

Characterization of CSOC 882, a novel immortalized ovarian cancer cell line expressing EGFR, HER2, and activated AKT

Daniel R. Scoles^{a,b,*}, James Pavelka^{a,b}, Ilana Cass^{a,b}, Hang Tran^a,
Rae L. Baldwin^c, Klara Armstrong^a, Beth Y. Karlan^{a,b}

^a Women's Cancer Research Institute at the Samuel Oschin Comprehensive Cancer Institute and Division of Gynecologic Oncology, CSMC Burns and Allen Research Institute, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Los Angeles, CA 90048, USA

^b Department of Obstetrics and Gynecology, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA, USA

^c Bristol-Myers Squibb, Los Angeles, CA, USA

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Abstract

Objective. Only a small number of comprehensively characterized immortalized ovarian cancer cell lines are available for laboratory studies on ovarian cancer. We describe a new ovarian cancer cell line that arose from primary culture of a stage IC, grade III ovarian carcinoma, designated CSOC 882.

Methods. We characterized CSOC 882 by karyotyping, growth modeling, immunohistochemical staining, immunoblotting, drug sensitivity testing, and xenografting in nude mice.

Results. CSOC 882 possessed an abnormal tetraploid karyotype including loss of one copy of chromosomes 2, 17, 19, and 21, and deletion of 8p21. Growth of CSOC 882 was best modeled using the logistic growth equation revealing an average doubling time of 31 h. CSOC 882 cells expressed vimentin, cytokeratin, p53, BRCA1, EGF receptor, HER2, androgen receptor, estrogen receptor α , and progesterone receptor, while no evidence of estrogen receptor β or factor VIII was observed. Some but not all CSOC 882 cells were positive for CA125 reflecting the primary tumor, which had patchy CA125 staining. Drug sensitivity assays demonstrated that CSOC 882 was more sensitive to cisplatin and carboplatin than SKOV3 and HCC1937 while CSOC 882 and SKOV3 were both sensitive to paclitaxel unlike HCC1937. CSOC 882 xenografts retained the original characteristics of vimentin, cytokeratin, and factor VIII labeling.

Conclusions. CSOC 882 is an immortalized cell line that has survived more than 130 passages in culture and retained molecular features of the primary tumor from which it was derived. Compared to the most common ovarian carcinoma cell lines, CSOC 882 is a unique resource for genetic and cellular research on ovarian cancer.

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Introduction

Ovarian cancer remains a disease that has few curative treatments or reliable prognostic markers. Advances in technology for characterizing the molecular and cellular pathways of ovarian cancer are therefore needed in order to develop improved ways for physicians to diagnose and treat women with

ovarian cancer. Such advancements include the establishment of new ovarian cancer cell lines that are immortalized, tumorigenic in mice, and retain the biological properties of primary ovarian cancer cells.

The availability of a wide variety of ovarian cancer cell lines that differ in their response to chemotherapeutic drugs and their expression of oncoproteins, tumor suppressors, and other signaling proteins is critical for the investigation of ovarian cancer pathways. Ideally investigators would have available a number of cell lines with differing expression patterns for proteins in signaling pathways important to ovarian cancer. Such proteins include the p53, BRCA1, and BRCA2 tumor

* Corresponding author. Women's Cancer Research Institute, Cedars-Sinai Medical Center, Room 290W, 8700 Beverly Boulevard, Los Angeles, CA 90048, USA. Fax: +1 310 423 9753.

E-mail address: scolesd@cshs.org (D.R. Scoles).

suppressors, which are often mutant in aggressive forms of ovarian cancer. HER2 and AKT activation is associated with rapamycin responsiveness and may reduce patient response to chemotherapy. Knowledge of steroid receptor status may also be beneficial to studies associating these to tumor progression. Because few primary cultures of ovarian carcinoma cells are efficiently grafted in mice, new characterizations of transformed cell lines that engraft more easily in mice than do primary cultures are critical for the investigation of ovarian cancer pathways *in vivo*. However, for ovarian cancer, there are relatively few well-characterized cell lines available. A limited number of ovarian cancer related oncoproteins and tumor suppressors have been described, and chemosensitivity has been investigated for only a few of these. In contrast there are approximately 200 cell lines that have been established from prostate tumors [1], and while the number of breast cancer cell lines has been described as few, at 65 it remains considerably higher than for ovarian cancer [2]. This demonstrates the need for the acquisition of new ovarian cancer cell lines for the effective investigation of ovarian cancer.

We regularly establish new primary ovarian carcinoma cell cultures from operative specimens at the time of our tumor banking activities. We discovered one ovarian carcinoma cell culture that was unique from the others in its ease of culture. With successive passage, we noted that this primary culture had characteristics like that of a cell line, remaining proliferative in culture beyond 130 passages. This study describes this spontaneously immortalized cell line, designated CSOC 882, including the expression of a number of relevant proteins, the rate of growth in culture, characteristics in as a xenograft tumor in nude mice, and sensitivity to cisplatin, carboplatin, and paclitaxel. Compared to the other described and available ovarian cancer cell lines, CSOC 882 has unique features advantageous for the study of ovarian cancer.

Materials and methods

Culture methods for CSOC 882

All cultures of CSOC 882 cells in the study included the use of McCoy's 5A medium with 10% FBS and 1% penicillin/streptomycin. Standard trypsin/EDTA was used in propagating cultures.

Rate of growth

To determine growth rate for CSOC 882, we plated equal numbers of cells in 12-well plates, allowed cells to grow for various times and counted cells in three wells per time point. Harvesting was conducted by rinsing wells three times with PBS, stripping with trypsin-EDTA, neutralizing the trypsin with McCoy's 5A media followed by centrifugation. The pellet was resuspended in 100 μ l McCoy's 5A media plus 100 μ l 0.4% Trypan blue. Live cells excluding Trypan blue were counted using a hemocytometer.

We determined the rates of growth by applying the natural and logistic growth models [4,5]. The logistic growth model described by the equation $\frac{dP}{dt} = rP(1 - \frac{P}{K})$, allows for modeling the growth of populations that are constrained by limitations on resources including contact inhibition, available nutrients, and available growth surface and is therefore suitable for growth of mammalian cells in culture. The variables in the equations include number of cells in the culture (population size) P , time t , the change in the population size over time dP/dt , a proportionality constant r , and the carrying capacity K determined by the specific limitations on the culture. Because CSOC 882 cells

are tumor cells that are not contact inhibited, we anticipated the possibility of a poor fit to the logistic growth equation. Therefore we also fitted the data using the natural growth model, which lacks the modifying factor $(1 - \frac{P}{K})$, which accounts for population growth constraints and from which the logistic growth model was derived. The natural growth model is described by the equation $\frac{dP}{dt} = rP$.

Antibodies

Antibodies used for immunoblotting included actin (Sigma #A-5441) and the following from Cell Signaling: HER-2 (#2242), EGFR (#2232), EGFR-p (Tyr1068) (#2234), AKT (#9272), and AKT-p (Ser473) (#4058). Antibodies used for immunohistochemical staining were mouse anti-vimentin (MAB3400, Chemicon International), mouse anti-cytokeratin AE1/AE3 (MAB3412, Chemicon International), mouse anti factor VIIIc (1199-234, Boehringer-Mannheim), rabbit anti-Her2/neu (#60 provided by Dennis Slamon), mouse anti-estrogen receptor α (sc-8005, Santa Cruz Biotechnology), rabbit anti-estrogen receptor β (sc-8974, Santa Cruz Biotechnology), rabbit anti progesterone receptor (sc-5638, Santa Cruz Biotechnology), mouse anti-p53 (Ab-6) (OP43, Calbiochem), and mouse anti-CA-125 (OC-125) (617-26, Signet).

Immunohistochemical staining

Cells were cultured on fibronectin coated glass for 48 h. Cells were fixed with methanol:acetone for 5 min then incubated with diluted primary rabbit antibodies overnight at 4°C. Visualization was accomplished by using the Vector ABC elite Peroxidase Kit (Vector), enhanced by DAB enhancer, and visualized with diaminobenzidine (DAB) (Biomed).

Images were taken with a 63 \times plan apo objective and phase contrast condenser in place.

Immunoblotting

Cultured cells were rinsed with ice-cold PBS and harvested on ice in Cell Lysis Buffer (Cell Signaling Technology) containing 1 \times protease inhibitor cocktail (Complete Mini, Boehringer-Mannheim). Equal amounts of protein were loaded onto 4–15% gradient Tris–glycine Ready Gels (Bio-Rad) and electrophoresed in XT Tricine Running Buffer (Bio-Rad). Proteins were transferred in 1 \times Tris/Glycine (Bio-Rad) to Immobilon-P PVDF membrane (Millipore). Membranes were incubated with primary antibodies for 1 h at room temperature, and then with secondary antibodies. Antibodies were diluted in TBS (Bio-Rad) with 0.5% Tween 20 and 5% milk. Detections were accomplished using ECL Western Blotting Detection Reagents (Amersham) and exposure to Hyperfilm-ECL (Amersham). Secondary antibodies were donkey anti-mouse-HRP or donkey anti-rabbit-HRP (#s 715-035-150 and 711-035-152, respectively, Jackson ImmunoResearch Laboratories, Inc.) Note that when phospho-AKT was detected, it was detected first and total AKT was detected after blots were stripped in stripping buffer (100 mM β -mecaptoethanol, 2% SDS, 62.5 mM Tris–HCl, pH 6.8) for 20 min at 60°C.

Cytogenetics

Cytogenetic analysis of CSOC 882 in passage 66 was accomplished by the G-banding method using a conventional Giemsa staining protocol [3]. We analyzed 10 metaphase spreads.

Xenograft analysis

We injected CSOC 882 cells into five nude mice. Injections included approximately 10 million cells suspended in 100 μ l media (lacking matrigel) per injection site into nude mice both intraperitoneally and subcutaneously.

Chemosensitivity

We determined the chemosensitivity of CSOC 882 to cisplatin, carboplatin, and paclitaxel comparatively to other cell lines with known chemosensitivity data including SKOV3 and HCC 1937. We conducted these assays in 96 well

plates by treating quadruplicate cultures with like doses of either cisplatin, carboplatin, or paclitaxel for 72 h, then determining cell abundances using the CellTiter 96® Non-Radioactive Cell Proliferation Assay Kit (Promega) following the vendor's protocol. Formazan production from MTT tetrazolium substrate was determined by measuring absorbance at 570 nm. Values were converted to a percentage of the highest absorbance.

Results

Clinical summary

The CSOC 882 cell line was derived from a tumor from a 55-year-old Filipino woman with no family history of breast or ovary cancer. Clinical findings included a 15 cm cystic mass adherent to the right anterior abdominal wall. Subsequent pathological evaluation determined the tumor to be a stage 1C, grade III ovarian carcinoma with clear cell features. There were no signs of metastases in peritoneum, lymph nodes, cecum, omentum, and other tissues. The patient received standard combination chemotherapy with carboplatin and paclitaxel following her surgery. Despite this, she developed a clinical recurrence 120 days post-op, and expired 39 days later.

Growth characteristics

In order to understand the rate of growth for CSOC 882, we determined the best fit of our CSOC 882 growth data using both the logistic and natural growth models. The observed growth for CSOC 882 cells best fit the logistic growth model, which is derived from the natural growth model by the inclusion of a term that accounts for constraints on population growth. In order to fit the logistic growth model, we made the assumption that the carrying capacity was equivalent to the highest value we observed for the 48-h time point when we halted the experiment when all wells at this time point had reached confluence. We assigned the carrying capacity to be $K=107,000$ cells. We also determined the population size at time zero to be $P_0=6333$ cells by plating three wells, letting cells adhere for 2 h, then subjecting the three wells to counting as described in Materials and methods. Application of the logistic growth model resulted in a value for the proportionality constant r of 0.09 with correlation coefficient r^2 of 0.999 for the curve with the best fit (Fig. 1A). Therefore, one can determine the number of cells at any time t by inserting these numbers into the solution of the logistic growth equation, which is $P_t = \frac{KP_0}{P_0 + (K - P_0)e^{-rt}}$. We also demonstrated a good fit ($r^2=0.97$) when we analyzed our data using the Gompertz equation. The natural growth model provided a good fit as well however with an even lower r^2 of 0.95 (Fig. 1B). In the logistic growth model, the doubling time is dynamic and defined by the time interval over which the initial cell population P_0 reaches the inflection point $K/2$, and is determined by the equation $t_2 = \frac{1}{r} \ln\left(\frac{K - P_0}{P_0}\right)$ [5]. For CSOC 882 cells, this value was $t_2=30.7$ h.

Proteins expressed in CSOC 882 cells

The initial immunohistochemical evaluation of the primary tumor demonstrated reactivity of tumor cells with antibodies

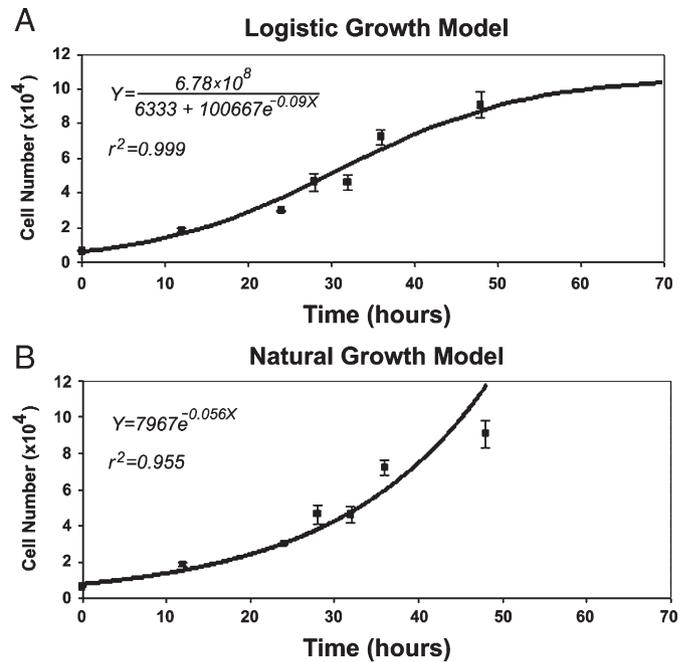


Fig. 1. Growth characteristics of CSOC 882. The growth pattern for CSOC 882 in our culture system was modeled with a better fit when using the logistic growth equation compared to the natural growth equation. Calculation of doubling time using the logistic growth model revealed that CSOC 882 cells double every 30.7 h in this system (see Materials and methods).

against cytokeratin, vimentin, and patchy staining with CA125. These features were retained in the CSOC 882 cell line, which demonstrated strong cytokeratin staining, strong vimentin staining, and only few cells positive for CA125 (Fig. 2). Additionally, we demonstrated no staining with Factor VIII consistent with CSOC 882 cells being of epithelial origin (Fig. 2). We also demonstrated by immunohistochemical staining that CSOC 882 is positive for p53, AR, ER α , BRCA1, and PR but negative for ER β (Fig. 2). These proteins were assessed by immunohistochemical staining because we have established systems for doing so in our laboratory for screening the primary cells that we regularly bank. We conducted further testing of HER2/AKT pathway proteins by the more sensitive method of immunoblotting to accurately compare the expression of these proteins in CSOC 882 to that in a variety of other ovarian cancer cell lines. Immunoblotting demonstrated that CSOC 882 cells are positive for HER2, EGFR, AKT, and phospho-AKT, but we were unable to detect the presence of EGFR phosphorylated on tyrosine 1068, which is known to activate the map kinase pathway (Fig. 3). A summary of the molecular characteristics of CSOC 882 is provided in Table 1.

Cytogenetics

Cytogenetic analysis of CSOC 882 was conducted on 10 cells in passage 66 revealing an abnormal pseudotetraploid karyotype with consistent numerical and structural changes. These included loss of one copy of chromosomes 2, 17, 19, and 21. Two deletions were observed on chromosome 8 at 8p21. There appeared to be one rearranged copy of the X chromosome

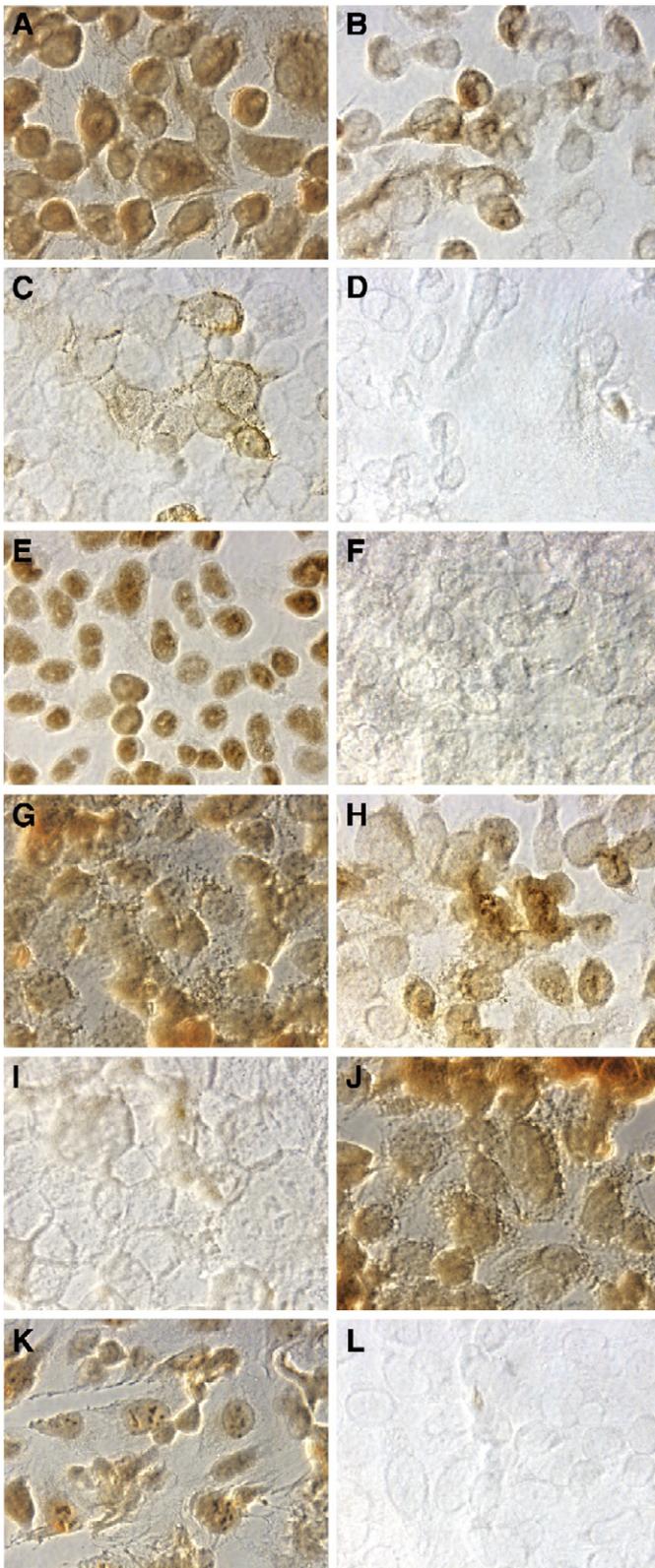


Fig. 2. Immunohistochemical staining of CSOC 882. Staining was conducted with antibodies to (A) vimentin, (B) pan-cytokeratin, (C) CA125, (D) Factor VIII, (E) p53, (F) p53 in CSOC 918, (G) androgen receptor, (H) estrogen receptor α , (I) estrogen receptor β , (J) progesterone receptor, (K) BRCA1, and (L) normal human serum in place of the primary antibody as a negative control. All are of CSOC 882 except as indicated for panel F.

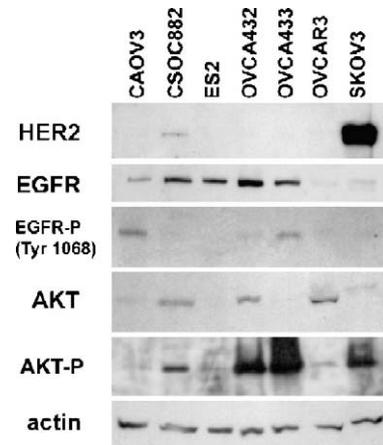


Fig. 3. The EGFR/HER2 pathway is activated in CSOC 882. Immunoblotting was used to assess proteins in the EGFR/HER2 pathway in CSOC 882 relative to six other ovarian carcinoma cell lines with loading normalized to actin. Immunoblotting demonstrated a signature for CSOC 882 of HER2 low, EGFR high, EGFR-P null, AKT low, and AKT-P high.

resulting from an X;13 translocation. The karyotype was determined to be $92\pm,XXX,t(X;13)(q13;q14),-2,del(8)(p21)\times 2,-17,-19,-21$.

Xenografting

We injected CSOC 882 cells into five mice both intraperitoneally and subcutaneously. One mouse died within a week of injection. Two developed an intraperitoneal mass and no subcutaneous mass. The remaining two mice had subcutaneous and intraperitoneal tumors (Figs. 4A and 4B). All mice were sacrificed in the seventh week post injection. A common feature among these mice was ascites, tumor adhesions to the spleens, intestines and livers, enlarged spleens, and liver metastases. We excised one 7 mm subcutaneous tumor (Fig. 4A) and established primary cells in culture for immunohistochemical evaluation. Immunohistochemical staining of these cells proved strong vimentin and cytokeratin positivity and no positivity for

Table 1
Molecular characteristics of CSOC 882

Protein	State	Method ^a
Vimentin	Positive	IHC
Cytokeratin	Low	IHC
CA125	Low	IHC
Factor VIII	Negative	IHC
P53	Positive	IHC
Androgen receptor	Positive	IHC
Estrogen receptor α	Positive	IHC
Estrogen receptor β	Negative	IHC
Progesterone receptor	Positive	IHC
BRCA1	Positive	IHC
EGFR	Positive	WB
Phospho-EGFR	Negative	WB
HER2	Low	WB
AKT	Positive	WB
Phospho-AKT	Positive	WB

^a IHC, immunohistochemical staining; WB, Western blotting.

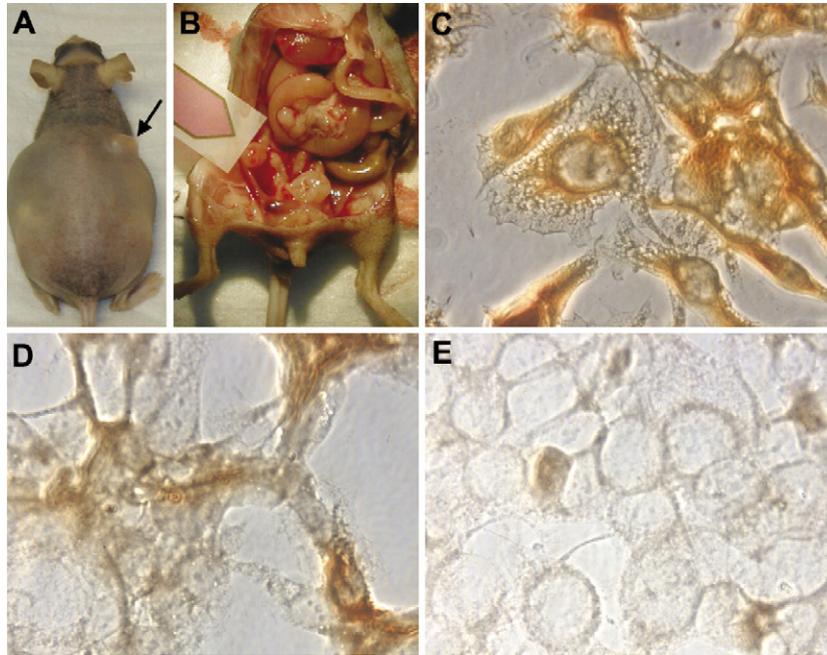


Fig. 4. CSOC 882 is a tumorigenic cell line. (A) Nude mouse with a subcutaneous CSOC 882 xenograft tumor. (B) Nude mouse with an intraperitoneal CSOC 882 xenograft tumor. A subcutaneous CSOC 882 xenograft tumor was excised and cultured *in vitro* then subjected to immunohistochemical staining with antibodies against (C) vimentin, (D) pan-cytokeratins, and (E) factor VIII.

Factor VIII (Figs. 4C–E). As our main interest was primarily tumorigenicity of CSOC 882, we only assessed these three antigens. This result demonstrated retention of the same vimentin, cytokeratin, and Factor VIII expression profile as for CSOC 882 (Fig. 2).

Chemosensitivity

SKOV3 is resistant to platinum chemotherapy and the breast cancer cell line HCC 1937 is resistant to paclitaxel [6,7]. Therefore we chose these two lines to which to compare the chemosensitivity of CSOC 882. The cellular response was best fit with a monophasic exponential decay model while the cellular responses to cisplatin and paclitaxel were biphasic so we modeled the latter using a biphasic exponential decay model. To accomplish this, we used the built in equations provided with the software package GraphPad Prism version 4. We demonstrated that SKOV3 was more resistant than HCC 1937, and that HCC 1937 was more resistant than CSOC 882 for both cisplatin and carboplatin (Figs. 5A and B). The IC₅₀ values for CSOC 882 in either cisplatin or carboplatin were approximately half of that for HCC 1937, and approximately a quarter of that for SKOV3 (Table 2). However, SKOV3 and CSOC 882 were both sensitive to paclitaxel while HCC 1937 was considerably more resistant (Fig. 5C, Table 2).

Discussion

During the course of our regular tumor banking activities, we noted one primary cell culture, CSOC 882, which stood out from the others by its aggressive growth. Further analysis of this cell culture demonstrated that it had established into a highly

proliferative cell line that retained the properties of the parental primary tumor, including heterogeneity of protein expression and tumorigenicity in mice. Our characterization of CSOC 882 demonstrated it to be unique among existing ovarian cancer cell lines.

Sensitivity of CSOC 882 cells to chemotherapeutic drugs

We showed that CSOC 882 was considerably more sensitive to platinum/taxol than SKOV3 with an IC₅₀ value for cisplatin of less than a quarter of that for SKOV3. This was not expected since CSOC 882 was derived from a patient who eventually succumbed to refractory progressive disease. The establishment of this platinum sensitive line from a patient who did not respond to platinum chemotherapy might be explained in terms consistent with the cancer stem cell theory. This theory hypothesizes that the bulk of a tumor consists of differentiated cells that are supported by lesser abundant but more highly metastatic undifferentiated progenitor cells [53] that may have greater drug resistance than their differentiated tumor cell counterparts [54]. Such progenitor cells typically have a low rate of proliferation and require specialized conditions for culture and would not be maintained in CSOC 882 cultures. It is therefore possible that platinum sensitive CSOC 882 cells are uncharacteristic of resistant metastatic cells that account for the platinum refractory progressive disease of this patient. However, CSOC 882 is not the most sensitive cell line that we have characterized as in more recent work we have demonstrated for CAOV3 cells a cisplatin IC₅₀ of 1.2 μ M [55]. While CSOC 882 is considerably more sensitive to cisplatin than SKOV3, it perhaps is best characterized as an ovarian carcinoma cell line with moderate sensitivity to cisplatin.

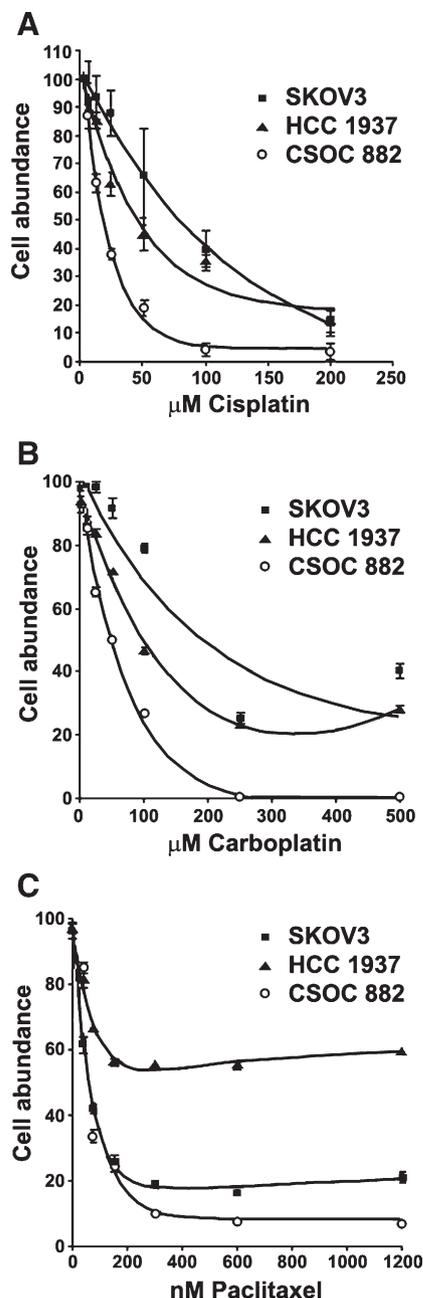


Fig. 5. Chemosensitivity of CSOC 882. The growth response of CSOC 882 cells cultured in the presence of (A) cisplatin, (B) carboplatin, or (C) paclitaxel was demonstrated using MTT proliferation assays in comparison to SKOV3 and HCC 1937 cells whose responses to these drugs are known. These assays demonstrated that CSOC 882 cells were more sensitive to cisplatin and carboplatin than SKOV3 and HCC 1937, and that HCC1937 were highly paclitaxel resistant unlike CSOC 882 and SKOV3. Cells were plated equally among the wells of a 96 well plate, recovered overnight then treated with the drugs as indicated, in quadruplicate. The dosewise treatments included 0, 3, 6.3, 12.5, 25, 50, 100, and 200 μM of cisplatin (A), 0, 10, 25, 50, 100, 250, 500 μM of carboplatin (B), and 0, 37, 75, 150, 300, 600, 1200 nM of paclitaxel (C). Data were analyzed using monophasic (A) and biphasic (B, C) exponential decay models. Values of r^2 ranged from 0.8 to 0.993.

Growth properties of CSOC 882 cells

CSOC 882 cells were easily cultured in standard media with no supplementary growth factors required. They appear as fast

growing cells with cuboid morphology. We investigated the growth properties of CSOC 882 cells by the application of three different growth models (logistic, Gompertz, and natural) and found that we could best predict growth with the logistic model. Our estimation of doubling time for CSOC 882 cells was 30.7 h and was not unlike that of other ovarian carcinoma cell lines ranging from 18 to 34 h [8–11]. The growth of CSOC 882 cells is that of an established immortalized cell line and CSOC 882 cells have undergone more than 130 passages in culture.

Molecular genetics of CSOC 882 cells

Like all cancers, several molecular mechanisms are altered during the development of ovarian carcinomas and their progression to a more malignant phenotype. Such changes include mutations that activate oncogenes, mutations that inactivate tumor suppressor genes, and chromosomal abnormalities resulting in gene deletion or loss of function. Chromosomal alteration in ovarian cancer has been reviewed in detail [12–14]. These studies demonstrated an exceptionally high number of cytogenetic abnormalities in ovarian cancer including alterations located in all chromosomes. CSOC 882 cells proved to be tetraploid with deletions of chromosomes 2, 17, 19 and 21. We also observed the translocation $t(X;13)(q13;q14)$ and noted that breakpoints at Xq13 and 13q14 have been observed previously in ovarian adenocarcinomas [12]. CSOC 882 cells also possess a deletion of 8p21. Deletions of 8p21 have been observed previously and are associated with increased malignant potential [15]. We find it of considerable interest that the *4EBP1* gene is located at 8p21. When 4EBP is phosphorylated by mTOR, 4EBP is released from eIF4e resulting in the initiation of protein translation. Loss of 4EBP deregulates eIF4e and is inversely correlated with the progression of intestinal tumors [16], and eIF4e overexpression has been observed in other women's cancers [17,18]. More work will be required to demonstrate whether 8p21 loss could be associated to CSOC 882 tumorigenesis or resistance to rapamycin.

Protein expression signature of CSOC 882

Consistent with an epithelial origin for CSOC 882 cells, 100% of cultures expressed vimentin and were negative for factor VIII. Epithelial cells are also expected to be positive for cytokeratins; however, we demonstrated only $\sim 40\%$ positivity for CSOC 882 using a pan cytokeratin antibody. Likewise, CSOC 882 cells were also only $\sim 20\%$ positive for CA125. The pattern of staining for CA125 entirely mimicked the pathology

Table 2
IC₅₀ values for CSOC 882, SKOV-3 and HCC 1937

Cell line	IC ₅₀ values ^a when cultured with		
	Cisplatin	Carboplatin	Paclitaxel
CSOC 882	18.9 μM	49.7 μM	66.8 nM
SKOV-3	79.8 μM	192.2 μM	56.3 nM
HCC 1937	45.5 μM	96.3 μM	Not achieved

^a We defined IC₅₀ as the inhibitory concentration effective for reducing the cell population by 50% over the 72-h culture period.

report showing that the primary tumor from which CSOC 882 cells were derived had patchy CA125 staining, and presurgical labs showing only a moderately elevated CA125 serum level for this patient of 54. We conclude that CSOC 882 cells are epithelial in origin and retain the protein expression signature of the primary ovarian carcinoma from which they were derived.

We assessed the expression of a number of tumor suppressors and oncoproteins in CSOC 882 cells by immunohistochemical staining. For each of p53, AR, ER α , ER β , PR, and BRCA1, a majority of the cells in the CSOC 882 culture shared the same pattern of staining. Each of these proteins was localized in the nucleus of CSOC 882 cells, except for ER β , which was not expressed. Positive expression of the hormone receptors AR, ER α , and PR was noted as well as positive staining for BRCA1 suggesting that CSOC 882 cells lack *BRCA1* mutations. P53 staining is normally not observed unless the *TP53* gene is mutated, and then its pattern of expression is considered prognostic in most cancers including ovarian cancer. Nuclear localization is associated with reduced survival [19,20] and may contribute to the aggressive growth of CSOC 882 cells.

The HER2 pathway is significant to ovarian cancer biology, so we used immunoblotting to compare the expression of HER2, total and active EGFR, and total and active AKT among seven selected ovarian carcinoma cell lines. Immunoblotting demonstrated that CSOC 882 cells highly expressed EGFR but weakly expressed HER2. To demonstrate constitutive activation of EGFR, we assessed EGFR with an antibody directed against tyrosine 1068 which has been implicated in the activation of AKT [21]. We were unable to detect phospho-EGFR in CSOC 882 cells, but we observed that AKT was highly phosphorylated.

CSOC 882 cells are tumorigenic in nude mice

CSOC 882 cells are easily engrafted in nude mice and therefore are useful in modeling ovarian cancer in mouse models. We previously investigated the xenografting of CSOC cell cultures in mice showing that 11 of 13 tested cultures established tumors in SCID mice, while we had no success

establishing CSOC xenografts in nude mice [22]. Comparatively, CSOC 882 is remarkably more tumorigenic than other primary cultures of ovarian carcinomas that we have studied and will likely prove to be a useful resource for investigations on ovarian cancer where mouse xenografts are required. We also showed that the expression of cytokeratin, vimentin, and factor VIII remained unchanged after passing CSOC 882 cells through nude mice lending further support for that CSOC 882 cells possess no contaminating fibroblasts.

CSOC 882 cells are unique among ovarian cell lines

While there are a number of ovarian cancer cell lines available to researchers, the number that are extensively characterized is relatively few in comparison to other tumor disorders including prostate cancer and breast cancer. Among these, the common ovarian carcinoma cell lines A2780, Caov3, OVCAR-3, PA-1, and SKOV3 have been cited hundreds of times in the literature and are extensively characterized. There exist approximately 10 other lines that have been cited moderately (20 or more times) in the ovarian cancer literature demonstrating a relatively significant impact on the study of the disease, and several others that have been used sporadically since the early 1980s. Many of the existing ovarian carcinoma cell lines were characterized by several laboratories over multiple years while few have been presented with a single comprehensive characterization such as the present description of the CSOC 882 cell line. None of the five most commonly studied ovarian carcinoma cell lines had precisely the same expression pattern and drug sensitivity as CSOC 882 (Table 3).

Our immunoblotting and immunohistochemical staining further demonstrated that CSOC 882 possess a unique pattern of expression of HER2 pathway proteins and other tumor suppressors and oncoproteins important to ovarian cancer biology highlighting CSOC 882 as a unique resource for ovarian cancer research. The data that we present are valuable for determining whether CSOC 882 may be useful for various molecular studies on ovarian cancer. For example, one particular unique use for CSOC 882 might be in the investigation of the effect of HER2 or EGFR knockdown on AKT activation. Both

Table 3
CSOC 882 compared to the five most-common ovarian carcinoma cell lines

Cell line	Tumor type	Molecular features ^a									Tumorigenic in mice	Chemosensitivity ^b	No. times cited	Citations
		CA125	p53	AR	ER	PR	HER2	EGFR	AKT-p	BRCA1				
CSOC 882	Adenocarcinoma	L	P	P	P	P	L	P	P	P	Y	CS, PS	N/A	N/A
A2780	Adenocarcinoma	N	W	–	N	–	P	N	P	–	Y	CS, PS	634	[23–29, 56]
Caov-3	Adenocarcinoma	N	M	–	P	P	N	P ^c	L ^c	P	Y	CS, PS	162	[30–35]
OVCAR-3	Adenocarcinoma	P	M	P	P	P	L ^d	L ^c	P	–	Y	CR, PS	497	[25,31,34,36–42]
PA-1	Teratocarcinoma	P	W	–	–	–	P	P ^c	–	P	Y	CS, PS	126	[8,31,32,43–48]
SKOV-3	Adenocarcinoma	N	Null	N	P	P	P	L ^c	P	P	Y	CR, PR	553	[32,34,41,49–52]

^a P, positive; N, negative; L, low; W, wild-type overexpressed; M, mutant; –, no data.

^b CS, cisplatin sensitive; CR, cisplatin resistant; PS, paclitaxel sensitive; PR, paclitaxel resistant. Note that the degree of sensitivity was not always consistent among the various authors. CAOV3 was highly cisplatin sensitive in experiments conducted in our laboratory (see text).

^c Fig. 3.

^d Constitutively phosphorylated.

^e Inferred by EGF binding that stimulated growth.

CSOC 882 and SKOV3 express HER2 but only CSOC 882 expresses EGFR which although is inactive, may effect HER2 mediated ATK signaling. An additional difference shown in Table 3 is the presence of p53 in CSOC 882 but absence in SKOV3. None of the seven ovarian carcinoma cell lines had identical expression patterns for HER2 pathway proteins and CSOC 882 is entirely unique in this group of cell lines.

In summary, the CSOC 882 cell line is comprised of highly prolific cells that express a number of hormone and growth factor receptors, possess an 8p21 deletion previously observed in other ovarian carcinomas, and are easily xenografted in nude mice. These cells retain the properties of the parental tumor from which they were derived including heterogeneity of CA125 and cytokeratin expression. The CSOC 882 cell line is unique among the common ovarian carcinoma cell lines including responses to chemotherapeutic drugs, and will likely prove to be a useful tool for investigating ovarian cancer.

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