J Cancer Res Clin Oncol (1997) 123:82–90

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ORIGINAL PAPER

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A new cell line from human undifferentiated carcinoma of the ovary: establishment and characterization

Received: 5 June 1996/Accepted: 9 September 1996

Abstract A cell line designated CUMO-2 has been established from an undifferentiated ovarian carcinoma. The s.c. injection of cells into nude mice gave rise to fast-growing tumors, while the i.p. route induced a peritoneal carcinomatosis with ascites. Histopathologically, the transplanted s.c. tumors closely resembled the original tumor, but tumors developed in the peritoneal cavity were highly anaplastic. The epithelial nature of the cells was confirmed by ultrastructural analysis. Sequential cytogenetic analyses on early and late passages revealed highly aneuploid tumor cells with consistent structural aberrations of chromosomes 1, 3, 8 and 11. CUMO-2 cells were found to produce CA 125 in vitro and in vivo. Cytosol estrogen receptor (ER) was found but progesterone receptor (PR) was not measured. HLA typing indicated the presence of DR8 and DQw4. A gonadotropin-releasing hormone (Gn-RH) analog inhibited cell growth and Gn-RH receptor mRNA was detected by reverse transcription/polymerase chain reaction in this cell line. Administration of transforming growth factor β1 inhibited both cell growth and *c-myc* mRNA expression. This cell line demonstrated a conformational band shift in exon 7 of the *p53* gene. It was a frameshift mutation.

Key words Human ovarian cancer · Permanent cell line · Cytogenetics · Transforming growth factor · Tumor- suppressor gene

This work was supported by a grant from the Korea research foundation, 1990

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Abbreviations *Gn–RH* gonadotropin-releasing hormone · *ER* estrogen receptor · *PR* progesterone receptor · *RT-PCR* reverse transcription/polymerase chain reaction · *TGF* transforming growth Factor · *SSCP* Single-strand conformation polymorphism · *MTT* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide

Introduction

Ovarian cancer remains a common, lethal disease. It is the fifth most common form of cancer in women (Richadson et al. 1985). Typically, the disease remains clinically silent until it is far advanced. Treatment success has been limited by the presence of advanced disease in the majority of patients at the time of diagnosis and by the development of resistance to currently available chemotherapeutic agents. Therefore it seems urgent to set up experimental models that can be used for exploring new modalities of treatment or for screening new agents.

Studies of ovarian cancer biology have been limited by the lack of relevant animal models and primary culture systems. To date, a limited number of established ovarian cancer cell lines have been described (Fuchtner et al. 1993; Buller et al. 1995) and these cell lines differ in morphology, karyotype, tumorigenicity, and tumor markers. Most of these were obtained from ascites and might lose many of the tumor-specific properties of the original tumor in prolonged in vitro culture.

As ovarian carcinomas represent many different histological types that have different spread patterns and different responses to therapy, cell lines of histologically different tumor types should be established to aid in the investigations. Primary ovarian carcinomas that are too poorly differentiated to be recognized as belonging to any of the specific common epithelial categories are classified as undifferentiated carcinoma.

The prognosis for patients with this aggressive tumor is poor, with overall 5- and 10-year survival rates of only 15% or less (Aure et al. 1971).

Therefore the establishment of a new undifferentiated ovarian cancer cell line that shows highly consistent tumorigenicity, stable chromosomal abnormalities, CA 125 production both in vitro and in vivo, the presence of steroid hormone and gonadobropin-releasing hormone (GnRH) receptors, sensitivities to GnRH and transforming growth Factor (TGFβ1), and mutation of the *p53* tumor-suppressor gene can lead to the elucidation of the pathogenesis of undifferentiated ovarian carcinoma and the development of new treatment approaches.

We describe here the establishment and characterization of a new human cell line, CUMO-2, which was derived from an undifferentiated ovarian carcinoma.

Materials and methods

Clinical findings

On 14 February 1989, we performed an exploratory laparotomy on a 57-year-old woman with ovarian cancer stage III. Surgery revealed a primary ovarian cancer extending to the omentum and rectal serosa. A tumoral specimen was taken from the cancerous bulky right ovarian mass, thus constituting previously untreated material. The histology was that of an undifferentiated ovarian carcinoma. The serum level of CA 125 was 490 U/ml at the time of tissue collection for cell line initiation. The patient subsequently received 2 cycles of cyclophosphamide (500 mg/m^2), doxorubicin (50 mg/m^2), and *cis*platin (60 mg/m^2), but her clinical condition deteriorated progressively. She died in June 1989.

Cell culture

Tumor specimens were held in ice-cold Waymouth's MB 752/1 medium supplemented with 100 IU/ml penicillin, 100 µg/ml streptomycin, and 15% fetal bovine serum for further processing. After mechanical dissociation with crossed scalpels, tumor fragments and cellular suspensions were collected, washed three times, and finally centrifuged. The sediments were resuspended in medium and seeded into 25-cm^2 tissue-culture flasks to initiate in vitro growth of the cells. Routine assays for *Mycoplasma* using a Gene-probe rapid-detection system (Gene-probe, San Diego, Calif.) were negative.

Morphology of the cultured cells

The cells grown in culture flasks were photographed by a phase-contrast microscopy and cells grown on slides were washed with phosphate-buffered saline and fixed in 95% ethanol. They were stained with hematoxylin/eosin, periodic acid/Schiff, mucicarmine, and Alcian blue. For transmission-electron-microscopic study, the tumor cells were seeded on tissue-culture-chamber slides (Lab-Tek, Naperville, Ill.) and fixed with 2.5% glutaraldehyde in 100 mM cacodylate buffer. They were then postfixed with a 2% osmium tetroxide in cacodylate buffer. Specimens were dehydrated through an ascending series of ethanol, infiltrated with 1:1 mixture of absolute ethanol and Epon 812, and infiltrated with pure Epon 812 overnight. After polymerization, thin sections were contrasted with uranyl acetate and lead citrate before being examined in a JEOL 1200 EX electron microscope.

Growth characteristics

Growth curves were constructed by seeding 1×10^5 cells into 25-cm^2 flasks and incubated for 13 days. The population doubling time was calculated from the growth curve. For studies of plating efficiency, 100 tumor cells were plated in each of the ten 25-cm^2 flasks. After 2 weeks, the colonies formed were fixed in 95% ethanol and stained with 0.5% crystal violet in 25% ethanol. The number of colonies (more than 50 cells) was counted.

Tumor xenografts

To analyze tumorigenicity, 5×10^6 cells were injected subcutaneously into the back of 20 mice and 5×10^6 cells were also injected intraperitoneally into 10, 4- to 6-week-old female nude mice (athymic nu/nu on BALB/c background). Samples comprising 1×10^5 cells were injected into the lateral tail veins of another 10 mice. Only cell suspensions with greater than 95% viability, as assessed by trypan blue dye exclusion, were used for injection.

Cytogenetic analysis

Sequential cytogenetic analyses at the 5th, 50th and 150th passages were carried out to monitor the extents of chromosomal evolution during cell-line establishment. Exponentially growing cells were exposed to colcemid (0.1 µg/ml) for 2 h. The cells were then exposed to a hypotonic solution consisting of a 1:4 mixture of 1% sodium citrate and 75 mM KCl for 30 min at 37 °C and fixed with ice-cold glacial acetic acid:methanol (1:3, v/v). Cell smears were prepared and stained with Giemsastain. A total of 30 spreads were photographed and the chromosome number in each spread was counted. G-banding was induced using the routine techniques published elsewhere (Pathak 1976).

CA 125 determination

Media, in which 2×10^5 cells/5 ml had been cultured for 5 days, were examined for CA 125 production. CA 125 was measured with the ELSA-CA 125 kit (CIS Bio International, France). The expected normal range for CA 125 concentration was below 35 U/ml. CA 125 levels were measured not only in the culture media but in the sera from mice xenografted with the CUMO-2 cells. The control medium was Waymouth's MB 752/1 with 10% fetal bovine serum. Triplicate assays were done to determine the CA 125 levels.

Hormone receptor assay

The cultured cells were analyzed for estrogen receptor (ER) and progesterone receptor (PR). Cell suspensions were centrifuged and the pellets, containing a minimum of 1×10^9 cells, were homogenized in a Polytron homogenizer (Brinkman, Swiss). For the ER and PR assay, Rianen [^{3}H]estrogen and progestin receptor assay kits (Du Pont Comp., Billerica, Mass.) were used respectively. The data, after correction for nonspecific binding, were analyzed using a Scatchard plot to determine the number of sites. Receptors were considered absent if less than 9 fmol ER or PR was present/mg protein.

HLA typing

HLA typing was performed on cultured cells using the allele-specific polymerase chain reaction (PCR) genotyping method (Saiki et al.

1986). The sequences of the locus-specific primers and allele-specific primers were designed according to Marsh and Bodmer (1992). HLA genotyping for three of the polymorphic class II loci (DRB1, DQA1, and DQB1) was conducted using two-step PCR. Each reaction mixture contained 0.1 µg cellular DNA, 15 pmol each locus-specific primer, 0.125 mM each dNTP, and 0.2 U Taq polymerase (Boehringer-Mannheim, Germany). The first amplification was carried out for 31 cycles. The locus-specifically amplified products were analyzed on 1.5% agarose gel and the reaction products were reamplified for 16 cycles with allele-specific primers.

Effect of Gn-RH on cell growth

To evaluate the effect of the GnRH analog [*D*-Trp^6]luleinizing-hormone-releasing hormone ([*D*-Trp^6]LHRH; Ferring AB, Sweden) on cell growth, exponentially growing cells were cultured in 96-well culture plates (1×10^3 cell/well). After 12 h, 10 µM of [*D*-Trp^6]LHRH was added to three replicate wells and cells were grown for another 36 h. The growth inhibition was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay kit (Chemicon, Calif.) and the percentage cytotoxicity was calculated. The absorbance was measured on an enzyme-linked immunosorbent assans (ELISA) plate reader (Dynatech MR 700 reader) with a test wavelength of 570 nm. The percentage cytotoxicity was calculated as follows: $(1 - A_{exp}/A_{control}) \times 100$, where A is the absorbance. The SD was always less than 10% of the mean.

Gn-RH receptor mRNA expression

Total RNA was extracted from CUMO-2 cells, using a commercial system (RNeasy total RNA kit) provided by Qiagen (Qiagen Inc., Germany). Reverse transcription (RT)-PCR was carried out using the RNA PCR kit (Perkin Elmer, N.J.) under the conditions recommended by the supplier. The product of reverse transcription (20 µl) was amplified in a 100-µl reaction containing 10 mM TRIS/HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 50 µM each dNTP, 2.5 U Taq polymerase, and 100 pmol each primer. The sequences of the oligonucleotide primers, upstream (5′-GACCTTGTCTGGAAAGATCC-3′) and downstream (5′-CAGGCTGATCACCACCATCA-3′), were synthesized according to the published human GnRH receptor cDNA sequence (Chi et al. 1993). We carried out 35 cycles of amplification: denaturation at 95 °C for 20 s, annealing/extension at 60 °C for 30 s, followed by a final extension for 7 min at 72 °C. The DNA product (10 µl) was run on 2% agarose gel, and bands were visualized by ethidium bromide staining on a UV transilluminator. A 123-bp DNA ladder (Gibco-BRL) was used as a size marker. The human ovarian carcinoma cell line, SK-OV3, was used as a positive control for GnRH receptor mRNA expression (Imai et al. 1994). The predicted fragment amplified by PCR was 319 bp.

Effects of TGFβ1 on cell growth and *c-myc* gene expression

To evaluate the effect of TGFβ1 on cell growth, exponentially growing cells were cultured in a 96-well culture plate (1×10^3 cells/well). After 12 h, 10 ng/ml human recombinant TGFβ1 (Genzyme, Cambridge, Mass.) was added to three replicate wells and cells were grown for another 24 h. The growth inhibition was measured using the MTT assay kit and percentage cytotoxicity was calculated. To determine the effect of TGFβ1 on the expression of the *c-myc* gene, 1×10^7 cells were incubated for 4 and 24 h in the presence of 10 ng/ml TGFβ1. Media of the control cultures contained no TGFβ1. Total RNA was extracted using a commercial system (RNeasy total RNA kit) provided by Qiagen (Qiagen Inc., Germany). An equal amount of RNA (10 µg) was electrophoresed through 1% formaldehyde/agarose gel and transferred to nylon membranes (Boehringer-Mannheim, Germany). The blots were hybridized with digoxigenin-labeled specific probes. A *c-myc* cDNA probe was prepared by PCR with digoxigenin-11-dUTP (Boehringer-Mannheim) using specific primers provided by Clontech (Palo Alto, Calif.). The blots were incubated with alkaline-phosphatase-conjugated anti-digoxigenin antibody and then hybridized probes were immunodetected with chemiluminescent substrate supplied by Boehringer-Mannheim. A full β-actin cDNA probe was used as an internal standard to check whether equal amounts of total RNA had been applied. Finally, reproducibility of our results was confirmed by repeating Northern analyses at least once.

Analysis of *p53* gene by PCR/single-strand-conformation polymorphism (SSCP)

To search for *p53* gene mutations by the PCR-SSCP method, four primer sets encompassing so-called "mutational hot spots": exon 4, exons 5 and 6, exon 7, and exons 8 and 9, were designed according to previously published sequences (Buchman et al. 1988). Extracted DNA was purified with a Gene Clean II kit (Bio 101 Inc., La Jolla, Calif.) for subsequent PCR. Each reaction mixture (5 µl) contained 50 ng DNA, 1.5 mM $MgCl_2$, 2 pmol each primer, 50 µM each dNTP, 0.037 MBq [a $-$ ^{32}P]dCTP (Amersham), and 0.125 U Taq polymerase. Amplification was carried out for 35 cycles of 30 s at 95 °C and 2 min at $(T_m - 5)$ °C. A 3-µl sample of each reaction mixture was then analyzed on 1.5% agarose gel containing ethidium bromide. The remaining 2 µl was diluted 50-fold with formamide dye solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol FF) followed by denaturation by boiling. A 2-µl sample of each mixture was electrophoresed on a 6% polyacrylamide gel containing 5% glycerol and the gels were exposed to X-ray film at -70 °C for 12–24 h. DNA from normal peripheral blood lymphocytes was used as negative control.

Direct DNA sequencing

Abnormal DNA fragments detected by SSCP analysis were eluted from a corresponding portion of the dried gel and amplified by the allele-specific PCR method (Suzuki et al. 1991). The products were sequenced by the dideoxy-DNA chain-termination method (Sanger et al. 1977), using 5′-^{32}P-labeled deoxyoligonucleotides as primers (Buchman et al. 1988) and the fmol DNA Sequencing System (Promega).

Results

Morphology of the original tumor

Histological examination of the original ovarian tumor specimen showed an undifferentiated ovarian carcinoma composed of sheets of anaplastic epithelial cells (Fig. 1A).

Establishment of the cell line

The CUMO-2 cell culture was started on 14 February 1989. After 14 days in the stationary period, favorable outgrowth was found, but no contamination by fibroblasts. Upon passage, the cells quickly became adherent to the flask and spread as a sheet of monolayered cells. The

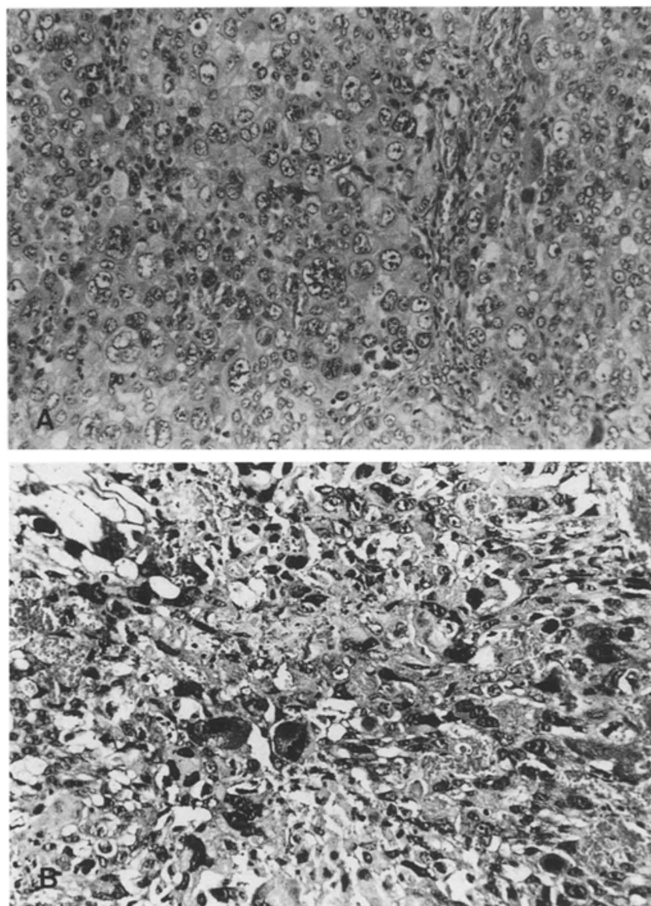

Fig. 1 **A** Histology of the original undifferentiated ovarian carcinoma showing marked nuclear pleomorphism, coarse granular chromatin pattern, and prominent nucleoli. Hematoxylin/eosin ×200. **B** Heterotransplanted CUMO-2-derived nude mouse tumor in peritoneal cavity. The carcinoma consists of highly anaplastic cells with hyperchromatic, large, and bizarre nuclei. Hematoxylin/eosin ×400

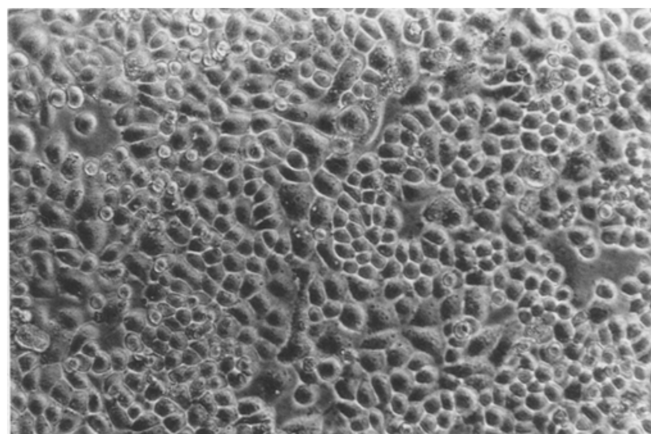

Fig. 2 Phase-contrast features of the monolayer-cultured cells revealing a sheet of polygonal, pleomorphic cells with a pavement-like arrangement. ×100

CUMO-2 cells grew well without interruption for more than 81 months and 230 serial passages were successively carried out.

Tumorigenicity and metastasis

The tumorigenic properties of this cell line are described in Table 1. Following s.c. injection, tumors developed in 16 of 18 mice (89%) within 2 weeks after the inoculation, while the i.p. route induced a peritoneal carcinomatosis associated with ascites in 6 of 8 mice (75%) within 3 weeks. Histopathologically, tumors developed in the peritoneal cavity were highly anaplastic (Fig. 1B). Following i.v. injection into the lateral tail vein, 5 of 7 mice (71%) had evidence of pulmonary metastasis at the time of termination.

Morphology of the cultured cells

Figure 2 is a phase-contrast micrograph of cultured CUMO-2 cells. In the monolayer cultures there were polygonal cells in a pavement-like arrangement, piled up without contact inhibition. The cytoplasms were stained positively by periodic acid/Schiff reagent and negatively by mucicarmine and Alcian blue. Ultrastructurally, cultured cells showed euchromatic nuclei with 2 or 3 nucleoli. Moderate numbers of mitochondria and numerous polysomes were scattered throughout the cytoplasm and an intracytoplasmic demosome was also found (Fig. 3A). CUMO-2 cells also showed the presence of certain epithelial characteristics such as the presence of demosomes with scattered bundles of tonofilaments (Fig. 3B).

Growth characteristics

Analysis of the growth curve of the CUMO-2 cells at passage 100 showed that the population doubling time

Table 1 Tumorigenic properties of CUMO-2 cells in nude mice

Injection site	No. of animals	Died unexpectedly	Tumor formations		
			Subcutaneous tissue	Peritoneum	Lung
s.c.	20	2	16/18 (89%)	–	–
i.p.	10	2	–	6/8 (75%)	–
i.v.	10	3	–	–	5/7 (71%)

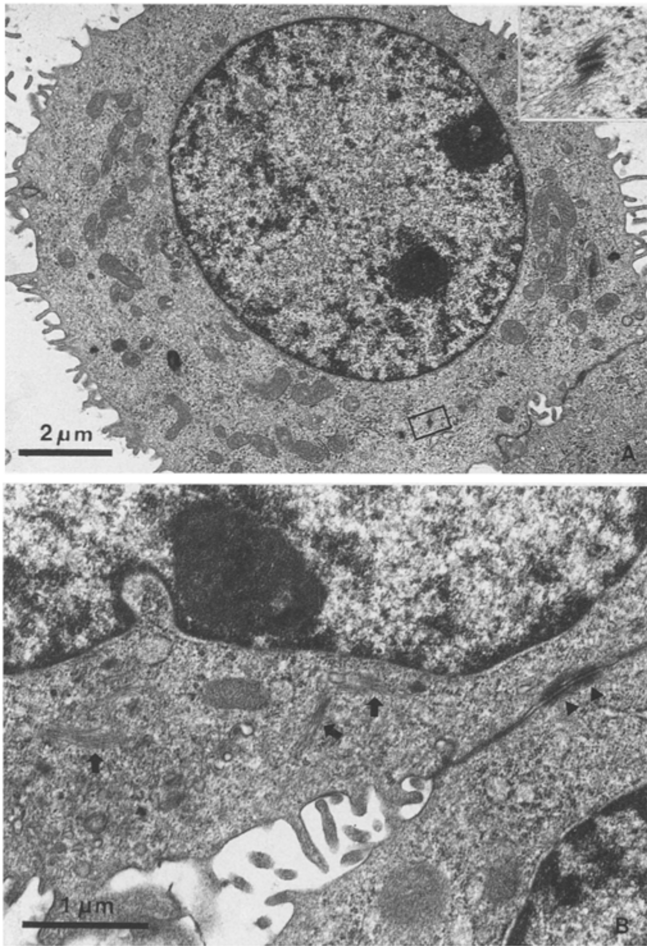

Fig. 3A, B Transmission electron micrographs illustrating representative characteristics of tissue-culture cells at passage 150. **A** Cells showing euchromatic nucleus with two nucleoli and cellular surface covered by numerous microvilli. Moderate numbers of mitochondria and numerous polysomes are scattered throughout the cytoplasm × 10 000 *Inset* higher magnification of rectangular area showed intracytoplasmic desmosome × 30 000) **B** Note scattered bundles of tonofilaments (*arrows*) and desmosomes (*arrow heads*) × 28 000

was approximately 50 h during exponential cell growth and the plating efficiency was 30%.

Cytogenetic analysis

The representative G-banded karyotype of this cell line is illustrated in Fig. 4. Repeated chromosome analyses on each passages revealed a highly aneuploid karyotype with numerous numerical and structural abnormalities. There were some cells with random gain or loss of a chromosome at each passage, but several consistently abnormal chromosomes were detected throughout all the passages. The overall chromosome counts per cell varied considerably over a large range with near-triploid modal numbers as shown in Table 2. All the chromosomes were involved in aneuploidy. The chromosomes 4, 18, 22, and X have only one or two copies in most of the cells, whereas the other chromosomes have more than three copies. Structural abnormalities of the chromosomes were exteremely common. Deletions and translocations were the most common rearrangement. The clonal markers observed in this cell line are summarized in Table 3. Those chromosomes most frequently involved in structural and numerical alterations were chromosomes 1, 3, 8, and 11. Structural aberrations of chromosome 1 were simple deletion of the short-arm region distal to 1p13 [marker (M) 1] and translocated derivatives of which those distal to the 1p32 regions were replaced with the chromosome of unknown origin (M2). Most of the cells showed simple short-arm deletion of chromosome 3, involving bands p12 → pter (M3). Whole-arm translocations between the long arm of chromosome 3 and the short arm of chromosome 5 were observed in all cells (M4). The isochromosome of 5p (M5) or the translocation derivatives of chromosomes 8 (M7) and 11 (M10) were also found in all cells, and the other markers (M6, M8, M9, M11, M12, and M13) were observed in more than two cells in metaphase analyzed. The deletion or translocation anomalies of the long arm of chromosome 6 [$6q^-$ or t(6;14)] were not found in this cell line.

CA 125 production

Control medium, i.e., not exposed to CUMO-2 cells, did not contain a detectable quantity of CA 125. However, the concentration of CA 125 in CUMO-2 cell culture media once the cells reached the confluent stationary phase was 200 U/ml. High levels of CA 125 (> 500 U/ml) were detected in the sera of tumor-bearing nude mice.

Hormone receptors

In CUMO-2 cells, ER (18 fmol/mg protein) was present but PR was not measured.

HLA typing

HLA class II phenotypes were DR8 and DQw4, and HLA class II allele specificities were DRBI*0802, DQAI*0101, DQAI*0201, and DQBI*04**.

Inhibition of cell growth by GnRH analog

The GnRH analog at concentration of 10 μM induced up to 40% reduction in cell number compared to control untreated cells.

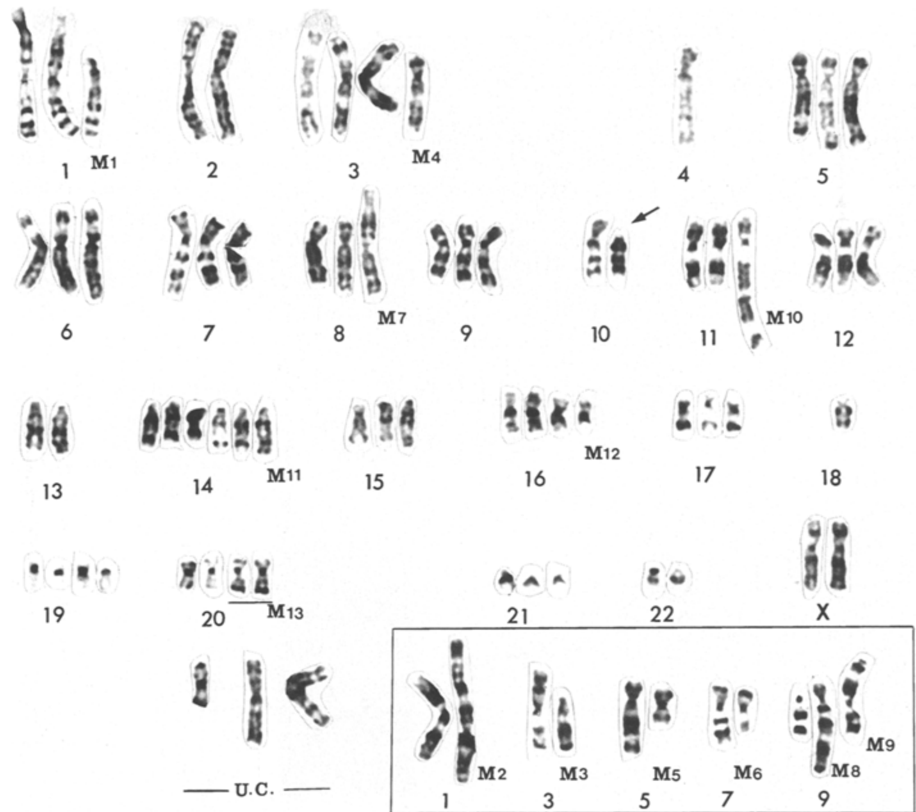

Fig. 4 A representative G–banded karyotype of CUMO-2 cells at passage 150. Identifiable clonal structural markers (M1–M13) are described in more detail in Table 3. Deletion of 10p seen in this karyotype (*arrow*) was not a clonal change. *Inset* additional examples of clonal markers from another cell *U.C.* unidentified marker chromosomes

Table 2 Distribution of chromosome numbers in 30 metaphase plates of CUMO-2 cells at passage 150

Range of chromosome numbers	No. of metaphases analyzed
50–60	4 (13%)
61–65	4 (13%)
66–69	8 (27%)
70–72	9 (30%)
> 72	5 (17%)
Total	30 (100%)

GnRH receptor mRNA expression

PCR amplification of first-strand cDNA from CUMO-2 and SK-OV3 cells was conducted with an oligonucleotide primer set. GnRH receptor mRNA was detected in CUMO-2 cells. The human ovarian carcinoma cell line, SK-OV3, also gave a predominant product (319 bp) identical to that obtained in CUMO-2 cells, as shown in Fig. 5.

Effects of TGFβ1 on cell growth and C-*myc* gene expression

After 24 h of treatment, TGFβ1 inhibited CUMO-2 cell growth as measured by MTT assay. The cytotoxicity was 28%. Control nontreated cells proliferated-progressively in vitro. We examined the effect of TGFβ1 on the expression of the proliferation-associated gene, c-*myc* in CUMO-2 cells. TGFβ1 suppressed c-*myc* mRNA expression after 24-h of exposure, as evaluated by Northern blot analysis (Fig. 6). These results show that TGFβ1 may play an antiproliferative effect on epithelial ovarian tumor growth by reducing the c-*myc* gene product.

Characterization of the SSCP band shift by direct sequencing

CUMO-2 cells demonstrated a conformational band shift in exon 7 of the gene (data not shown). To confirm and determine the specific nature of this mutation, an abnormal SSCP band shift was sequenced. It was a frameshift mutation (a one-base-pair deletion in codon 254) (Fig. 7), which would result in the premature formation of a stop codon and ultimately a truncated protein.

Discussion

Minor areas of differentiation, manifested by the presence of occasional endometrial-type glands, mucinous

Table 3 Clonal markers in CUMO-2 cells

Markers	Description
M1	del (1) (qter→p12:)
M2	der (1)t(1;?)(1qter→1p32::?)
M3	del(3) (qter→p12:)
M4	t(3q;5p) (5pter→cen→3qter)
M5	i(5p)
M6	del(7) (pter→q22:)
M7	der(8)t(1;8) (1pter→1p13::8p11→8qter)
M8	der(9)t(9;?) (9pter→9q34::?)
M9	der(9)t(3;9) (3pter→3p12::9p13→9qter)
M10	der(11)t(11:?) (11pter→11q23::?)
M11	$14q^+$
M12	del(16)(pter→q13:)
M13	$20q^+$

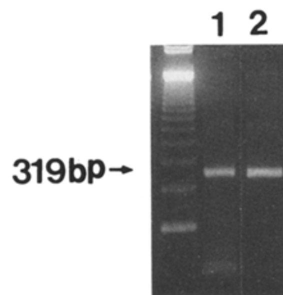

Fig. 5 Polymerase chain reaction (PCR) amplification of first-strand cDNA from two ovarian carcinoma cell lines, CUMO-2 (*lane 1*) and SK-OV3 (*lane 2*). Oligonucleotide primers, upstream and downstream (5′-GACCTTG TCTGGAAAGATCC-3′ and 5′-CAGGCTGATCACCACC ATCA-3′) were used

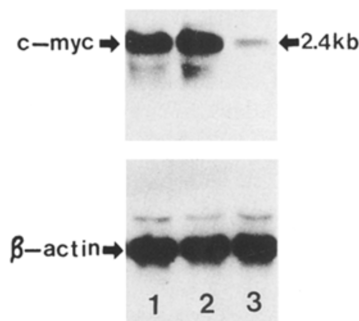

Fig. 6 Northern blot analysis of c-*myc* mRNA isolated from CUMO-2 cells treated with 10 ng/ml TGFβ1 for 4 (*lane 2*) and 24 h (*lane 3*). *Lane 1*, c-*myc* expression in nontreated control

droplets, or psammoma bodies, are not considered sufficient evidence to classify this tumor more specifically. This cell line showed periodic-acid/schiff positive staining of the cytoplasm.

Subcutaneous injection of cells into nude mice produced rapidly growing primary tumors within 2 weeks. Along with tumor aggressiveness, metastasis to the lung was observed in some of the sacrified animals after i.v. injection. Another point of interest is the induction of peritoneal carcinomatosis in nude mice after i.p. injection of cells. The mouse peritoneum is of great interest in human ovarian cancer studies because of its similarity to the site where the disease occurs in women.

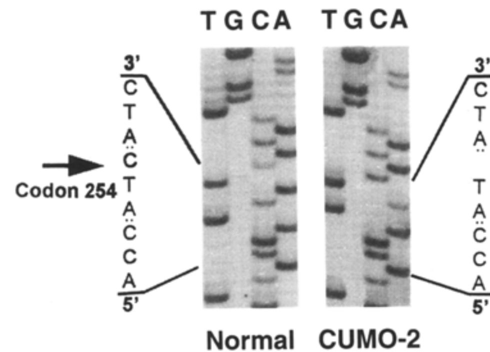

Fig. 7 Nucleotide sequence analysis of frameshift mutation detected by PCR/single-strand-conformation polymorphism analysis in CUMO-2 cells. PCR products were subjected to direct sequencing analysis by the dideoxy-DNA chain-termination method. This showed a one-base pair deletion in codon 254

This cell line has a karyotype similar to other ovarian cancer cell lines with many rearranged chromosomes including structural abnormalities of chromosomes 1, 3, 8, and 11 (Sheer et al. 1987). This cell line also demonstrated several unusual marker chromosomes that were not frequently reported in other ovarian cancers or cell lines such as isochromosomes [i(5p)] or whole-arm translocations [t(3q;5p)]. Abnormalities of chromosome 1 have been identified in most malignancies (Olah et al. 1989) and appear to be related to a common event to neoplasia. Rearrangements of chromosome 1 had certain regions (particularly involving the region distal to band 1p32) that were more often involved than others. It is noticeable that the CUMO-2 cell line also demonstrated deletion or translocation involving the same region. Deletion of the short arm of chromosome 3 has previously been reported in solid ovarian tumors as well as in ascitic fluid and cell lines (Sheer et al. 1987). Abnormalities of 3p, usually deletions involving bands 3p13–21, have been described in ovarian tumors or cell lines. The deletion or translocation anomalies of the long arm of chromosome 6 [$6q^-$ or t(6;14)], which were reported previously as one of the most frequently involved clonal markers in ovarian carcinoma (Wake et al. 1980), were not found in this cell line. Recently there have been many reports on anti-oncogenes, which may play a role in carcinogenesis (Knudson 1993). It has been suggested that the loss of a normal allele (loss of heterozygosity) by the deletion of a specific region of a chromosome results in the expression of an abnormal allele which has a transforming activity. In this cell line, it is interesting to note that the structural aberrations on chromosomes 1, 3, 5 and 11 include many antioncogene sites like NB1 (1p36), VHL (3p25), APC (5q21), and MEN1 (11q13). Thus, to delineate the relationship between specific chromosomal abnormalities and cancer-associated genes in this cell line, further molecular genetic studies

are needed. The karyotype of the cultured cells may undergo considerable changes in vitro (Kunzmann and Holzel 1987; Smith et al. 1989) and this cell line also showed some evidence of numerical and structural instability of chromosomes through successive passages. However, we noticed that some chromosomal abnormalities were consistent from early to late passages. Since the cultured cells were examined quite early at passage 5, it is likely that many of these markers found in this passage were present in the original tumor. It is remarkable that the consistent marker chromosomes of this cell line include many of the structural abnormalities that were reported previously to be frequently involved in primary ovarian cancer (Panani and Ferti-Passantonopoulou 1985). Some of these are ubiquitous and may form part of the genetic background for cancer development in general.

These consistent chromosomal abnormalities are also part of the background for tumor progression in vitro. The cytogenetic stability of this cell line over 6 years increase its usefulness as a model for further investigation into human ovarian malignancy.

In ovarian carcinoma, ER and PR are present on 63% and 48%, of cells respectively (Slotman and Rao 1988). Nonetheless the significance of their presence in the pathogenesis of epithelial ovarian tumors has not yet been elucidated. Hamilton et al. (1983) have described a human ovarian carcinoma cell line (NIH:OVCAR-3) that contains estrogen and androgen receptors. The original histological type of NIH:OVCAR-3 was that of an undifferentiated carcinoma. This agrees with the report of Schwartz et al. (1985) that poorly differentiated neoplasms have a greater chance of being positive for the ER than do better differentiated tumors. CUMO-2 cells, an undifferentiated ovarian carcinoma cell line that has been grown in long-term tissue cultures, has proved to contain ER and may become a model for studying the actions of steroid hormones in ovarian cancer.

The antitumor action of GnRH in breast and prostate cancers has been shown to result from a desensitization or down-regulation of GnRH receptors in the pituitary, with a consequent decline in gonadotropin secretion and gonadal steroid production. But there are indications that GnRH suppresses the growth of the cancer cells in vitro and that the specific binding sites for GnRH are demonstrated in a breast tumor (Eidne et al. 1987). A similar autocrine system based on GnRH has been proposed for the pancreas (Fekete et al. 1989) and prostate cancers (Qayum et al. 1990). These findings have raised the question whether a GnRH-dependent autocrine mechanism might also-exist in human ovarian tumors, which could be used for novel therapeutic approaches. To elucidate a possible direct effect of GnRH on human ovarian cancer, we checked whether it inhibits tumor cell growth and found that the cells were quite sensitive to Gn-RH. Although we demonstrated the expression of Gn-RH receptor in this cell line, the nature of the Gn-RH binding sites (low affinity/high capacity and/or high affinity/low capacity) has to be determined by future research. The expression of GnRH receptor in this cell line may provide a rationale for the further exploration of a direct antitumor effect of GnRH.

TGFβ1 is an important modulator of cell growth and differentiation (Roberts and Sporn 1990). TGFβ1 exerts its specific effects on target cells through interaction with specific cell-surface receptors, but signal-transduction pathways are poorly understood as yet. It has been reported that TGFβ1 treatment of several different cell lines results in the rapid down-regulation of c-*myc* RNA and protein levels (Mulder et al. 1988; Pietenpol et al. 1990). Because c-*myc* expression is required for cell growth (Pietenpol et al. 1990), it has been suggested that one mechanism by which TGFβ1 inhibits cell-cycle progression is through suppression of the c-*myc* gene product. c-*myc* has also been implicated in the novel signal transduction pathways involved in apoptosis (Kimura et al. 1995). To date, results concerning its role in apoptosis are not only extremely complex but, in many cases, highly contradictory. Some investigators found that up-regulation of c-*myc* expression caused apoptosis (Askew et al. 1993), while others suggested that down-regulation may be mandatory for the induction of apoptosis (Alnemri et al. 1992).

Further research is necessary to clarify the relationship between c-*myc* expression and apoptosis in response to the inhibitory effect of TGFβ1. We investigated whether TGFβ1 inhibition of epithelial ovarian cancer cell growth occurs as a consequence of down-regulation of c-*myc* gene expression. The results reported here indicate that c-*myc* is down-regulated in CUMO-2 cells in response to TGFβ1 and TGFβ1 may act as a negative growth regulator of epithelial ovarian carcinoma cells in vitro. Further study of this potent growth inhibitor will provide insights into ovarian cancer biology and suggest potential therapeutic strategies.

The alterations within the coding sequences of the *p53* tumor-suppressor gene are among the most frequent genetic changes detected in human cancers. Yaginuma and Westphal (1992) reported that frequent *p53* gene abnormalities in human ovarian carcinoma cell lines suggested that inactivation of *p53* functions may play a significant role in human ovarian carcinogenesis. The mutation disclosed in this cell line was in exon 7. This is in good agreement with the pattern generally observed in most tumor types, in which mutations are found in exons 5–9, with an apparent preference for exons 5, 6, and 7, which are known to code for highly conserved amino acid blocks.

These results suggest that chromosome 1, 3, 8, and 11 abnormalities and *p53* gene mutation may work together in ovarian carcinogenesis, and GnRH and TGFβ1 can be used as adjuvant treatment modalities in epithelial ovarian carcinoma.

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