Establishment and Characterization of a Novel Cancer Cell Line (SMOV-1), Derived from the Ascitic Fluid of a Woman with Ovarian Cancer Who Had Never Responded to Chemotherapeutic Drugs

Tomohiro Iida,[1]* Kyoko Yonamine,[1] Hisakiyo Takeuchi,[1] Kazuhiko Hayashi,[1] Takashi Meguro,[2] Toshihito Shinagawa,[3] and Akira Amemiya[4]

[1]Department of Obstetrics and Gynecology and the [2]Research Laboratory of St. Marianna University Yokohama City Seibu Hospital, Yokohama, Japan, and Departments of [3]First Division of Pathology and [4]Obstetrics and Gynecology, St. Marianna University School of Medicine, Kawasaki, Japan

Background: A novel cell line, designated SMOV-1, was established from the ascitic fluid of a woman with ovarian cancer. This report describes the process of establishment and characterization of this cell line.
Methods: The case involved a woman who had undergone a surgical intervention, due to a serous papillary adenocarcinoma of the ovary. She received the combination chemotherapy of PAC (cisplatin + doxorubicin + cyclophosphamide) 6 times after the surgery. However, as a result of malignant pleural effusion during chemotherapy, she died 7 months after surgery. Before surgery, ascitic fluid was removed from the patient for cell culture, after obtaining her consent. The cells were successfully subcultured, and designated SMOV-1.
Results: The cell line gave a pavement-stone pattern, without contact inhibition. SMOV-1 could also be transplanted into nude mice, and the tissue showed reconstruction of the original tumor. The chromosome number of the SMOV-1 cells was 57, and the DNA index was 2.28. The population-doubling time of the SMOV-1 cells was 61.4 hours. Cultured SMOV-1 cells were still capable of producing the tumor-associated antigens CA 125, CA 19-9, and sTn (sialosyl-Tn).
Conclusion: We propose that this novel cell line is of possible use for the investigation of drug resistance in ovarian cancer.

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Key words: ovarian cancer, in vitro, tumor marker, drug resistance

INTRODUCTION

Ovarian cancer is uncommon in Japan.[1] However, because ovarian cancer is usually asymptomatic in its early stages, and because there are no screening programs of proven usefulness, 70% or more of patients present with widespread disease at the time of diagnosis.[2] Therefore, most patients with ovarian cancer must be given anticancer drugs as adjuvant therapy prior to or after laparotomy. Cisplatin-based combination chemotherapy has become available, and over 70% of patients with ovarian cancer initially respond to this treatment.[3] However, not all ovarian cancers are equally sensitive to this drug combination, and the efficacy of treatment is severely limited by the development of drug resistance.[4] In this report, we present a novel cancer cell line, derived from the ascitic fluid of a woman with ovarian cancer who had never responded to treatment with anticancer drugs.

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MATERIALS AND METHODS

Case History

The patient was a 49-year-old Japanese woman who complained of decreasing urination, abdominal distention, and mild constipation. Important findings were limited to the abdomen and pelvis. There was an obvious presence of ascitic fluid. The pelvic examination revealed a fixed adnexal mass that could not be separated from the uterus, and obvious nodulation in the posterior cul-de-sac. The level of serum CA 125 was 508 U/mL. She was admitted to our hospital on December 30, 1993. The ascitic fluid was removed for cytologic examination by puncture of the abdominal cavity on January 9, 1994, and was used for cell culture, after obtaining patient consent. A laparotomy (bilateral salpingo-oophorectomy + hysterectomy + partial omentectomy) was performed on January 18, 1994. Her

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disease was staged as IIIc, according to the International Federation of Gynecology and Obstetrics (FIGO) nomenclature (1988), serous papillary adenocarcinoma of the ovary, after exploratory surgery. She received 6 courses of the PAC regimen (cisplatin + doxorubicin + cyclophosphamide) after surgery. In spite of this, she acquired a malignant pleural effusion during the chemotherapy. We administered doxorubicin to her right pleural cavity and cisplatin + etoposide intravenously. Despite our efforts, she died on 21 August 1994, without having displayed any favorable response to the anticancer drugs.

Establishment of the Cell Line

We isolated the cells by centrifugation (1000 rpm, 5 minutes) from the ascitic fluid. The cells were plated in tissue culture flasks (Falcon; Becton Dickinson, Franklin Lakes, NJ, USA) with Eagle's MEM (minimum essential medium) (Nissui, Tokyo, Japan, + 15% fetal bovine serum, from JRH Biosciences, Lenexa, KS, USA) at 37°C in a water-jacketed carbon dioxide gas (CO_2) incubator. Immortalized adherent cells were soon observed, and they have been successfully subcultured since 16 January 1994. At that time, the cells were Monocloned, using the limiting dilution method, and designated SMOV-1.

Morphologic Characteristics

We used a phase-contrast microscope for daily observation of the SMOV-1 cells. After the 15th passage, the SMOV-1 cells were cultured in Lab-Tek chamber slides (Nunk, Naperville, IL, USA), fixed with 90% ethyl alcohol (EtOH), and stained by the Papanicolaou method.

Cell Proliferation

A 5×10^5 aliquot of SMOV-1 cells was cultured in triplicate in a 60-mm cell-culture dish (Falcon, Becton Dickinson), containing 4 mL of culture medium. Cells were harvested every 72 hours, treated with 0.1% trypsin + 0.02% EDTA, and counted, using a hematocytometer, until 15 days after inoculation. The supernatant of the cultured medium was stored at –20°C for later analysis of its constituents. The saturation density of the SMOV-1 cells was estimated and calculated, using a similar method, at 25 days after the seeding.

Transplantation of SMOV-1 into Nude Mice

A 6×10^6 aliquot of SMOV-1 cells was inoculated subcutaneously into 12-week-old, female BALB/c AnN Crj-nu/nu mice (2 sites each × 3 mice = 6 sites; mice from Nihon Charles River, Yokohama, Japan).

Karyotypic Characteristics

SMOV-1 cells (15th passage) in the metaphase were stained by the G-band method,[5] and the number of chromosomes was counted in each cell. The karyotype of SMOV-1 was expressed according to the International System for Human Cytogenetic Nomenclature (ISCN) 1991 convention.[6]

Flow Cytometric Analysis

Cell suspensions of SMOV-1 were fixed in iced, 70% EtOH and stained with propidium iodide (50 μg/mL in phosphate-buffered saline (PBS), from Sigma, St Louis, MO, USA), along with normal human lymphocytes. We analyzed them for their DNA-index,[7] using an EPICS-753J flow cytometer (Coulter Electronics, Hialeah, FL, USA).

Products

The tumor-associated antigens in the stored supernatants of the cultured medium were measured (CA 125,[8] CA 19-9,[9] and sialosyl-Tn (sTn)[10] using a Centocor kit, Toray Fuji Bionics, Tokyo, Japan; RIA (radioimmune assay) using an Otsuka kit, Otsuka Phamaceutical, Tokushima, Japan). The results were expressed using the following formula: Products = (concentration × 4)/cell number, where products are expressed as units/cell number per 72 hours, and concentration is units per milliliter.

RESULTS

Morphologic Characteristics

Light micrographs of the original tumor tissue showed a papillary configuration and acinar formation, which are commonly recognized characteristics of serous papillary adenocarcinoma of the ovary (Fig. 1A). Phase-contrast micrographs of SMOV-1 showed a pavement-stone–like pattern, without contact inhibition (Fig. 1B). The edges of the cells were polygonal, cytosomes were present in moderate amounts with vacuoles, and the chromatin of the nucleus showed a fine granular pattern, using the Papanicolaou stain (Fig. 1C).

Cell Proliferation

The SMOV-1 cells showed about 72 hours of delayed growth, prior to logarithmic proliferation. The population-doubling time was 61.4 hours in the logarithmic growth phase (Fig. 2). The saturation density of SMOV-1 was $2.43 \pm 0.31 \times 10^5$ cells per cm^2.

Transplantation of SMOV-1 into Nude Mice

On the 50th day after inoculation, tumors appeared at 3 of 6 sites in the mice. The maximal tumor size was under 2 mm in diameter at that time. The tumors also had the characteristics of an adenocarcinoma, like the resected, original tumor tissue (Fig. 3). However, they never became larger as a result of the rejective reaction of the nude mice.

Karyotypic Characteristics

The chromosome number of the SMOV-1 cells was 57. One uncharted (marker) chromosome was also seen (Fig. 4).

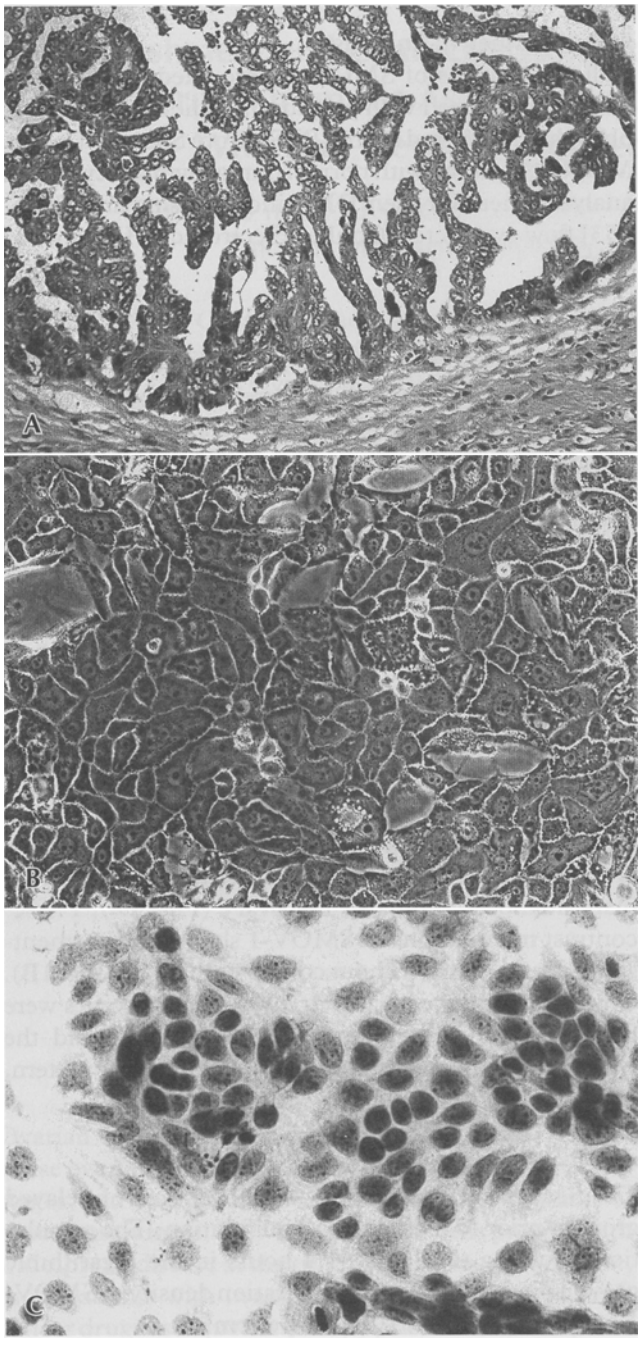

Fig. 1. Photomicrographs of the original tumor tissue in a 49-year-old woman with ovarian cancer: (**A**) light micrograph shows a papillary configuration and acinar formation, indicating a serous papillary adenocarcinoma of the ovary (hematoxylin and eosin stain; original magnification, × 50); (**B**) phase-contrast micrograph shows a pavement-stone pattern, without contact inhibition (original magnification, × 50); (**C**) Papanicolaou staining shows the edges are polygonal, the cytosomes are present in moderate amounts, with vacuoles, and the chromatin of the nucleus shows a fine granular pattern (original magnification, × 100).

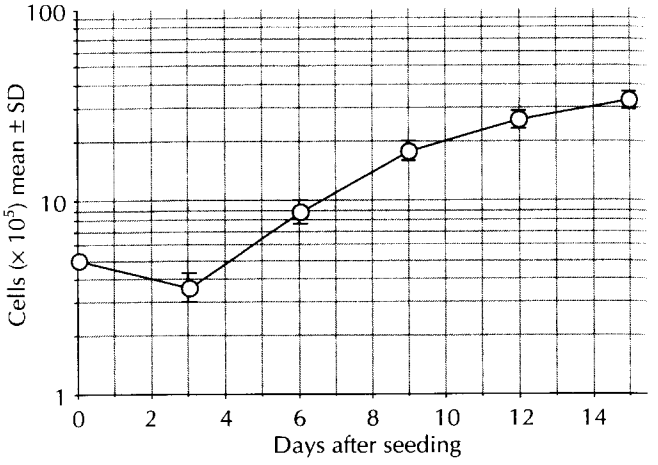

Fig. 2. Growth curve of SMOV-1 cells. The population-doubling time was 61.4 hours in the logarithmic growth phase.

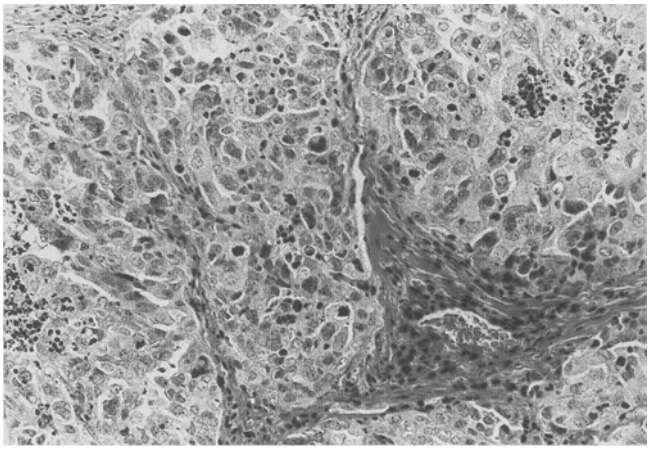

Fig. 3. Morphologic characteristics of SMOV-1 xenograft in nude mice (hematoxylin and eosin stain; original magnification, × 50). The transplanted cells resulted in an adenocarcinoma, with all the characteristics of the resected, original tumor tissue.

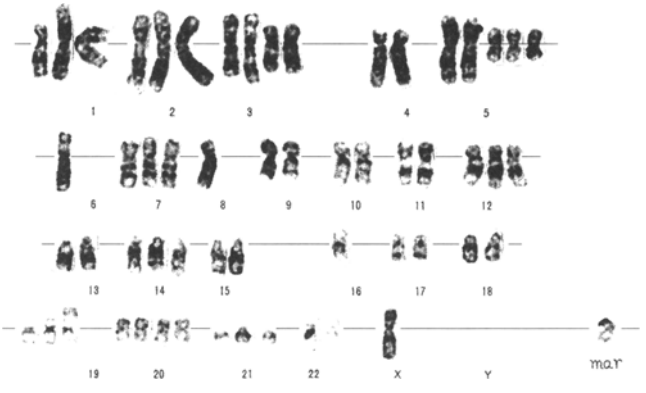

Fig. 4. Chromosomal analysis, using the G-bang method on SMOV-1 cells. The chromosome number was 57. Karyotype was as follows: 57,X +add(1)(p22)x2 +add(3)(p13)x2 +add(5)(q11)x3 der(5)t(5;8)(p11;q11)x2,–6,+7,-8,+12,+14,–16, +add(19)(p13), +20x2, +21,+ mar.

Flow Cytometric Analysis

The channel number of the $G_0 + G_1$ peak of SMOV-1 was 68.14, while that of normal human lymphocyte is 29.84. Therefore, the DNA index of SMOV-1 was calculated to be 2.28 (68.14/29.84).

Products

The maximum levels (in U/10^5 cells per 72 hours) of the 3 tumor-associated antigens were 546.1 for CA 125; and 7.08 for CA 19-9, and 3.77 for sTn (Fig. 5). The maximum accumulation of CA 125 and CA 19-9 was seen in the stationary phase of growth. However, the maximum level of sTn was seen in the delay period, before the start of the growth phase.

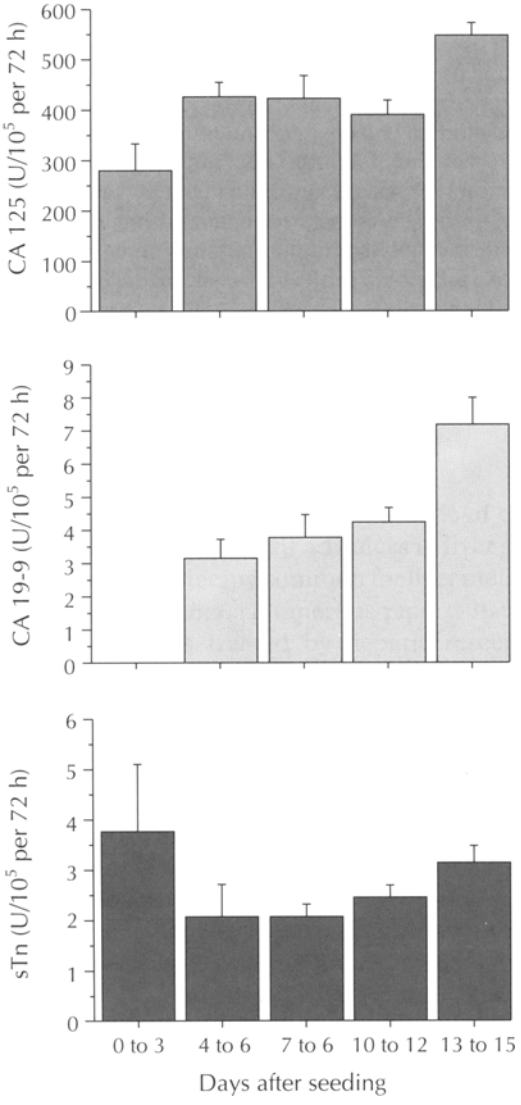

Fig. 5. Products of SMOV-1 cells. The maximum production (in units/10^5 cells per 72 hours) of 3 tumor-associated antigens was 546.1 for CA 125, and 7.08 for CA 19-9, and 3.77 for (sialosyl-Tn (sTn). Mean + SD.

DISCUSSION

SMOV-1 may be considered an established cell line for several reasons. Infinite growth was established, and SMOV-1 exhibited a lack of contact inhibition. We were able to produce a histologic reconstruction of the original tumor on inoculation into nude mice. There was also the presence of an abnormal karyotypic and DNA aneuploidy, and the ability to produce multiple, tumor-associated antigens.

Resistance to chemotherapeutic drugs has been a common feature of the natural history of ovarian cancer. This is all the more a problem when one considers that the majority of epithelial ovarian cancers are in the advanced stage when a diagnosis is made. The standard therapy for a woman with advanced, epithelial ovarian cancer is PAC, or similar, cisplatin-based combinations. However, long-term disease control with this regimen occurs in less than 10% of women with stage III disease, and less than 5% of those with stage IV disease.[11]

Paclitaxel is a compound extracted from the bark of the Pacific yew tree (Taxus brevifolia). Its use results in the multiplication of microtubules, and cancer cells cease to divide in metaphase when treated with this drug.[12] In clinical trials in 1989, paclitaxel was reported to produce partial or complete responses in 30% of patients with advanced ovarian cancer.[13] Recently, the Gynecologic Oncology Group reported that the incorporation of paclitaxel into first-line therapy for ovarian cancer improved the duration of progression-free survival, and of overall survival.[14] However, a major problem for the future will be whether the use of paclitaxel therapy will continue to improve the long-term survival of patients with ovarian cancer. Therefore, understanding and circumventing drug resistance is a major focus of ongoing research into ovarian cancer.

In general, mechanisms postulated to explain the development of resistance to chemotherapy may be divided into 2 types, on the basis of whether they involve host factors or cellular factors. Many of the normal functions of host factors may be restored by an alternation in drug administration. For ovarian cancer, as well as for many other solid tumors, cellular factors play a larger and more interesting role than host factors. Cell lines that display drug resistance are useful in investigations of the mechanisms underlying cellular factor resistance, and are eagerly sought after. The SMOV-1 cell line is unique in the sense that the potentiality of drug resistance arose spontaneously, unlike the usual drug-resistant cell lines, transformed or cloned from drug-sensitive cell lines.

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