Basic Research Paper

Establishment and biological characteristics of a human ovarian carcinoma cell line originated from peritoneal effusion

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Background and Objective: Establishing ovarian cancer cell lines will benefit researches on biological characteristics of ovarian cancer stem cells and antitumor drugs. This study was to establish a human ovarian carcinoma cell line from peritoneal effusion, and explore its biological characteristics. Methods: Cells were isolated from peritoneal effusion of an ovarian carcinoma patient, purified and cultured in vitro. The morphology of the cells was observed under electron microscope. The growth curve of cells was drawn to calculate cell doubling time (TD). Cell karyotype was analyzed. The levels of sex hormone, and the expression of CA125 and CA19-9 in cell culture supernate were detected by radioimmunoassay. The cells were transplanted into nude mice to observe tumor formation. Results: A new ovarian carcinoma cell line was established, which had been maintained in vitro for over 100 passages in two years. Abnormal nuclei were observed under electromicroscope. TD was 40.8 h. The karyotype of the cells was hyperdiploid with 63–120 chromosomes. The level of estradiol in cell culture supernate was 45.0 pg/mL; the level of testosterone was 0.03 ng/mL; pregnandione was undetected; the level of cells was 4.49 U/mL; the level of CA19-9 was 4.09 U/mL. Poorly differentiated ovarian adenocarcinoma was formed subcutaneously in nude mice after transplantation. Conclusion: The new ovarian carcinoma cell line is proved to be an immortalized and malignant cell line.

Among gynecological tumors, ovarian carcinoma is often accompanied with early lymph node metastasis to the pelvic and abdominal cavities, which finally leads to formation of peritoneal effusion. At present, it is assumed that the seeds of malignant tumor metastasis are cancer stem cells, which are closely related to metastasis and clinical drug resistance. In this study, peritoneal effusion was draw from the abdominal cavity of a patient with clinically advanced ovarian carcinoma (complicated with peritoneal effusion) after systematic chemotherapy, and cells were isolated from the peritoneal effusion, purified and consecutively passaged to establish a ovarian carcinoma cell line. Given its source and sampling features, this ovarian carcinoma cell line will provide a new experimental material for studies on drug resistance, metastasis and cancer stem cells of ovarian carcinoma.

Materials and Methods

Cell source, main instruments and reagents. Peritoneal effusion (100 mL) was drawn from an advanced ovarian carcinoma patient after systematic chemotherapy using paclitaxel combined with carboplatin. The ovarian carcinoma was confirmed as poorly differentiated adenocarcinoma by pathology. Main instruments, RPMI-1640 culture media and trypsin were purchased from Hyclone Company. MTT reagent was purchased from Sigma Company. Nude mice were purchased from Shanghai Laboratory Animal Center of Chinese Academy of Sciences [animal license No. SCXX(Hu)2002-0010]. Electron microscope (model TM100) was purchased from Hitachi Company (Japan).

Cell culture and cell line establishment. Cell deposit was collected from peritoneal effusion after centrifugation, re-suspended in RPMI-1640 culture media, added with separation solution, and centrifuged at 800 × g for 25 min. Then, the tumor cell layer was collected and washed with RPMI-1640 culture media, added with 100 U/mL penicillin, 100 μg/mL streptomycin, 2 × 10^{-4} mol/L ME and 15% fetal bovine serum at 37°C in 5% CO2 and under saturated humidity. Cells were digested with a mixture of 0.25% trypsin and 0.02% EDTA. After differential velocity adherent-wall purification, cells were passaged two to three times per week. Cells of different generations were stored in liquid nitrogen (-196°C) and analyzed for cellular biological characteristics.

Cellular morphologic observation. Growth status and manner of cells were observed under a phase-contrast microscope. When digested by trypsin-EDTA mix, cells were collected, centrifuged, then small cell gobbets were fixed in 2.5% glutaraldehyde and osmic acid, embedded in Epon812 and undergone ultra-thin
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section. Slices were stained and observed under an electron microscope.

Cell growth status examination with trypan blue staining. Cells (1 × 10^4/mL) were seeded into 24-well plates. Four wells of cells were stained by trypan blue every day to count live cells and draw cell growth curves. Slope b of regression equation was calculated from the curve and population-doubling time DT was calculated according to the formula DT = lg2/b.

Karyotype analysis of cells. After passage, cells were cultured for 48 h and added with Colchicine (1 ng/mL) for further 2-hour culture. Chromosomes were prepared for karyotype analysis according to the conventional method.

Tumor formation examination. Cells of the 51st generation were digested by trypsin. Single cell suspension (1.0 × 10^7/mL) was prepared in serum-free culture media, and subcutaneously injected into the axillae of 6-week old nude mice (0.25 mL per mouse). After the mice were fed under bacteria-free condition for 3–4 weeks, tumor masses were collected for pathologic examination with HE staining.

Detection of both related markers and hormones in cells by radioimmunoassay. Estradiol, testosterone and pregnendione were examined in cells of the 80th generation by radioimmunoassay. CA125 and CA19-9 were detected using chemo-luminescence method according to the reagent kit protocol.

Results

Process of cell line establishment. Various types of cells were present in primary culture of peritoneal effusion cells, mainly spindle cells. The cells grown in a non-clonal manner; some cells grown in the epithelium-like manner. After the primary culture cells were purified by the differential velocity adherent-wall method and passaged for four weeks, two epithelium-like cell clones appeared. The clones were further purified for amplification culture and were passaged more than 110 generations in two years.

Cellular morphology. Under an inverted phase-contrast microscope, ovarian carcinoma cells displayed clonogenic growth, epithelioid polygon in shape, and arrangement of rounded pebbles. When cultured in high density, cells merged in layers (Fig. 1). Round, oval-round or irregular cells, imbalanced ratio of nuclei to cytoplasm, clear nuclear membrane, obvious nucleolus, high nuclear atypia, side aggregation of some nuclei, and double nuclei were observed under a transmission electron microscope (Fig. 2).

Cell growth curve and doubling time. Cell growth reached a plateau phase when cultured for three days. The doubling time of cells was 40.8 h.

Karyotypes of cells. The karyotype of ovarian cancer cells was polyploidy with 63–120 chromosomes. The karyotype of most cells were hyperdiploid with abnormal structure, such as dicentromere (Fig. 3).

Tumor formation. Cells were injected subcutaneously into the axillae of four nude mice (2.5 × 10^6 cells/mouse). Palpable masses appeared in the inoculation sites two weeks after injection, and enlarged to diameters of 1-2 cm in the 3rd and 4th week. Histopathologic examination showed that the cells in the masses were poorly differentiated ovarian adenocarcinoma cells, which were similar to those in the original solid tumor (Fig. 4).
Detection of estradiol, testosterone, pregnendione, CA125 and CA19-9. In the culture supernatant of the 80th generation of ovarian cancer cells, the level of estradiol was 45.0 pg/mL, testosterone was 0.03 ng/mL, pregnendione was undetectable, the level of CA125 was 4.49 U/mL and CA19-9 was 4.09 U/mL.

Discussion

Ovarian carcinoma is one of the three common gynecological malignant tumors and its mortality is the leading one among gynecological tumors, which were mainly resulted from multiple organ failures solicited by peritoneal effusion and multiple-metastasis in the abdominal and pelvic cavities. In recent years, most ovarian carcinoma cells can be killed by platin-based chemotherapy combined with surgery, but the prognosis has not been obviously improved yet. Some researchers have suggested that a few cancer cells in tumor tissues, which possess potential of self renewing, proliferation and differentiation and serve as cancer stem cells, might be the origin of tumor recurrence, metastasis and chemoresistance.2,3 Present studies on cancer stem cells suggest that most solid tumors consist of both cancer stem cells and comparatively differentiated tumor cells, indicating cancer stem cells are seeds for establishing cell lines. Further studies indicate that cancer stem cells are cell seeds for tumor metastasis and possess natural characteristics of drug resistance,2-4 that is, chemotherapeutic drugs can inhibit proliferation of cancer cells but cannot kill cancer stem cells. Therefore, we think that the possibility of establishing ovarian carcinoma cell lines could be increased using samples from metastatic tissues after intervention of chemotherapeutic drugs, where high ratio of cancer stem cells may be obtained for cell culture. Most advanced ovarian carcinoma patients have peritoneal effusion and need to receive chemotherapy. Hence, peritoneal effusion formed from metastasis of advanced ovarian cancer and chemotherapy become two subtle clinical procedures for screening and concentrating cancer stem cells. First, peritoneal effusion indicates the presence of metastatic ovarian carcinoma cells-cancer stem cells. Second, most non-cancer stem cells in ovarian carcinomas are killed after chemotherapy while the proportion of cancer stem cells in the remaining cells would be increased.

It is not difficult to obtain clinical samples of malignant tumors such as ovarian carcinoma, but establishing an immortalized ovarian carcinoma cell line in vitro still is a long, complicated and difficult process which has been proved by many experiments. Based on the theory of cancer stem cells discussed above, we drawn peritoneal effusion from a clinically advanced ovarian cancer patient with peritoneal effusion after chemotherapy for cell culture of ovarian cancer cells. We successfully established the cell line by only sampling once. Except accidental factors, both sample choosing and timing for cell culture are important success factors.

At present, this ovarian cancer cell line possesses important features of immortalized cell lines. The features include consecutive passages of more than 110 generations in about two years, hyperdiploid karyotype, high capability of tumor formation named GXOV-01, certain endocrine function of estradiol and testosterone, and so on. Pregendione is undetectable in the cells after passaged for 80 generations, possible due to long-term in vitro culture conditions which were unfavorable for maintaining its endocrine function. Both expression and secretion of malignant epithelial tumor markers, such as CA125 and CA19-9, are detectable in culture supernatant, indicating the cell line owns biological characteristics of malignant tumor cells and is a novel ovarian carcinoma cell line. Based on the specific background of establishing this ovarian carcinoma cell line, it may be useful for investigating the biological characteristics of ovarian carcinoma stem cells, anti-cancer drug screening, drug resistance in ovarian carcinoma and metastasis.

References