142641	chr12	12q24.33	3.45816
<i>GALNT9</i>	NM_001122636	chr12	12q24.33	3.45816
<i>LOC100130238</i>	NR_024563	chr12	12q24.33	3.45816
<i>LOC645277</i>	NM_001195520	chr12	12q24.33	3.45816
<i>P2RX2</i>	NM_012226	chr12	12q24.33	3.45816
<i>PGAM5</i>	NM_001170543	chr12	12q24.33	3.45816
<i>POLE</i>	NM_006231	chr12	12q24.33	3.45816
<i>PXMP2</i>	NM_018663	chr12	12q24.33	3.45816
<i>NOC4L</i>	NM_024078	chr12	12q24.33	3.45816
<i>AKAP17A</i>	NM_005088	chrX	Xp22.33	3.44566
<i>ASMT</i>	NM_004043	chrX	Xp22.33	3.44566
<i>E2F7</i>	NM_203394	chr12	12q21.2	3.33575
<i>BEAN1</i>	NM_001136106	chr16	16q21	3.29383
<i>CDH5</i>	NM_001795	chr16	16q21	3.29383
<i>MTAP</i>	NM_002451	chr9	9p21.3	3.0744
<i>BCL8</i>	NR_027992	chr15	15q11.1-15q11.2	3.0515
<i>CXADRP2</i>	NR_024387	chr15	15q11.1-15q11.2	3.0515
<i>GOLGA6L6</i>	NM_001145004	chr15	15q11.1-15q11.2	3.0515
<i>GOLGA8C</i>	NR_027411	chr15	15q11.1-15q11.2	3.0515
<i>HERC2P3</i>	NR_036432	chr15	15q11.1-15q11.2	3.0515
<i>LOC646214</i>	NR_027053	chr15	15q11.1-15q11.2	3.0515
<i>LOC727924</i>	NR_015416	chr15	15q11.1-15q11.2	3.0515
<i>NF1P1</i>	NR_028506	chr15	15q11.1-15q11.2	3.0515
<i>OR4M2</i>	NM_001004719	chr15	15q11.1-15q11.2	3.0515
<i>OR4N4</i>	NM_001005241	chr15	15q11.1-15q11.2	3.0515
<i>POTEB</i>	NM_207355	chr15	15q11.1-15q11.2	3.0515
<i>OR4N3P</i>	NR_028067	chr15	15q11.1-15q11.2	3.0515
<i>REREP3</i>	NR_033735	chr15	15q11.1-15q11.2	3.0515
<i>MYCN</i>	NM_005378	chr2	2p24.3	3.05049
<i>MYCNOS</i>	NR_026766	chr2	2p24.3	3.05049

not related to the original biological characteristics of the epithelial ovarian carcinoma.

The CA5171 cells had mutations in *PIK3CA*, *PTEN*, and *TP53*, but wild types in *KRAS*, *BRAF*, and *CTNNB1*. Undifferentiated ovarian carcinoma is classified as type II tumor with frequent *TP53* mutations.^{17,18} The Cancer Genome Atlas Project reported that mutations in *TP53* are predominant in at least 96% of high-grade ovarian cancer samples, and that the *RB1* and *PI3K/RAS* pathways are deregulated in 67% and 45%, respectively.¹⁹ In addition to *TP53* mutations, the CA5171 cells also had *PIK3CA* and *PTEN* mutations which are usually noted more frequently in type I tumors. Personalized medicine is the trends for managing cancer, and specific gene mutations or dysregulated signal pathways are potential targets for the development of new drugs. CA5171 cells harboring the specific mutations of *PIK3CA*, *PTEN*, and *TP53* could be a tool for developing target therapy for ovarian carcinoma.

The CA5171 cells contained gene copy number gains especially in the ch12q24.33 region and from ch15q11.1 to

ch15q11.2. Among these loci, several genes have been associated with cancer transformation. *LRRN2* has been reported to be overexpressed in malignant gliomas, and the encoded protein may function as cell-adhesion molecules or as signal transduction receptors.²⁰ *PAK7* has been reported to be implicated in the regulation of cytoskeletal dynamics, proliferation, and cell survival signaling, and overexpressed in pancreatic, colorectal, and gastric carcinoma.²¹⁻²³ *NRP1* has been reported to affect cell survival, migration, and attraction. It is overexpressed in gastrointestinal tract, pancreatic, hepatic, prostate, lung, breast, and ovarian carcinoma.²⁴ *PDE4D* has been reported to encode the protein with 3', 5'-cyclic-AMP phosphodiesterase activity, and overexpressed in melanoma, ovarian, endometrial, and gastric cancers.²⁵ *GALNT9* has been reported to be involved in mucin-type O-linked glycosylation in the Golgi apparatus. Its expression could be a prognostic marker in neuroblastoma.²⁶ *CDH* may play a role in regulating vascular morphology and stability, and overexpressed in melanoma.²⁷ *MYCN* and *MYCNOS* have been associated with a variety of tumor.^{28,29}

Figure 4. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays of the CA5171 cells to cytotoxic drugs. A, The cells were treated with paclitaxel (0.005, 0.05, 0.5, or 5 $\mu\text{mol/L}$) for 24 (left column), 48 (middle column), or 72 hours (right column). The median lethal dose (LD_{50}) of paclitaxel after treatment for 48 hours was about 0.005 $\mu\text{mol/L}$. The LD_{50} of paclitaxel after treatment for 72 hours was less than 0.005 $\mu\text{mol/L}$. B, The cells were treated with doxorubicin (0.005, 0.05, 0.5, or 5 $\mu\text{mol/L}$) for 24 (left column), 48 (middle column), or 72 hours (right column). The LD_{50} of doxorubicin after treatment for 24 or 48 hours was between 0.5 and 5 $\mu\text{mol/L}$. The LD_{50} of doxorubicin after treatment for 72 hours was between 0.05 and 0.5 $\mu\text{mol/L}$. C, The cells were treated with cisplatin (0.05, 0.5, 5, or 50 $\mu\text{mol/L}$) for 24 (left column), 48 (middle column), or 72 hours (right column). The CA5171 cells were resistant to cisplatin.

The CA5171 cells showed epithelial–mesenchymal transition (EMT) properties. The EMT plays a crucial role in physiological processes, including tissue differentiation, organogenesis, and tissue repair. Dysregulation of EMT can promote antiapoptosis, tumor invasion, migration, and metastasis.³⁰ The loss of *E-cadherin* expression is an important step in EMT. *SNAIL*, *ZEB*, and *TCF3* are direct repressors of *E-cadherin* transcription, and *Twist1* is an indirect repressor. These factors are also potential regulators of cell adhesion and movement.³⁰ *SNAIL1* and *SNAIL2* have been correlated with disease relapse and survival in patients with ovarian carcinoma.³⁰ *ZEB* has been reported to be overexpressed and associated with ovarian tumor invasiveness.^{31,32} *Twist* has been associated with tumor metastasis.³³ High *ZEB1* expression in ovarian carcinoma might promote EMT and tumor progression by repressing genes contributing to the epithelial phenotype while activating

those associated with the mesenchymal phenotype.³⁴ Loss of *E-cadherin* expression impairs the cell–cell adhesion, which allows detachment of cells, nuclear localization of β -catenin, and tumor invasion.³⁵ Overexpression of transcription factors *SNAIL1*, *TWIST1*, and *ZEB1*, which regulate the EMT, promotes tumor cell invasion of peritoneal tissues and metastasis.³⁶ The CA5171 cells expressed *SNAIL1*, *ZEB1*, *TCF3*, and *TWIST1*, but not *E-cadherin*. The EMT characteristics may be a reason for the invasion and migratory abilities.

In conclusion, CA5171 is a new cell line of undifferentiated epithelial ovarian adenocarcinoma with specific mutations of *PIK3CA*, *PTEN*, and *TP53*. The cells were sensitive to paclitaxel and doxorubicin but resistant to cisplatin in vitro and in vivo. The cells also showed EMT properties which may be related to the invasion and migration potentials. The CA5171 cell line is a new novel tool for ovarian cancer research.

Figure 5. The in vivo mice xenograft experiments. A, The representative picture of the mice inoculated with CA5171 cells 34 weeks after tumor inoculation. B, The tumor growth rates in the mice with inoculating different numbers of CA5171 tumor cells. C, The microscopic picture of a tumor in hematoxylin and eosin stain (100 $\times$ in left side and 400 $\times$ in right side). D, The change of tumor volumes after treated with respective cytotoxic drug in mice. The tumor volume increased in the control and cisplatin groups, but decreased in the paclitaxel and doxorubicin groups.

Authors' Note

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Declaration of Conflicting Interests

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Supplemental Material

The online supplemental figures are available at <http://rs.sagepub.com/supplemental>

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin.* 2009;59(4):225-249.
2. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin.* 2010;60(5):277-300.
3. Agarwal R, Kaye SB. Ovarian cancer: strategies for overcoming resistance to chemotherapy. *Nat Rev Cancer.* 2003;3(7):502-516.
4. Kurman RJ, Shih Ie M. The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. *Am J Surg Pathol.* 2010;34(3):433-443.
5. Kuo KT, Mao TL, Jones S, et al. Frequent activating mutations of PIK3CA in ovarian clear cell carcinoma. *Am J Pathol.* 2009;174(5):1597-1601.
6. Lu TP, Lai LC, Tsai MH, et al. Integrated analyses of copy number variations and gene expression in lung adenocarcinoma. *PLoS One.* 2011;6(9):e24829.
7. Aoki D, Suzuki N, Susumu N, et al. Establishment and characterization of the RMG-V cell line from human ovarian clear cell adenocarcinoma. *Hum Cell.* 2005;18(3):143-146.

8. Nishikawa Y, Yoshida Y, Kawahara K, Kurokawa T, Tajima K, Kotsuji F. Establishment of a novel human ovarian cancer cell line with high anchorage-independent growth ability. *Int J Oncol*. 2003;23(6):1679-1686.
9. Scoles DR, Pavelka J, Cass I, et al. Characterization of CSOC 882, a novel immortalized ovarian cancer cell line expressing EGFR, HER2, and activated AKT. *Gynecol Oncol*. 2007;104(1):120-128.
10. Alama A, Barbieri F, Favre A, et al. Establishment and characterization of three new cell lines derived from the ascites of human ovarian carcinomas. *Gynecol Oncol*. 1996;62(1):82-88.
11. Hirte HW, Kaiser JS, Bacchetti S. Establishment and characterization of four human epithelial ovarian carcinoma cell lines. *Cancer*. 1994;74(3):900-906.
12. Provencher DM, Finstad CL, Saigo PE, et al. Comparison of antigen expression on fresh and cultured ascites cells and on solid tumors of patients with epithelial ovarian cancer. *Gynecol Oncol*. 1993;50(1):78-83.
13. Mobus V, Gerharz CD, Press U, et al. Morphological, immunohistochemical and biochemical characterization of 6 newly established human ovarian carcinoma cell lines. *Int J Cancer*. 1992;52(1):76-84.
14. Wong WS, Wong YF, Ng YT, et al. Establishment and characterization of a new human cell line derived from ovarian clear cell carcinoma. *Gynecol Oncol*. 1990;38(1):37-45.
15. Langdon SP, Lawrie SS, Hay FG, et al. Characterization and properties of nine human ovarian adenocarcinoma cell lines. *Cancer Res*. 1988;48(21):6166-6172.
16. Hamilton TC, Young RC, McKoy WM, et al. Characterization of a human ovarian carcinoma cell line (NIH: OVCAR-3) with androgen and estrogen receptors. *Cancer Res*. 1983;43(11):5379-5389.
17. Kurman RJ, Shih Ie M. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer—shifting the paradigm. *Hum Pathol*. 2011;42(7):918-931.
18. Banerjee S, Kaye SB. New strategies in the treatment of ovarian cancer: current clinical perspectives and future potential. *Clin Cancer Res*. 2013;19(5):961-968.
19. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. *Nature*. 2011;474(7353):609-615.
20. Almeida A, Zhu XX, Vogt N, et al. GAC1, a new member of the leucine-rich repeat superfamily on chromosome band 1q32.1, is amplified and overexpressed in malignant gliomas. *Oncogene*. 1998;16(23):2997-3002.
21. Giroux V, Iovanna JL, Garcia S, Dagorn JC. Combined inhibition of PAK7, MAP3K7 and CK2alpha kinases inhibits the growth of MiaPaCa2 pancreatic cancer cell xenografts. *Cancer Gene Ther*. 2009;16(9):731-740.
22. Gong W, An Z, Wang Y, et al. P21-activated kinase 5 is overexpressed during colorectal cancer progression and regulates colorectal carcinoma cell adhesion and migration. *Int J Cancer*. 2009;125(3):548-555.
23. Gu J, Li K, Li M, et al. A role for p21-activated kinase 7 in the development of gastric cancer. *FEBS J*. 2013;280(1):46-55.
24. Wild JR, Staton CA, Chapple K, Corfe BM. Neuropilins: expression and roles in the epithelium. *Int J Ex Pathol*. 2012;93(2):81-103.
25. Lin DC, Xu L, Ding LW, et al. Genomic and functional characterizations of phosphodiesterase subtype 4D in human cancers. *Proc Natl Acad Sci USA*. 2013;110(15):6109-6114.
26. Berois N, Gattolliat CH, Barrios E, et al. GALNT9 gene expression is a prognostic marker in neuroblastoma patients. *Clin Chem*. 2013;59(1):225-233.
27. Berx G, van Roy F. Involvement of members of the cadherin superfamily in cancer. *Cold Spring Harb Perspect Biol*. 2009;1(6):a003129.
28. Bordow SB, Norris MD, Haber PS, Marshall GM, Haber M. Prognostic significance of MYCN oncogene expression in childhood neuroblastoma. *J Clin Oncol*. 1998;16(10):3286-3294.
29. Jacobs JF, van Bokhoven H, van Leeuwen FN, et al. Regulation of MYCN expression in human neuroblastoma cells. *BMC Cancer*. 2009;9:239.
30. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell*. 2009;139(5):871-890.
31. Peinado H, Olmeda D, Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat Rev Cancer*. 2007;7(6):415-428.
32. Zhou XM, Zhang H, Han X. Role of epithelial to mesenchymal transition proteins in gynecological cancers: pathological and therapeutic perspectives [published online August 29, 2014]. *Tumour Biol*. 2014.
33. Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev Cell*. 2008;14(6):818-829.
34. Hurt EM, Saykally JN, Anose BM, Kalli KR, Sanders MM. Expression of the ZEB1 (deltaEF1) transcription factor in human: additional insights. *Mol Cell Biochem*. 2008;318(1-2):89-99.
35. Schmalhofer O, Brabletz S, Brabletz T. E-cadherin, beta-catenin, and ZEB1 in malignant progression of cancer. *Cancer Metastasis Rev*. 2009;28(1-2):151-166.
36. Davidowitz RA, Selfors LM, Iwanicki MP, et al. Mesenchymal gene program-expressing ovarian cancer spheroids exhibit enhanced mesothelial clearance. *J Clin Invest*. 2014;124(6):2611-2625.