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Characterization of a Novel Cell Line (HCH-3) Derived from a Human Ovarian Clear Cell Carcinoma

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Abstract

Objective: Established cell lines are important materials helping a medical basic research. The reports listed in detail of the cell line derived from an ovarian clear cell carcinoma were only 14, so far. Owing to little information, the establishment of malignant tumor cell line with individual characteristics is especially significant to research this disorder. Therefore, this study was carried out to establish and characterize a new human cell line derived from ovarian clear cell carcinoma.

Methods: The cell line HCH-3 was established from a left ovarian tumor of a 41-year-old woman. Characters of the cell line investigated included morphology, chromosome analysis, heterotransplantation, tumor markers, chemosensitivity, and cancer genes.

Results: This cell line has been growing well for 206 months and subcultured more than 50 times. Monolayer cultured cells were multipolar in shape, showing a cobble stone appearance and a tendency of multilayering without contact inhibition. They showed a human karyotype with a modal chromosomal number in the hypotetraploid range. The cells could be transplanted into the subcutis of SCID mice and made tumors looking like the original tumor. HCH-3 cells demonstrated both CA 125 and CA19-9 which were detected immunohistochemically in the original tumor and the heterotransplanted tumor. The cells were not sensitive to agents generally managed in the treatment of gynecological cancers by MTT assay. KRAS and TP53 mutations were discovered in hotspot locations of 50 cancer genes.

Conclusion: HCH-3 is an ovarian clear cell carcinoma cell line in which CA 125 and CA19-9 expression have been clarified. Mutations were discovered in KRAS and TP53 genes. This newly established cell line may be helpful in basic study on ovarian clear cell carcinoma, the etiology of which is not yet entirely recognized.

Keywords: Ovarian clear cell carcinoma; Cell line; MTT assay; CA125; CA19-9; Hot spot; Point mutation

Introduction

Ovarian clear cell carcinoma is a variant of epithelial ovarian cancer and has a poor outcome for women with advanced stage [1,2]. Late reviews recommend that most clear cell carcinomas are related with endometriosis, and endometriosis-associated ovarian cancer tends to occur in younger women, 5-6 years earlier than high-grade serous adenocarcinoma [3,4]. And so, it was thought important to use an ovarian clear cell carcinoma cell line for clinical and basic study of this disease. We report here the establishment and characterization of a new human cell line (HCH-3) of ovarian clear cell carcinoma that expresses both CA 125 and CA 19-9 and has point mutations in hotspots of KRAS and TP53.

Materials and Methods

We performed an abdominal simple total hysterectomy, bilateral salpingo-oophorectomy, and omentectomy on a 41-year-old woman with left ovarian cancer International Federation of Gynecology and Obstetrics (FIGO) stage Ic 17 years ago. The patient gave informed consent for act of this research. Intraoperative diagnosis of frozen section was an ovarian adenocarcinoma. Measurement of preoperative serum tumor marker levels showed that CA 125 was 1.417 U/ml (normal level <35 U/ml), CA 19-9 was 181 U/ml (<37 U/ml), and carcinoembryonic antigen (CEA) was 0.5 ng/ml (<2.5 ng/ml). She was dealt with twice with 100 mg cisplatin (CDDP) and 400 mg etoposide (VP-16) intraperitoneally, and 5 times with 450 mg carboplatin (CBDCA), 60 mg pirarubicin hydrochloride (THP), and 400 mg cyclophosphamide (CPA) intravenously. Her clinical condition was good after surgery and chemotherapy for at least 5 years.

Culture techniques and media: Tissue section of ovarian tumor was finely minced with a couple of sharp edges in a dish including sans serum Ham's F-12 medium (Flow Laboratories Inc., McLean, VA, USA), mixed gradually with an attractive stirrer in a 0.25% trypsin arrangement (Flow Laboratories Inc.), centrifuged at 70 g for 5 min, and put in culture medium at 37°C in a humidified chamber with 5% CO_2 and 95% air. Cells were refined in Ham's F-12 medium in addition to 20% precolostrum infant calf serum (Mitsubishi Chemical Industries Ltd., Tokyo, Japan) with kanamycin. At that point, subcultures were passaged with 0.1% trypsin and 0.02% ethylenediamine-tetraacetic acid (EDTA) arrangement like clockwork. Six months after the essential culture, the centralization of precolostrum infant calf serum in the way of life medium was lessened from 20% to 10%.

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Morphology of the original tumor and cultured cells: Living cells developed in culture flagons were seen with a stage differentiate magnifying instrument. For histological conclusion, the first tumor was fixed in 10% formalin, implanted in paraffin, and cut into 4 µm-segments which were recolored with hematoxylin-eosin (HE) and occasional periodic acid Schiff (PAS) stains. Monolayer refined cells on slides were fixed in 90% ethanol and recolored by Papanicolaou's technique [5].

Growth characteristics: Qualities of cells were inspected in passages 8-10. Suspensions of 1×10^5 cells were plated in 35 mm plastic dishes and hatched for 15 days. Next, the quantity of cells from two dishes was measured every other day utilizing a programmed cell counter (Coulter CounterR, Coulter Electronics, Luton, England). The population doubling time and saturation density were resolved from the development bend. For examinations of plating efficiency, 1×10^2 and 2×10^2 single-suspension cells were set into five 60 mm plastic dishes each and refined for 21 days. Plating efficiency was computed by the proportion of the quantity of colonies (more than 10 cells) to the aggregate number of placed cells. For the mitotic record, monolayer cells were refined for 5 days and responded with 1×10^{-7} M colcemid (Demecolcine Solution, Wako Pure Chemical Industries, Osaka, Japan) for 4 h, put in a 0.2% KCl solution for 15 min, and afterward fixed well-ordered in a methanol: acidic corrosive arrangement (3:1). After air-drying, the cells were recolored with Giemsa, and the quantity of mitotic cells in 1,000 cells were measured.

Chromosome analysis: Histograms of chromosome number distribution were decided using 50 metaphase plates. Their karyotypes were analyzed in 10 cells according to the International System for Human Cytogenetic Nomenclature.

Heterotransplantation: Twelve million cells (passage 9) were infused subcutaneously into the dorsal district of 5-week-old SCID mice (SCID/Os, Shionogi, Osaka, Japan). At the point when the tumors had developed to 5-10 mm in distance across following a month, they were resected and treated for morphological reviews. For histology, the expelled tumors were fixed in 10% formalin, inserted in paraffin, and recolored with HE and PAS. For electron microscopy, the piece of the first tumor was fixed by plunging in a blend of 1.25% glutaraldehyde and 1% paraformaldehyde supported with phosphate buffered saline (PBS), pH 7.4, at 4ºC for 3 h. In the wake of washing with PBS, the tumor was post fixed with 1% osmium tetroxide at 4ºC for 1 h, then washed in PBS, dehydrated in evaluated convergences of ethanol, and installed in Epon 812. Areas 0.5 µm thick were cut with a 6000 ultramicrotome (Sorvall, Du Pont, CT, USA) and recolored with toluidine blue. Ultrathin segments showing light gold obstruction shading were cut from the comparing territories in the toluidine blue-recolored areas, twofold recolored with uranyl acetic acid derivation and lead citrate, and saw under a JEM-100SX electron magnifying instrument (JEOL, Tokyo, Japan) at 80kV [6].

Tumor markers: Medium in which 2×10^6 cells/5 ml were cultured for a week was examined for α fetoprotein (AFP), CA 125, CA 19-9, CA 72-4, CEA, human chorionic gonadotropin (HCG), squamous cell carcinoma (SCC) antigen, and tissue polypeptide antigen (TPA) by radioimmunoassay or chemiluminescent immunoassay.

Immunohistochemical stainings: Deparaffinized 4-µm segments of the first and heterotransplanted tumors on glass slides were recolored immunohistochemically utilizing the universal Immuno-enzyme Polymer (UIP) technique (Envision pack; DAKO, Glostrup, Denmark). Slides were plunged in 0.03% hydrogen peroxidase and absolute methanol to square endogenous peroxidase, washed with PBS, and after that warmed in an autoclave for 20 min. In the wake of cooling, the slides were brooded with essential counter acting agent at room temperature for 40 min and after that treated with Envision (DAKO) at room temperature for 30 min, trailed by hatching with diaminobenzidine (DAB) for 5-10 min. Antibodies against CA 125 (DAKO) and CA 19-9 (DAKO) were utilized to distinguish tumor markers, and antibodies against human estrogen receptor α (Nichirei Bioscience Inc., Tokyo, Japan) and human progesterone receptor (Nichirei) were utilized to identify hormone receptors. Antibodies against Hepatocyte nuclear factor-1β (HNF-1β) [7] (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and Annexin IV [8] (Santa Cruz Biotech), which are occasionally communicated in ovarian clear cell carcinoma, were likewise treated.

Chemosensitivity assays: The impacts of actinomycin D (ACD, MSD KK, Tokyo, Japan), doxorubicin (ADM, Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan), 5-fluorouracil (5-FU, Kyowa Hakko Kirin Co.), mitomycin C (MMC, Kyowa Hakko Kirin Co.), CBDCA (Bristol-Myers K.K., Tokyo, Japan), CDDP (Bristol-Myers), VP-16 (Bristol-Myers), paclitaxel (PTX, Bristol-Myers) [9], and irinotecan SN-38 (CPT-11, Yakult Honsha Co., Ltd., Tokyo, Japan) [10], which are regularly figured out how to treat gynecological malignancies [11], on the refined cells were inspected by 3-(4,5-dimethyl-2-thiazolyl)- 2,5-diphenyl-2H tetrazolium bromide (MTT) assay. The agents were fused in culture medium and utilized immediately. For MTT assay, 5×10^3 cells in 50 µl medium were seeded in octaplicate into each well of 96-well microwell plates. For persistent agent exposure studies, different weakened agents in 50 µl were put in after 48 h of brooding. The wells were hatched for 72 h after the option of agents, after which MTT (50 µl of 2 mg/ml; Wako Pure Chemical Industries, Ltd, Osaka, Japan) was added to each well, and the plates were brooded for 4 h more. The medium was then disposed of, 150 µl of dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO, USA) was added to each well, and the plates were shaken for 5 min. The optical density was then measured at 570 nm on a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA), and the viable grouping of the middle lethal dosage was resolved from the measurements reaction bend (EC50: measurements of agent required to reduction last cell number or optical density in MTT assay to half of control) [12].

Mutational analysis

DNA was separated from living cells utilizing DNA extraction Kit (PureLinkTM Genomic DNA Mini Kit, Invitrogen, Life Technologies, Carlsbad, CA, USA). Somatic mutations (substitutions, insertions or deletions) were judged utilizing the Ion AmpliSeqTM Cancer Hotspot Panel v2, intended to increase 207 amplicons covering ~2800 COSMIC transformations from the 50 most typically educated oncogenes and tumor suppressor genes (Ion Torrent, Life Technologies) (Table 1) [13,14].

Results

Histopathology of the original tumor: Light microscopy showed the original tumor to be a clear cell carcinoma, cells of which had clear cytoplasm with large nuclei and prominent nucleolei, and a partial hobnail appearance (Figure 1).

Establishment of the cell line: Tissue pieces from the original tumor were cultured and after a 2-month stationary period, certain out-growths developed. Primarily, mixture of spindle-shaped fibroblasts and epithelial cells was observed, but fibroblasts faded from the cultures upon passaging the cells, which were named HCH-3. The HCH-3 cells

UGO Gene Symbol	Description	Gene ID
ABL1	ABL proto-oncogene 1, non-receptor tyrosine kinase	25
AKT1	v-akt murine thymoma viral oncogene homolog 1	207
ALK	anaplastic lymphoma receptor tyrosine kinase	238
APC	adenomatous polyposis coli	324
ATM	ataxia telangiectasia mutated	472
BRAF	v-raf murine sarcoma viral oncogene homolog B	673
CDH1	cadherin 1, type 1, E-cadherin (epithelial)	999
CDKN2A	cyclin-dependent kinase inhibitor 2A	1029
CSF1R	colony stimulating factor 1 receptor	1436
CTNNB1	catenin (cadherin-associated protein), beta 1	1499
EGFR	epidermal growth factor receptor	1956
ERBB2	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2	2064
ERBB4	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 4	2066
EZH2	enhancer of zeste homolog 2	2146
FBXW7	F-box and WD repeat domain containing 7, E3 ubiquitin protein ligase	55294
FGFR1	fibroblast growth factor receptor 1	2260
FGFR2	fibroblast growth factor receptor 2	2263
FGFR3	fibroblast growth factor receptor 3	2261
FLT3	fms-related tyrosine kinase 3	2322
GNA11	guanine nucleotide binding protein (G protein), alpha 11 (Gq class)	2767
GNAQ	guanine nucleotide binding protein (G protein), q polypeptide	2776
GNAS	GNAS complex locus	2778
HNF1A	HNF1 homeobox A	6927
HRAS	Harvey rat sarcoma viral oncogene homolog	3265
IDH1	isocitrate dehydrogenase 1 (NADP+)	3417
IDH2	isocitrate dehydrogenase 2 (NADP+), mitochondrial	3418
JAK2	Janus kinase 2	3717
JAK3	Janus kinase 3	3718
KDR	kinase insert domain receptor (a type III receptor tyrosine kinase)	3791
KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	3815
KRAS	Kirsten rat sarcoma viral oncogene homolog	3845
MET	met proto-oncogene	4233
MLH1	mutL homolog 1	4292
MPL	myeloproliferative leukemia virus oncogene	4352
NOTCH1	Notch 1	4851
NPM1	nucleophosmin (nucleolar phosphoprotein B23, numatrin)	4869
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog	4893
PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	5156
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha	5290
PTEN	phosphatase and tensin homolog	5728
PTPN11	protein tyrosine phosphatase, non-receptor type 11	5781
RB1	retinoblastoma 1	5925
RET	ret proto-oncogene	5979
SMAD4	SMAD family member 4	4089
SMARCB1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1	6598
SMO	smoothened, frizzled class receptor	6608
SRC	v-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog	6714
STK11	serine/threonine kinase 11	6794
TP53	tumor protein p53	7157
VHL	von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase	7428

Table 1: List of the 50 genes targeted by the Ion AmpliSeqTM Cancer Hotspot Panel v2 (Ion Torrent).

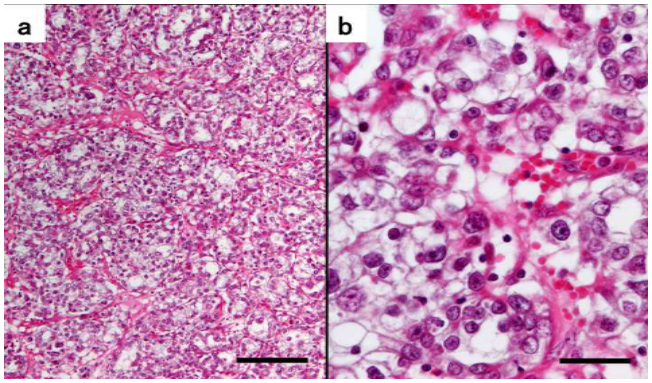

Figure 1: Histology of the original tumor. The left ovarian tumor is a clear cell carcinoma, with cells harboring clear cytoplasm and a partial hobnail appearance, as shown by hematoxylin and eosin (HE) staining. (a, bar=200 μm; b, bar=50 μm).

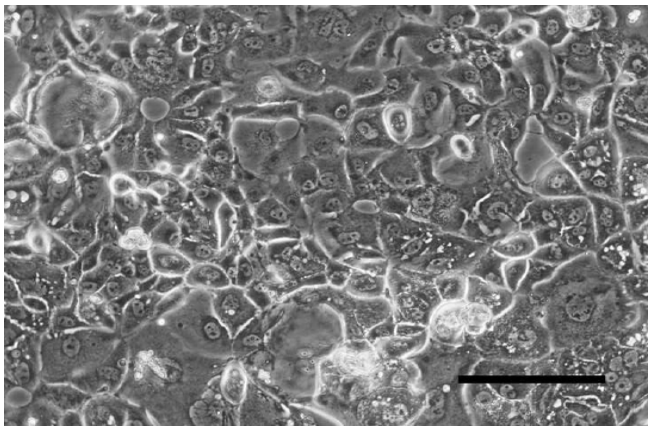

Figure 2: Phase contrast microscopy. The cells show a cobble stone appearance with multilayering. (bar=200 μm).

grew well, without contact inhibition, for more than 206 months, and more than 50 serial passages were successively performed. They continue to demonstrate constant growth.

Morphology of the cultured cells: The cultured cells grew in monolayer and looked to be epithelial, showing a cobble stone appearance. Multilayering of cells was definitely noted even after they reached confluency (Figure 2). The cells were multilateral and showed neoplastic structures such as unusual aggregation of chromatin granules, thickened nuclear membrane, and multiple prominent nucleoli. Multinucleated giant cells were also observed (Figure 3).

Growth Characteristics: The growth curve was analyzed in passage 8 of the HCH-3 cell line. Three days after culturing, the cells increased logarithmically (Figure 4). The population doubling time, saturation density, plating efficiency, and mitotic index were 82 h, 9.7×10^4 cells/cm^2, 0.8%, and 10.2%, respectively.

Chromosome Analysis: This cell line showed a modal chromosome number that was in the hypotetraploid range (78-87) (Figure 5). Chromosomal analysis revealed the following abnormalities; 80-86<4n>, XXXX, del (X) (p21) [7], -2[8], -3[4], -4[9], add (4) (q31) [5], -5[3], -6[10], -7[4], I (8) (q10) [10], -9[7], add (9) (p11) [3], -10[4], -11[3], -12[8], add (12) (p11) [2], -13[10], -13[8], add (13) (p11) [9], -14[10], -14[4], -15[4], -16[4], -17[3], -18[9],-19[7], -21[9], -21[3], +4-7mar (Figure 6).

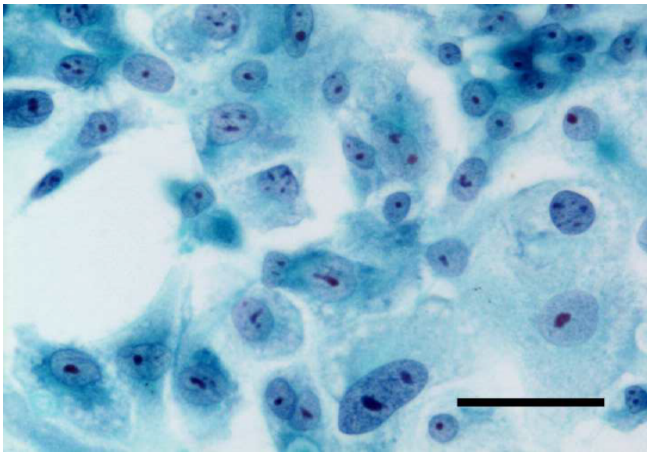

Figure 3: Cytopathology of cultured HCH-3 cells. Multinucleated giant cells with polygonal cytoplasm are seen by Papanicolaou stain. (bar=50 μm).

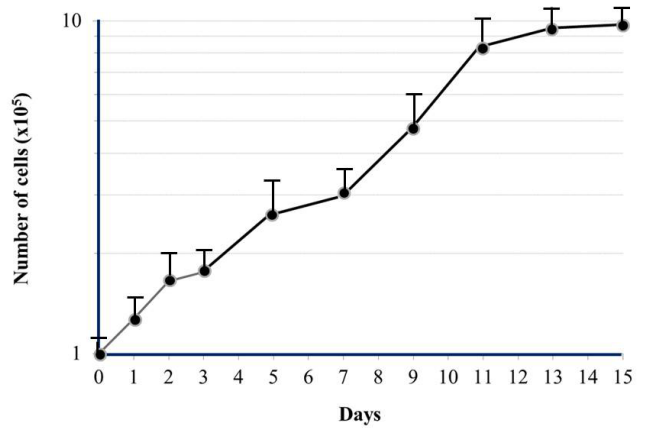

Figure 4: Growth curve of HCH-3. The cells increased logarithmically.

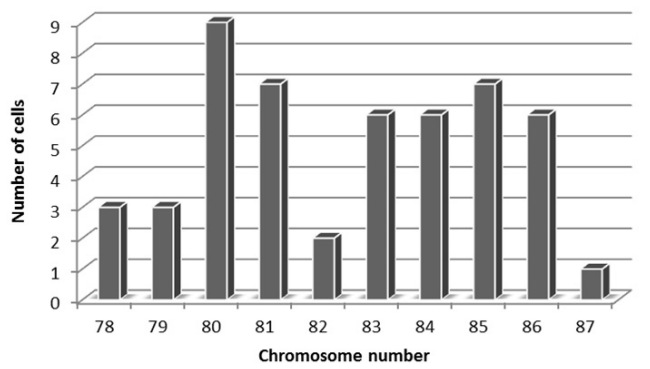

Figure 5: Distribution of chromosomal numbers in HCH-3 (8th generation). The modal number is in the hypotetraploid range.

Heterotransplantation: Histologically, the transplanted tumors were clear cell carcinomas, i.e. with cells showing clear cytoplasm, which strongly looked like the original tumor (Figure 7). They were also positive for PAS stain. Electron microscopy showed that neighboring cells had desmosome-like junctions. The cells had notched nuclei, rich mitochondria in the cytoplasm, and many microvilli on the cell surface (Figure 8). These characters suggested that the cells were epithelial in origin.

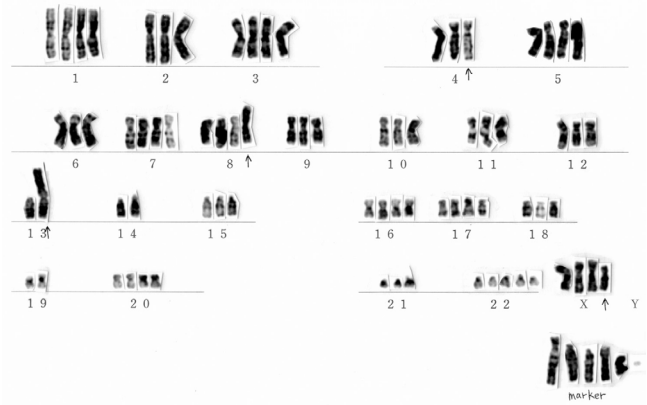

Figure 6: Karyotype of HCH-3 (8th generation). Chromosomal analysis revealed various abnormalities.

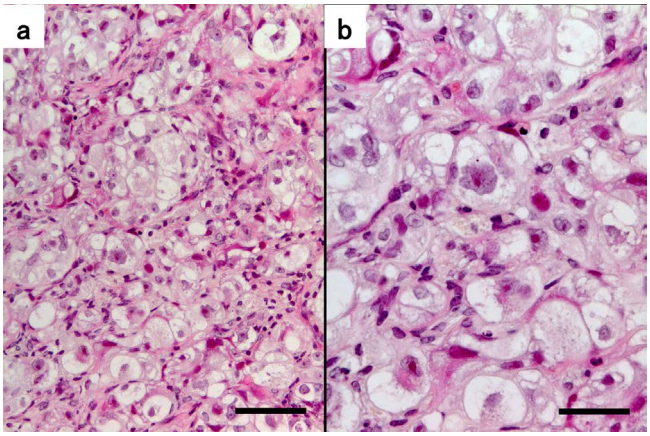

Figure 7: Micrograph of the tumor transplanted into SCID mice. It shows a clear cell carcinoma with large nuclei and prominent nucleolei, strongly looking like the original tumor. (a, bar=100 μm; b, bar=50 μm).

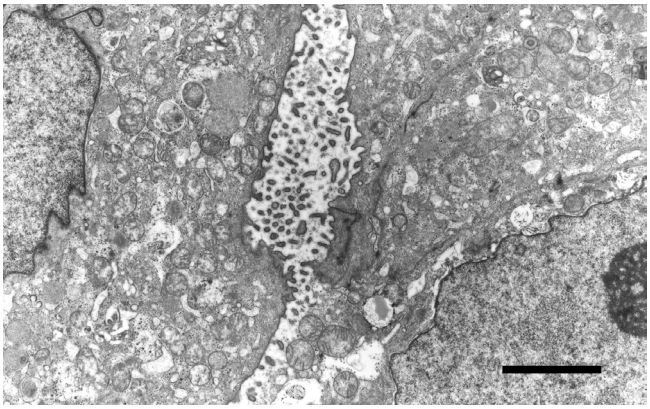

Figure 8: Electron micrograph of the tumor transplanted into SCID mice. The cells have many microvilli on the surface and rich mitochondria in the cytoplasm, and are connected with desmosome-like junctions. (bar=3 μm).

Tumor markers: The following tumor markers were positive in the culture medium: CA 19-9, 18 U/ml and TPA, >2,000 U/L. In contrast, they were negative for: AFP, <1 ng/ml; CEA, <0.5 ng/ml; CA 125, <5 U/ml; CA 72-4, 4.4 U/ml; HCG-β, <0.1 ng/ml; and SCC antigen, <0.5 ng/ml.

Immunohistochemical stainings: CA 125, CA 19-9, HNF-1α, and Annexin IV were proved immunohistochemically in cancer cells from the original tumor and transplanted tumors (Figures 9 and 10). The expression levels of the cancer antigens (CA125 and CA 19-9) in transplanted tumor were slightly weaker than those in original tumor. Estrogen and progesterone receptors were not detected.

Chemosensitivity: The EC50 values of anti-cancer agents for HCH-3 cells (Figure 11) are listed in Table 2.

Mutational analysis: The amount of 4.3 μg DNA was extracted from 2 x 10^6 living cells. Two variants were discovered in hotspot locations of 50 cancer genes and 11 of called variants outside of hotspot locations were found (Table 3).

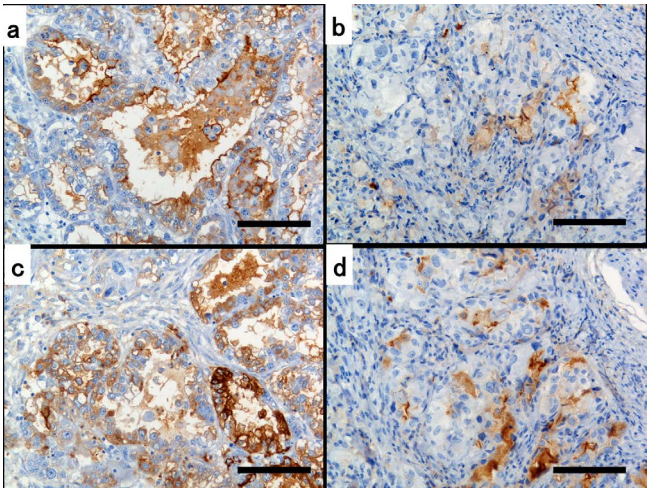

Figure 9: Immunohistochemical stainings of the original tumor (a, c) and heterotransplanted tumor (b, d) for CA 125 (a, b) and CA 19-9 (c, d). Cancer cells are positive for CA 125 and CA 19-9 (a, b, c, d; bar=100 μm).

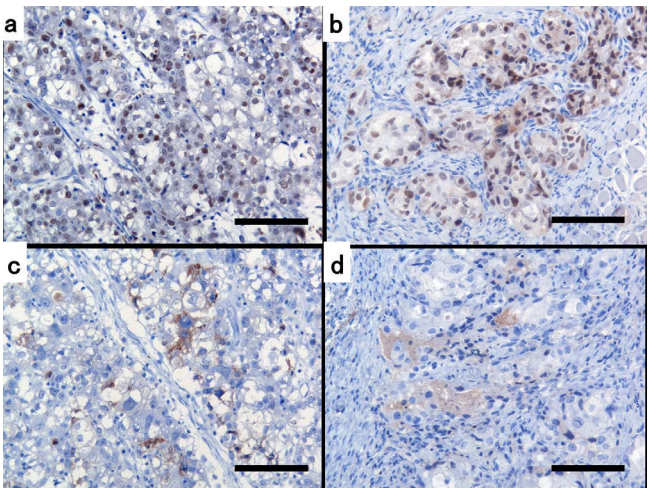

Figure 10: Immunohistochemical stainings of the original tumor (a, c) and heterotransplanted tumor (b, d) for HNF-1α (a, b) and Annexin IV (c, d). Cancer cells are positive for HNF-1α and Annexin IV (a, b, c, d; bar=100 μm).

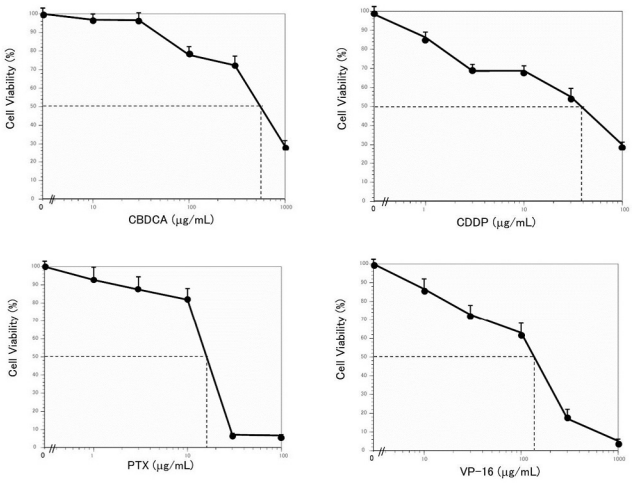

Figure 11: Chemosensitivity of HCH-3 cells. HCH-3 cells are considered no-sensitive to CBDCA, CDDP, PTX and VP-16.

Drug	EC50 (mg/mL)	PPC (mg/mL)
ACD	>100	0.08
ADM	0.88	0.4
CBDCA	555.6	37.1
CDDP	38.3	8.5
CPT-11	2.99	0.05
5-FU	1129	15.3
MMC	22.4	2.4
PTX	16	11.8
VP-16	136.4	13

Table 2: Chemosensitivity using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay. EC50, effective concentration for 50% kill; PPC, peak plasma concentration taken intravenously; ACD, actinomycin D; ADM, doxorubicin; CBDCA, carboplatin; CDDP, cisplatin; CPT-11, irinotecan hydrochloride; 5-FU, 5-fluorouracil; MMC, mitomycin C; PTX, paclitaxel; VP-16, etoposide.

Discussion

In this report, we established a different cell line, HCH-3, of human clear cell carcinoma of the ovary by culturing tissue parts from a removed tumor, and to get credible proof that this cell line truly exhibits the original tumor and disease, we studied its biological characteristics. We discovered that these cells indicated the following features: 1) histology of the original tumor was clear cell carcinoma and positivity for PAS, HNF-1α, and Annexin IV stainings; 2) viable in culture for over 206 months; 3) neoplastic, pleomorphic, and pile up easily without contact inhibition; 4) chromosomes were of a human karyotype with aneuploid distribution; and 5) transplantable into SCID mice and made tumors which histologically similar to the original tumor.

Notwithstanding the advancements in refined expertise, it is as yet difficult to build up a cell line, mainly as a result of blend with fibroblasts, which as a rule grow sooner than epithelial cells and bolster their detachment from the plate. To defeat this, we exploited the way that fibroblasts isolate sooner than epithelial cells by trypsin, and we erased each fibroblast and most epithelial cells, leaving just a couple of colonies of epithelial cells to extend. The cells slowly expanded well

S.No	Chromosome	Position	Ref Allele	Variant	Allele Type	Frequency	Variant Type	Allele Source*	Allele Name	Gene Symbol	Coverage	AA Ref	AA Variant
1	chr2	212812097	T	C	Heterozygous	33	SNP	Novel	---	ERBB4	1623	unknown	unknown
2	chr4	1807894	G	A	Homozygous	100	SNP	Novel	---	FGFR3	1912	T	T
3	chr4	55141055	A	G	Homozygous	100	SNP	Novel	---	PDGFRA	1964	P	P
4	chr4	55980239	C	T	Homozygous	100	SNP	Novel	---	KDR	1516	unknown	unknown
5	chr5	112175770	G	A	Homozygous	100	SNP	Novel	---	APC	1989	T	T
6	chr5	149433596	TG	GA	Homozygous	100	MNP	Novel	---	CSF1R	1919	unknown	unknown
7	chr9	21971106	G	A	Heterozygous	33.8	SNP	Novel	---	CDKN2A	1993	R	C
8	chr10	43613843	G	T	Homozygous	100	SNP	Novel	---	RET	1996	L	L
9	chr11	108155167	T	C	Heterozygous	32.3	SNP	Novel	---	ATM	1999	D	D
10	chr12	25398284	C	T	Heterozygous	34.4	SNP	Hotspot	COSM521	KRAS	1981	G	D
11	chr13	28610183	A	G	Heterozygous	49.9	SNP	Novel	---	FLT3	1966	unknown	unknown
12	chr16	68835678	G	A	Heterozygous	43.5	SNP	Novel	---	CDH1	1999	R	Q
13	chr17	7578389	G	A	Homozygous	100	SNP	Hotspot	COSM11090	TP53	1987	R	C

Ref, Reference; AA, Amino Acid; *Allele Source, whether a variant is hotspot: Novel or Hotspot;

Table 3: Variants out side of hotspot locations of 50 cancer genes.

Cell line	Age	Materials	Chromosome Number	DT	Transplantability	Immunotaining	Characteristics
HUOCA-II (1987)	51	ovary	46 (mode)	24, 28	Yes	-	tumor angiogenesis factor
RMG-I (1988)	34	ascites	47 (mode)	60	Yes	BFP, ferritin, PLAP	-
OCC1 (1990)	47	ascites	70-77	36, 38	Yes	-	production of CA125
RMG-II (1991)	53	ascites	hypertetraploid	58	No	CA125, TPA, MA602-1, MA602-6	production of CA125, TPA, MA602-1, MA602-6
OVISE (1995)	40	metastatic tumor	62 (59-65)	60	Yes	CA125, CA19-9, EGFR, ER(-), PgR(-)	production of CA19-9, CA125, TPA
OVTOKO (1995)	78	metastatic tumor	78 (76-83)	70	Yes	EGFR, ER(-), PgR(-)	-
JHOC-5 (1999)	47	ovary	74-85	52	No	-	CA125
JHOC-6 (1999)	43	recurrent tumor	46-49	70	Yes	-	CA125
SMOV-2 (1999)	46	tumor	85-92	48.2	Yes	-	p53 mutation (-)
TAYA (2002)	43	ascites	69-74	50	No	-	p53 Exon5 point mutation, PTEN mutation (-)
RMG-V (2005)	52	ascites	83 (77-85)	15.5	No	-	-
TU-OC-1 (2013)	65	ovary	64-69	38.4	Yes	-	PIK3CA mutation (+)
TU-OC-2 (2016)	68	ovary	84 (41-96)	37.5	No	ARID1A (-)	PIK3CA mutation (-), p53 mutation (-)
HCH-1 (2016)	67	ovary	39-44	48.7, 66.4	Yes	CA125, CA19-9, HNF-1beta, Annexin IV, ER(-), PgR(-)	production of CA19-9, CA125, TPA
HCH-3	41	ovary	78-87	82	Yes	CA125, CA19-9, HNF-1beta, Annexin IV, ER(-), PgR(-)	production of CA19-9, TPA, KRAS mutation (+), TP53 mutation (+)

DT; doubling time (hour).

Table 4: Cell lines from ovarian clear cell carcinoma.

to make evident provinces, and the principal subculture was made 3 months after the primary culture.

Some ovarian clear cell carcinoma cell lines have been applied for basic study, but only 14 of them have had their characteristics explained in detail in the literature (Table 4) [15-26]. HCH-3 cells had the longest doubling time in these cell lines.

Tumor markers are helpful not only in detecting ovarian cancer but also in noticing tumor recurrence or evaluating therapy. In this patient, CA 125 and CA 19-9 were particularly helpful tumor markers because her serum levels were preoperatively high. In the original and transplanted tumors, both were immunohistologically positive for CA 125 and CA 19-9, while this was also demonstrated in two previously reported cell lines (OVISE [19], HCH-1 [26]). However, contradictorily, the HCH-3 cells did not produce much of CA 125 and CA 19-9 in the culture media *in vitro*. And so, it signifies a good model for the research of ovarian cancers that express tumor markers.

The ovary is believed a target organ of steroid hormones, but OVISE [19], OVTOKO [19], HCH-1 [26] and HCH-3 possess neither estrogen nor progesterone receptors. As the mechanism of action and localization of steroid hormone receptors are still mostly unfamiliar, HCH-3 can be handled as a cell line without hormone receptor for research on receptors.

Not only surgery but also chemotherapy is very important processes for the therapy of ovarian cancer. The CAP (cyclophosphamide, ADM, CDDP) treatment protocol had been generally managed for therapy of ovarian cancer; but, PTX and CBDCA have been lately used although they are not always efficient. Therefore, CPT-11 has been managed for platinum- and taxane-resistant epithelial ovarian cancer [27]. With a specific end goal to assess the impacts of chemotherapeutic meds on HCH-3 cells, chemosensitivity to a board of medicines was resolved utilizing the MTT assay, which is as yet considered a quick and exact strategy for screening for agents responsiveness of refined cells. *In vitro* affectability was characterized as over half development hindrance at

pinnacle plasma focuses. Along with this criterion, we verified that HCH-3 cells were not sensitive to agents generally managed in the treatment of gynecological malignant tumors using MTT assay.

Emerging evidence have shown that epigenetic mechanisms including DNA methylation, histone modifications, chromatin remodeling as well as non-coding RNAs, play a critical role in the initiation and development of human carcinomas [28]. Particularly for the ovarian cancer, recent study has shown that histone H3K9 methyltransferase G9a serves as a hallmark of its aggression and promotes its peritoneal metastasis [29]. Together with another key histone H3K9 methyltransferase GLP, G9a play an essential role in the establishment and maintenance of H3K9me1/2 at euchromatin [30]. Mechanistically, G9a suppressed the expression of a set of tumor suppressors, including CDH1, DUSP5, SPRY4, and PPP1R15A in ovarian carcinomas through ectopic establishment of H3K9me2 at their promoters [31].

As recent study revealed that G9a also interacts with DNA methyltransferases (DNMTs) and is involved in the maintenance of DNA methylation at particular loci [32], investigation of the patterns of both histone and DNA methylation in normal cells as well as ovarian carcinoma cells might shed light on the obscure mechanisms that required for genesis and progression of this cancer in humans.

On the other hand, various genetic alterations discovered in ovarian cancer. The frequency of driver gene mutations in the altered subtypes (serous, mucinous, endometrioid, clear cell) is controversial. The PIK3CA [33] gene was discovered to be specifically mutated in ovarian clear cell carcinoma. Then ARID1A [34] mutation and the consequent loss of expression are often detected. Rechsteiner et al. [35] reported that TP53 mutations occurred frequently not only in high-grade serous carcinomas (58.7%), but also in mucinous carcinoma (57%) and clear cell carcinoma (52%). KRAS mutations were chiefly recognized in mucinous carcinoma (57%) and were concurrently with TP53 mutations (36%).

As a molecular analysis, somatic mutations were evaluated using Panels of 50 most frequently reported oncogene and tumor suppressor genes. HCH-3 had KRAS and TP53 variants in hotspot locations of 50 genes including PIK3CA, and 11 of called variants outside of hotspot locations. Inappropriately, ARID1A was not contained within this Panel. Thus, a future molecular classification of ovarian cancer should consider the mutational status of all subtypes and may be clarified using new methods for sequencing.

Conclusion

HCH-3 is an ovarian clear cell carcinoma cell line in which CA 125 and CA19-9 expression have been clarified. Mutations were discovered in *KRAS* and *TP53 genes*. As it is difficult to establish a cell line from the malignant tumor individually, the cell line that we established and characterized would be very helpful in basic study on ovarian cancer, specifically clear cell carcinoma, the etiology of which is not yet entirely recognized.

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