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CHARACTERIZATION OF NEWLY ESTABLISHED CELL LINE HUOA FROM A HUMAN OVARIAN SEROUS CYSTADENOCARCINOMA

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Synopsis We have recently established a cell line, HUOA, of human ovarian serous cystadenocarcinoma. The cultured cells are various shapes ; cylindrical, spindle-shaped, roundish, or polygonal. They are rich in cytoplasm and PAS-positive. They proliferate to form multi-layers without contact inhibition, and they easily form mirror ball-like cysts. These cysts free themselves to float in the culture medium. Cell growth is comparatively slow, and the population doubling time is about 80 hours. The plating efficiency is 16%. The cell line has been cultured for 5 years with more than 100 successful passages and is still proliferating. The HUOA line can be transplanted subcutaneously into nude mice and forms a serous cystadenocarcinoma similar to the original tumor, but when transplanted into the intraperitoneal cavity, the HUOA line forms floating cysts similar to those in vitro. We regard this formation of cyst as an expression of differentiation of the cystadenocarcinoma cells.

Key words: Cell line • Ovary • Cyst formation

Introduction

Several cell lines of ovarian adenocarcinoma have been established[2)5)6)8)10)~12)], and the HUOA line that we established has several similar characteristics to the ovarian adenocarcinoma ; however, the HUOA line differs in that mirror ball-like cysts easily form in vitro as well as in vivo. A mirror ball-like cyst is made by a single layer of cell walls. Therefore, we are going to report on the course of establishment of the cell line, especially on the mechanism of formation of mirror ball-like cysts, and the cytobiological properties of this line.

Materials and Methods

1. Materials

A 60-year-old Japanese woman underwent hysterectomy and bilateral salpingooophorectomy on February 20, 1981. Tumors existed in both ovaries, and the tumors were cultured. The tumors were stained by hematoxylin and eosin (HE), periodic acid-Schiff (PAS), mucicarmine and alcian blue.

2. Culture techniques and culture media

The tumor tissues were rinsed twice with culture medium supplemented with antibiotics and minced with a sharp pair of scissors in growth medium. The small fragments were dissociated with dispase [600 Pronase Unit (PU)/ml : Gohdo-Shusei Co., Tokyo] for 30 minutes at 37℃. Then the dissociated cells were centrifuged at 800g for 10 minutes. The sediments were resuspended in Ham's F-12 growth medium (Grand Island Biological Co., N.Y.) supplemented with 15% fetal calf serum (Flow Labotories, Md.), and 4×10^5 viable cells were incubated at 37℃ in a humidified atmosphere containing 5% CO_2. The cells were transferred to a 1 : 2 dilution, using 600 PU dispase/ml.

3. Growth characteristics

The growth characteristics such as growth curve (GC), population doubling time (DT), saturation density (SD) and plating efficiency (PE) were examined by previously reported techniques[6)].

4. Morphology

The HUOA cells were fixed with 95% ethanol solution and stained with Papanicolaou stain. The cells were also fixed with 10% formalin solution and stained with PAS, mucicarmine, or alcian blue. For transmission electron microscopy, the cells were fixed with 2.5% glutaraldehide in 0.1M phosphate buffer, pH 7.3 for 30 minutes and post-fixed with 1% OsO_4 in 0.1M Millonig's buffer at pH 7.3

for 15 minutes. The cells were dehydrated with graded ethanol, and embedded in Epon-Araldite resin. Polymerization of the resin was done at 40℃ for 1 week. The ultrathin sections were stained with lead citrate and uranyl acetate and then observed with JEOL 100B electron microscopy.

5. Chromosome analysis

The distribution of the chromosome number and G-band karyotypes were studied by previously reported techniques[6].

6. Heterotransplantation

1×10^5, 1×10^6, 1×10^7, and 1×10^8 cells were transplanted subcutaneously in 3 BALB/c 6 week old nude mice (Japan clea Co., Tokyo), and the culture grafts were examined 8 weeks after transplantation. 1×10^7 cells were also transplanted into the intraperitoneal cavity of nude mice, and the tumors were examined 4 weeks after transplantation.

7. Tumor marker production

Carbohydrate-antigen (CA-125), Tissue polypeptide antigen (TPA), alpha-fetoprotein (AFP), carcino-embryonic antigen (CEA), tumor antigen-4 (TA-4), ferritin and β_2-microglobulin were examined. 5×10^5 cells were cultured in serum-free Ham's F-12 medium for 2 days, and the amounts of the tumor markers in the conditioned media were measured by RIA.

8. Hormone receptor assay

Estradiol-17β and progesterone receptors were assayed by the method of Horwitz and McGuire[3] with slight modification as reported previously[4].

Results

1. Pathology of culture materials

The original tumor was diagnosed as well differentiated papillary serous cystadenocarcinoma (Fig. 1). The cytoplasms strongly stained with PAS, weekly stained with alcian blue, and was negative with mucicarmine. Mirror ball-like cysts were not detected.

2. Establishment of cell line and growth characteristics

Initially, both epithelial colonies and fibroblastic cells were present, but the epithelial colony grew more rapidly in the primary cultures. Pure epithelial cells were isolated from the primary cultures by the colony isolation technique (Okumura's method)[7]. Then, the HUOA (human Umino Ovarian adenocarcinoma cell) line was established which grew for more than 5 years with more than 100 successful passages and still showing stable growth. The growth characteristics such as GC, SD and PE were examined at passages 5, 20, 40 and 100 as shown in Table 1. Average DT is about 80 hours and PE is about 16%.

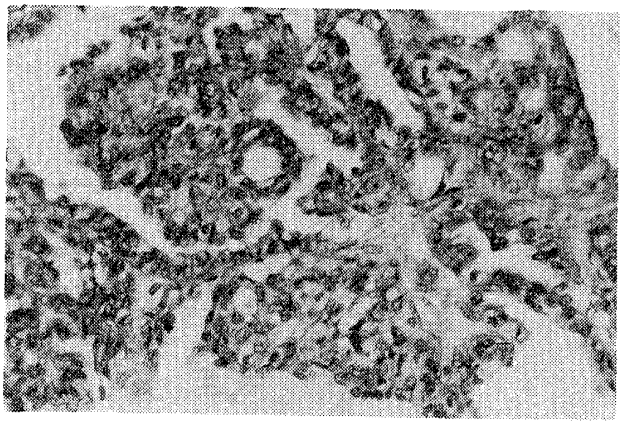

Fig. 1. The original tumor (culture material) was interpreted as well differentiated serous cystadenocarcinoma. HE stain, ×150.

Table 1. Growth characteristics of HUOA cell line

PN	DT (hr)	SD (10^4/cm^2)	PE(%)
5	87	3.35	18
20	80	3.42	16
40	78	3.42	17
100	77	3.55	20

PN=passage number, DT=population doubling time, SD=saturation density, PE=plating efficiency; 500 single suspended cell were placed into 6 cm plastic dishes and cultured for 10 days. PE was determined by the ratio of the number of colonies to the total number of inoculated cells.

3. Morphology of cultured cells

The cultured cells were various shapes (Fig. 2a), such as cylindrical, spindle-shaped, roundish, or polygonal and rich in cytoplasm which was strongly positive for PAS, weekly positive for alcian blue and negative for mucicarmine. The PAS positive substance was digested with diastase. The cultured cells appeared as a pavement or jigsaw puzzle-like arrangement and grew in multilayers without contact inhibition. The cells formed mirror ball like-cysts which were usually found floating in the culture medium (Fig. 2b). Initially, parts of the monolayer cell sheet would swell to form domes

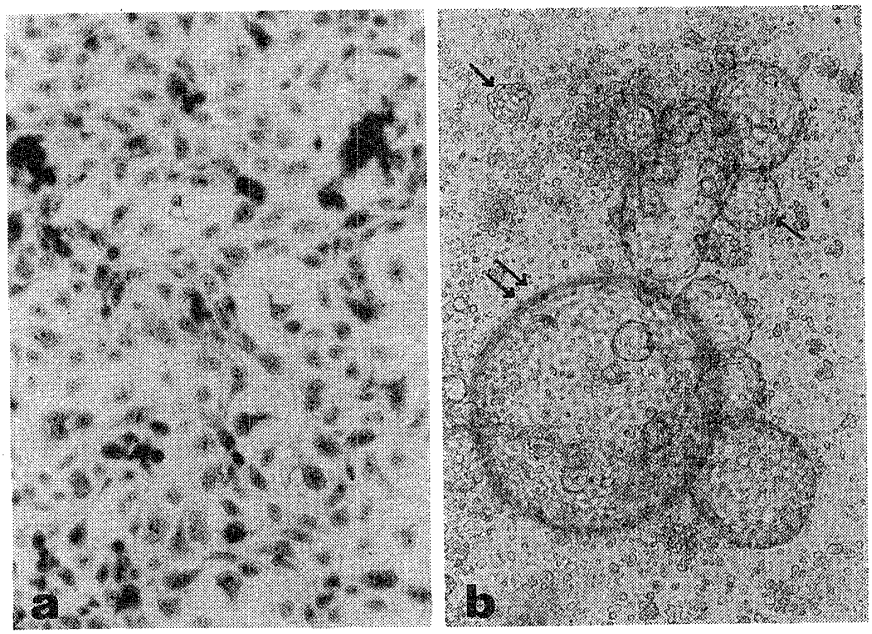

Fig. 2a. The cultured cells (passage 10) were cylindrical, spindle, polygonal in shape and nuclei showed neoplastic and pleomorphic features. Papanicolaou stain, ×150. 2b. The cells formed hemi-cyst (single arrow) and mirror ball-like cyst (double arrow). Phase contrast microscopy, ×100.

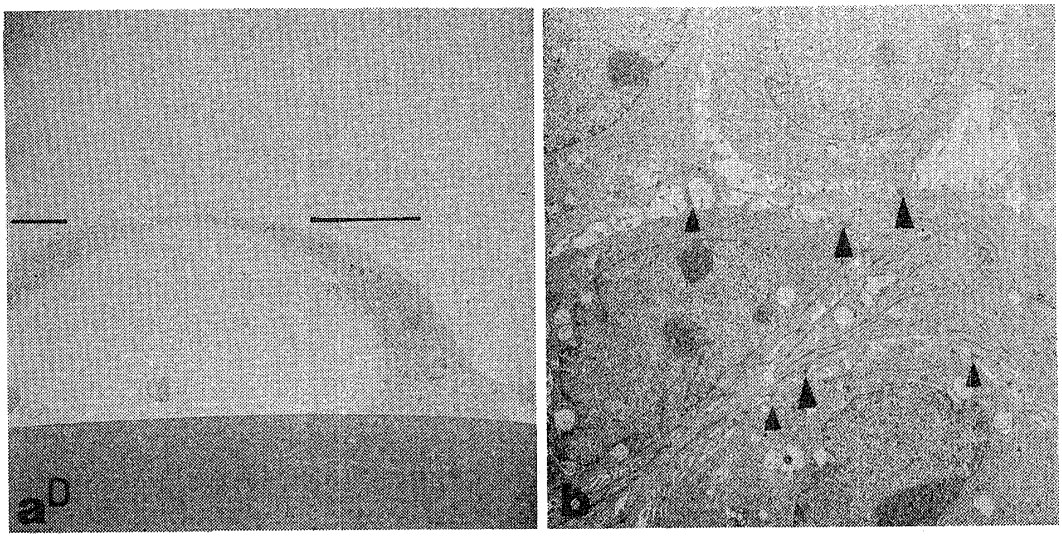

Fig. 3a. The monolayer cell sheet swelled in domes. Microvilli protruded into the dome. D indicates the bottom of plastic dish. ×2,000. 3b. The section tangenital to the surface of the dome (line of Fig. 3a). The adjacent cells were united each other by desmosomes (arrow capital) and formed dome. Transmission electron microscopy, ×3,000.

(Fig. 3a), and then cells along the margin migrated to the center of the domes to form a monolayer cell sheet along the bottom of the domes, and these domes became the mirror ball-like cysts. The cells contained well developed Golgi apparati and many vesicles, and contacted each other by desmosomes (Fig. 3b). Also microbilli were found protruding into the cavity.

4. Chromosomal analysis

Chromosomal analysis was done at passages 5, 20, 40 and 100. The chromosomal number ranged from 75 to 108, and the modal number was stable at

the hyper-tetraploid (92~95) range (Fig. 4). G-band karyotypes are shown in Fig. 5. The abnormal chromosomes (3p−, 4q+, 6q−, 7p+) and the marker chromosomes were present in every cell.

5. Heterotransplantation

Cells numbering more than 1×10^7 produced tumors in the subcutis and in the intraperitoneal cavity of nude mice, while cells less than 1×10^6 did not. The grafts were interpreted as well differentiated serous cystadenocarcinoma (Fig. 6a). The hemi-cysts and mirror ball-like cysts were observed at the surface of the abdominal tumors (Fig. 6b). The cytoplasm and the lumen were PAS positive.

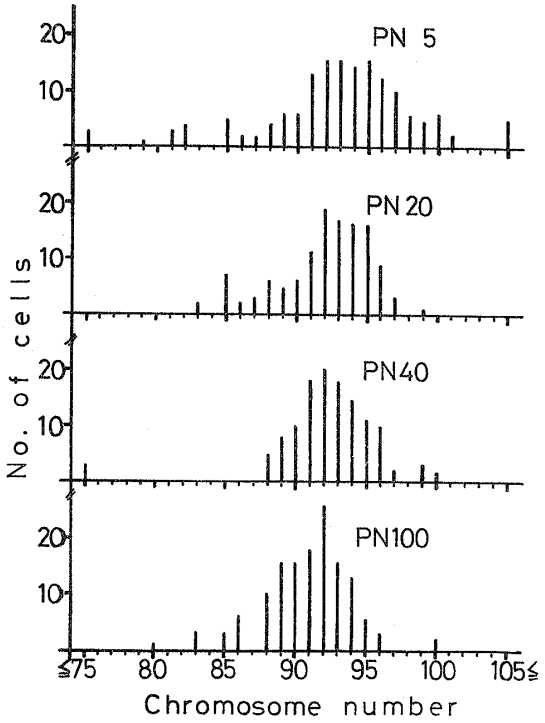

Fig. 4. Distribution of chromosome number of HUOA line. The chromosomal number was widely distributed, and the modal number was stable at the 82~92 range.

6. Tumor marker production

CA-125 (52 U/ml), TPA (3,200 U/ml), HCG (5.6 ng/ml), ferritin (9.6ng/ml) were detected in the conditioned medium, which AFP, CEA, TA-4 and β_2-microglobulin were not detected.

7. Hormone receptor assay

Estradiol-17β and progesterone receptors were not detected in the cultured cells.

Discussion

Several cell lines of ovarian adenocarcinoma had been established[2)5)6)8)10)~12)]. These cell lines were; 1) polygonal or spindled shaped, 2) PAS-positive, 3) epithelial-like, 4) forming adenocarcinoma in nude mice, 5) many cells produced CA-125, 6) used in preparation of monoclonal antibody, such as OC125[1)], 7) used to judge the effectiveness of anticarcinoma agents[6)], etc. However, formation of mirror ball-like cysts was not included among the general properties of the cell lines, except those of ovarian clear cell adenocarcinoma cell line HAC2[9)] and ovarian adenoacanthoma cell line HMOA[5)], in which the mirror ball-like cysts were

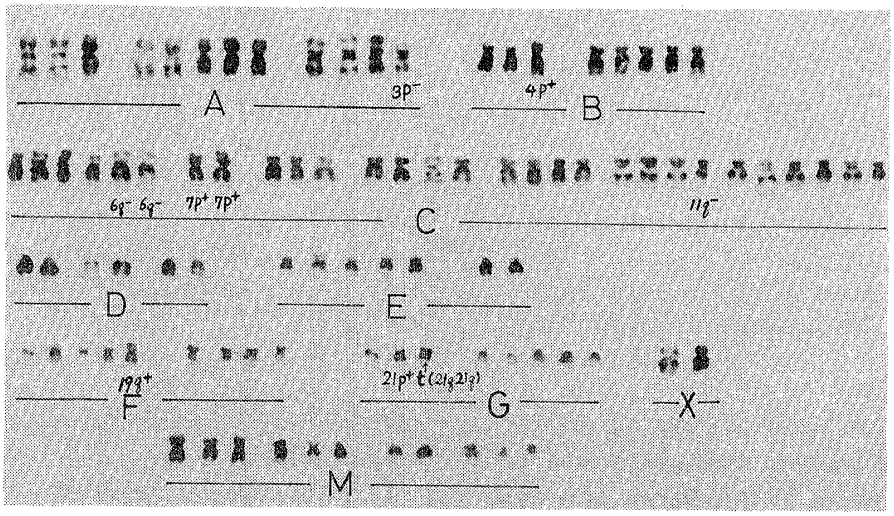

Fig. 5. G-band karyotype of HUOA (passage 10). The abnormal constructions (3p−, 4q+, 6q−, 7p+) and the marker chromosomes were present in 100% of cells. Giemsa stain, ×1,000.

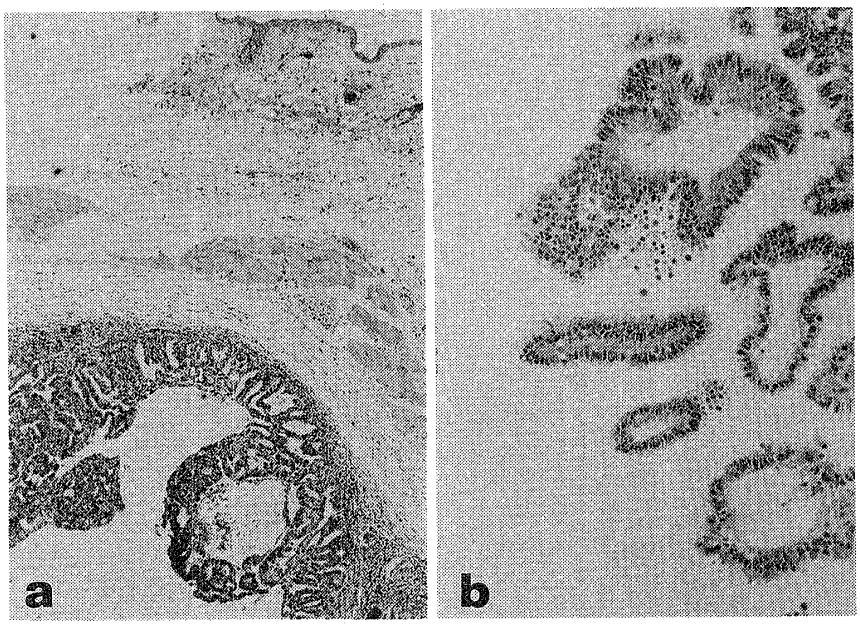

Fig. 6a. The histology of a tumor formed by HUOA cells from the 10th passage and interpreted as well differentiated serous cystadenocarcinoma. 6b. The intraperitoneal tumor was interpreted as well differentiated serous cystadenocarcinoma. Hemi-cyst and mirror ball-like cyst were observed on the surface of the tumor. HE stain, ×100.

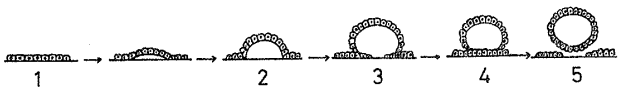

Fig. 7. The schematics of histogenesis of mirror ball-like cyst. (1) monolayer cell sheet, (2) dome or hemi-cyst, (3) migration of cells from the periphery to center, (4) mirror ball-like cyst, (5) liberation of cyst.

formed after confluence. But the HUOA cells easily form mirror balls before confluence, as their unique features. Therefore, we examined the formation of mirror ball-like cysts in experimental systems *in vitro* as well as *in vivo*. It was found *in vitro* that a part of the monolayer cell sheet would swell in domes, when the domes were filled with PAS-positive substance. This substance was digested by diastase. These phenomena could be explained by the production of glycogen by the cells and rising of pressure inside the domes. Then cells from the margin proliferated and migrated to form a monolayer sheet of cells at the bottom of the domes which then formed mirror balls. These balls were liberated into the culture solution (Fig. 7). Similar formation of mirror balls was observed on the surface of tumors formed by transplantation of the cells into the intraperitoneal cavity of nude mouse. Successive stages of a bulging sheet of cells, hemi-cyst, and mirror balls on the tumor surface were observed and a large amount of PAS-positive substance was contained in the domes.

We would like to consider the mirror ball formation as a differentiation of the adenocarcinoma cells or adenocarcinoma tissue. This is supported by the facts that 1. the mirror balls resemble glandular cavities and many microvilli are found on the surface of cells forming cavity of cyst, especially on the inner surface, 2. Golgi apparatus is developed on the cells and many secretion granules are found, and PAS-positive substance are found in the cytoplasm and cavity, and 3. these findings are the same as those found in differentiation of glandular cells.

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概要 ヒト卵巣漿液性嚢胞腺癌細胞株（HUOA line）を60歳日本婦人の卵巣癌手術材料より新たに樹立した．培養細胞は円柱形，紡錘形，類円形，多菱形と多彩で，細胞質は比較的豊富でPAS強陽性である．接触阻止が見られず容易に多層性増殖を示すが，最も特徴的なことは，cyst（mirror ball＝MB）を容易に形成し培養液中に浮游してくることである．そこで，MBの形成機序をin vitroとin vivoの実験系で検討した．in vitroではmonolayer cell sheetの一部がドーム状に盛上る．この時，空洞内はPAS陽性物質で充満されている．この現象は細胞がPAS陽性物質を産生分泌し内腔の圧が高まつてドーム状に盛上がるものと解釈される．また内腔面には多数のmicrovilliが見られる．さらにドームの底面のplastic dish上でドームの辺縁より細胞が増殖migrateして細胞のmonolayer sheetを形成し，MBを形成して培養液中に浮游してくるものと考えられる．株細胞の増殖は比較的緩やかで，倍加時間は約80時間である．またコロニー形成率は16%である．培養開始後，5年になるが，今日まで100回以上の継代に成功し，今も安定した増殖をしている．HUOA lineはヌードマウス皮下への移植が可能で元腫瘍類似の漿液性嚢胞腺癌を形成した．またヌードマウスの腹腔に移植するとin vitroの場合と同様にcystを形成し腹腔に遊離してきた．このcystの形成は腺癌細胞の分化の表現と考えたい．