Establishment and characterization of two cell lines (HEC-155, HEC-180) derived from uterine papillary serous adenocarcinoma

T. Iida[1], M. Hamano[2], N. Yoshida[1], K. Yonamine[1], K. Hayashi[1], K. Kiguchi[1], B. Ishizuka[1], Y. Nishimura[3], T. Arai[3], M. Kawaguchi[3], H. Hata[4], J. Watanabe[5], H. Kuramoto[3, 4]

[1]Department of Obstetrics and Gynecology, St. Marianna University School of Medicine, Kawasaki
[2]Tissue Culture Center, [3]Department of Clinical Cytology, Graduate School of Medical Sciences, Kitasato University
[4]Department of Obstetrics and Gynecology, [5]Department of Pathology, School of Medicine, Sagamihara (Japan)

Summary

Uterine papillary serous adenocarcinoma (UPSC) is an uncommon histologic subtype of endometrial cancer that characteristically behaves aggressively with a poor prognosis. We established two novel cell lines derived from UPSC designated HEC-155 and HEC-180. Both cell lines have been growing steadily in monolayer cultures for over ten years. Overexpression of p53, Ki67 and p27 was detected in both cell lines by immunohistochemistry. Using a DNA sequencing technique, a point mutation of p53 was detected in exon 8, codon 286 in HEC-155 and in exon 6, codon 195 in HEC-180. These newly established cell lines should be useful for investigating the characteristics of UPSC.

Key words: Uterine papillary serous carcinoma (UPSC); Cell line; p53 mutation.

Introduction

Uterine papillary serous carcinoma (UPSC) is an uncommon histologic subtype of endometrial cancer that characteristically behaves aggressively and has a worse prognosis than uterine endometrioid adenocarcinoma (UEC) [1-3]. The causes of these characteristics have yet to be determinded in detail. An in vitro model of endometrial carcinoma, the HEC-1 cell line, was first established from a typical endometrioid adenocarcinoma in 1968 [4]. However, cell lines originating from UPSC have been very rare in the literature.

In this study, we report the establishment of two cell lines (HEC-155, HEC-180) derived from UPSC. The morphology, cytogenetics, growth characteristics and immunoreactivity to hormone receptors and cancer-associated gene products were investigated.

Materials and Methods

Case 1 (HEC-155)

A 56-year-old female with primary endometrial cancer underwent a simple hysterectomy and bilateral salpingo-oophorectomy at the Kitasato University Hospital on January 24, 1989. The tumor material was obtained from the original uterine tumor with informed consent (Figure 1). Following a diagnosis of Stage IIIa cancer, the patient received postoperational chemotherapy. However, she sustained recurrence and expired four years and ten months after initial treatment.

Case 2 (HEC-180)

A 64-year-old female diagnosed with endometrial carcinoma underwent a radical hysterectomy and bilateral salpingo-oophorectomy with pelvic lymphadenectomy at the Kitasato University Hospital on May 14, 1991. The tumor material was obtained from the original uterine tumor with informed consent (Figure 2). Following a diagnosis of Stage IIIa endometrial carcinoma, the patient received postoperative chemotherapy. She expired due to another cause without recurrence seven years and nine months after initial treatment.

Primary culture

The cells of both carcinomas were cultured using the same procedure. The tumor tissues from the original endometrium were minced into pieces 2-3 mm in size with scissors, and dispersed into single cells with a 0.25% trypsin and 0.02% EDTA solution at room temperature. After being washed in 5 ml of Eagle's minimum essential medium (Eagle's MEM; Nissui Phamac. Co., Tokyo, Japan) containing 10% fetal bovine serum (FBS; JRH Biosciences, Lenexa, KS), the cells were incubated in tissue culture dishes (60 mm in diameter; Falcon; Becton Dickinson Co., Franklin Lakes, NJ) at 37°C in a 5% CO_2 atmosphere.

Morphological Examination

The cultured cell lines were observed by phase-contrast microscopy.

Cell proliferation

Growth curves were calculated by seeding $5x10^5$ cells (HEC-155) or $2x10^5$ cells (HEC-180) in a 60 mm culture dish containing 5 ml of Eagle's MEM supplemented with 10% FBS. The cells in the culture dishes were harvested using the trypsin-EDTA solution. The viable cells were enumerated in triplicate.

Karyotypic analysis

For the chromosome preparation, cultured cells at the 210th passage (HEC-155), 181st passage (HEC-180) were treated with colcemide (0.2 μg/ml) for two hours at 37°C, and then a hypotonic KCl solution (0.2%) for 20 minutes. The cells were fixed for ten minutes in methanol: acetic acid (3: 1). After the

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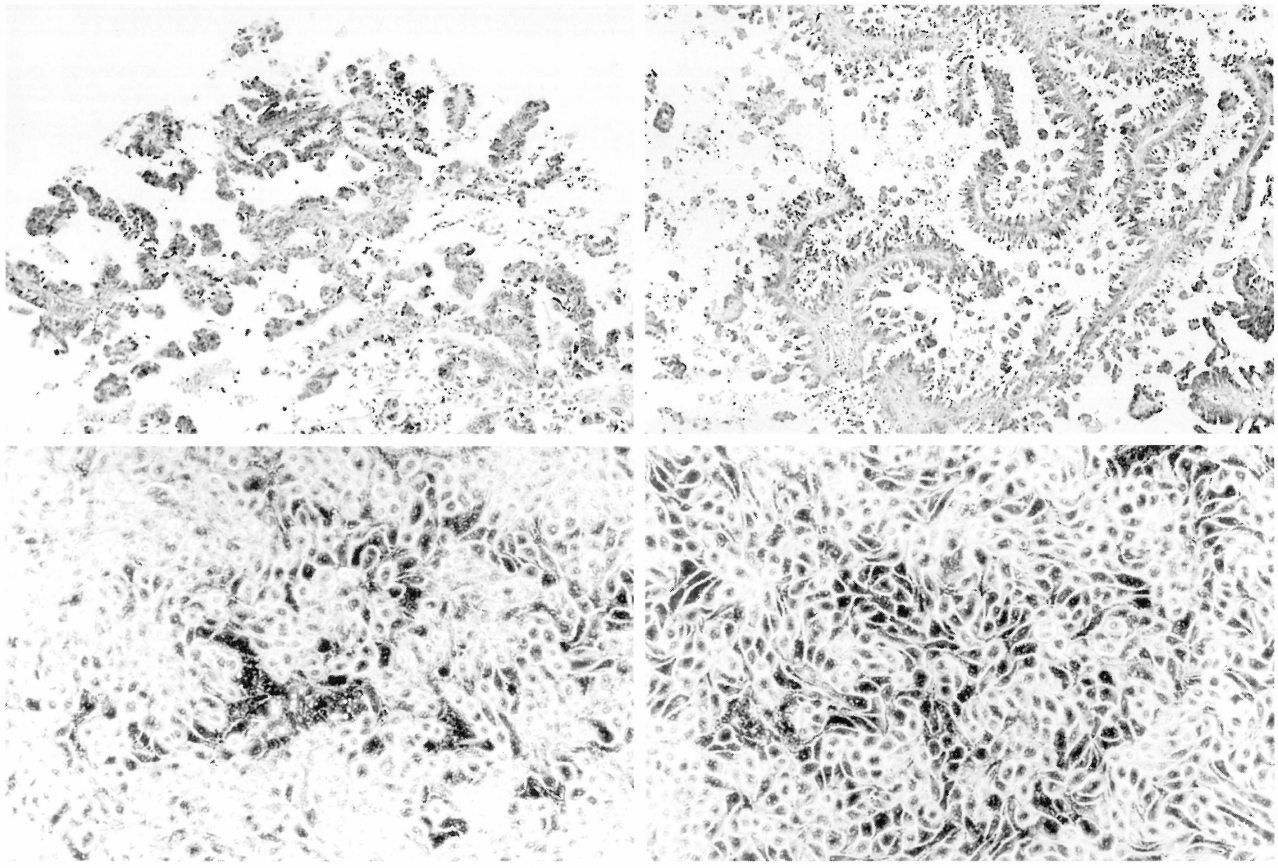


Figure 1. — Histopathology of the original serous papillary adenocarcinoma of HEC-155 from the uterine body (HE stain x 100).
Figure 2. — Histopathology of the original serous papillary adenocarcinoma of HEC-180 from the uterine body (HE stain x 100).
Figure 3. — Phase contrast microscopic findings of HEC-155 forming a monolayered sheet with a pavement-like arrangement of tumor cells and marked piling-up (x 200).
Figure 4. — Phase contrast microscopic findings of HEC-180 also forming a monolayered sheet with a jigsaw puzzle-like arrangement of polygonal cells and moderate degree of piling up (x 200).

fixation, they were added drop-wise to glass slides, dried over an alcohol lamp, and stained with a conventional Giemsa stain 5), allowing for the karyotype of the cell lines to be expressed.

Xenotransplantation

The tumorigenic properties of the two cell lines were assayed in three female nude mice or SCID mice. The mice were injected subcutaneously on the back at two sites per mouse with 1 X 10^7 viable cells. The sizes of the growing tumors were measured once a week until two months after the innoculation.

Immunohistochemistry

Both original tumors and cultured cells were treated with monoclonal antibodies, including those for the estrogen receptor (Novocastra, Benton Lane, UK, x40) progesterone receptor (Novocastra, x40) p53 (Dako Cytomation, Glostrup, Denmark, x80) p21 (CALBIOCHEM, San Diego, CA, x100) Ki67 (Dako Cytomation, x100) p27 (Novocastra, x80) Cyclin A (Novocastra, x100) Cyclin D1(Oncogene, San Diego, CA, x80) Cyclin E (Novocastra, x40) C-erbB2 (Novocastra, x40) Bcl-2 (Dako Cytomation, x200). Biotinylated goat antimouse immunoglobulin and antirabbit immunoglobuline (DAKO LSAB kit; Dako Cytomation) were used as secondary antibodies. Immunostaining was performed using peroxidase-DAB (diaminobenzidine). The labeling indices were calculated as follows; under a 400-power microscope, three fields were chosen at random and the total number of cells was compared with the number of positive cells and the result expressed as a percentage. The labeling indices were compiled into four categories: No positive cell = (–), 1 to 4% = (±), 5 to 49% = (+), over 50% = (++)

Mutational analysis of the p53 gene

DNA from both cell lines was prepared and purified by the phenol chloroform method for analysis. Mutations in exons 5, 6, 7 and 8 of the p53 gene were investigated using the PCR-SSCP method 6).

Olygonucleotide primer sets were as follows:

exon5	5'-TGTTCACTTGTGCCCTGACT-3'
	5'-CAGCCCTGTCGTCTCTCCAG-3'
exon6	5'-TGGTTGCCCAGGGTCCCCAG-3'
	5'-GGAGGGCCACTGACAACCA-3'
exon7	5'-CTTACCACAGGTCTCCCCAA-3'
	5'-AGGGGTCAGCGGCAAGCAGA-3'
exon8	5'-TTGGGAGTAGATGGAGCCT-3'
	5'-AGTGTTAGACTGGAAACTTT-3'

After 35 cycles of amplification, the PCR products were sequenced directly with a Sequence-Pro kit (TOYOBO, Osaka Japan).

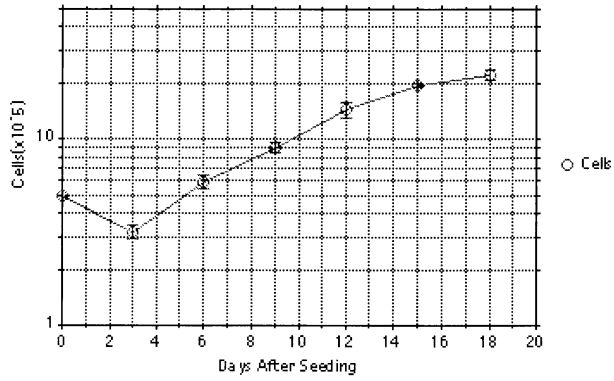


Figure 5. — Growth curve of HEC-155. The population doubling time in the logarithmic phase was 84 hours.

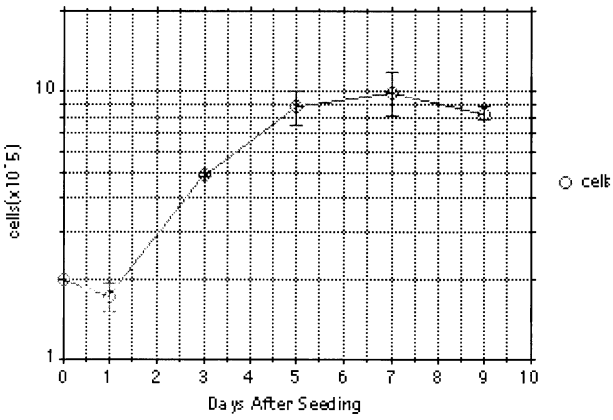


Figure 6. — Growth curve of HEC-180. The population doubling time in the logarithmic phase was 36 hours.

Results

Culture history

The culture medium was renewed twice a week. The epithelial tumor cells grew steadily, having been subcultures every two weeks. There was no fibroblastic outgrowth from the start of the primary culture. No cloning was necessary in either cell line. The cells were in their 206th passage (HEC-155) and 177th passage (HEC-180) on April 1, 2003.

Morphology of cell lines

Cells of both lines have a small polygonal shape and grow in multiple layers without contact inhibition. HEC-155 forms a monolayered sheet with a pavement-like arrangement and marked piling-up (Figure 3). In contrast, HEC-180 forms a monolayered sheet with a jigsaw puzzle-like pattern of polygonal cells and less marked piling-up.

Cell proliferation

The population doubling times of HEC-155 and HEC-180 cells in the logarithmic growing phase were 84 hours (Figure 5) in the 75th passage and 36 hours (Figure 6) in the 33rd passage, respectively.

Karyotypic analysis

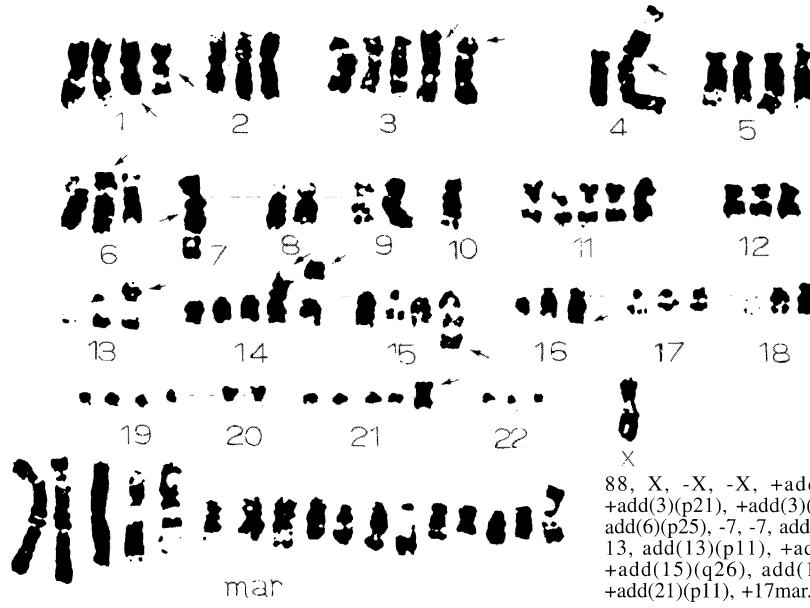
HEC-155

The number of chromosomes ranged from 52 to 63, with a mode of 58. The karyotype, which was human in type, was as follows:

62, XX, add(1)(q42), +2, +add(3)(q21), +5, +5, +6, +7, add(11)(q23)x2, -13, add(14)(q11), -15, add(18)(p11), add(21)(p11), +mar. (Figure 7).

62, XX, add(1)(q42), +2, +add(3)(q21), +5, +5, +6, +7, add(11)(q23)x2, -13, add(14)(q11), -15, add(18)(p11), add(21)(p11), +11mar.

Figure 7. — Karyotype of HEC-155.



88, X, -X, -X, +add(1)(q11), add(1)(q32), +add(3)(p21), +add(3)(p25), -4, add(4)(p11), +5, add(6)(p25), -7, -7, add(7)(q22), -8, -9, -10, +11, -13, add(13)(p11), +add(14)(p11), +i(14)(q13), +add(15)(q26), add(16)(q24), +19, -20, +21, +add(21)(p11), +17mar.

Figure 8. — Karyotype of HEC-180.

HEC-180

The number of chromosomes was between 67 to 94, and the mode was 75. The karyotype, which was human in type, was as follows:

88, X, -X,-X, +add(1)(q11), add(1)(q32), +add(3)(p21), +add(3)(p25), -4, add(4)(p11), +5, add(6)(p25), -7, -7, add(7)(q22), -8, -9, -10, -10, +11, +11, -13, add(13)(p11), +add(14)(p11), +i(14)(q13), add(15)(q26), add(16)(q24), +19, -20, +21, +add(21)(p11), +17mar. (Figure 8).

Xenotransplantation

Neither cell line was successfully transplanted to either nude mice or SCID mice.

Immunohistochemistry

Both cell lines and their original tumors reacted positively to the various antibodies listed in Table 1. The p53 and Ki67 expression was marked. However, p27 expression was more predominant in the cell lines than the original tumors. In contrast, bcl-2 expression was diminished in the cell lines compared to the original tumors.

Table 1. – *Immunohistochemical features of HEC-155, HEC-180 and the original tumors.*

	ER	PR	p53	p21	Ki67	p27	CyclinA	CyclinD1	Cyclin E	C-erbB2	Bcl-2
Original tumor	+	+	++	±	+	+	±	N-C+	+	++	++
HEC-155	–	+	++	±	++	++	+	+	+	++	+
Original tumor	+	+	++	±	+	±	+	N-C+	+	++	++
HEC-180	–	++	++	±	++	++	–	±	±	±	–

N: nucleus; C: cytoplasm.

Mutations of the p53 gene

Point mutations were identified in exon 8 of the p53 gene in HEC-155 and in exon 6 in HEC-180 (Table 2).

Table 2. – *p53 gene mutations. The sequence analysis for p53 gene mutations revealed a point mutation in exon 8, codon 286 in HEC-155 cells and exon 6, codon 195 in HEC-180 cells.*

	exon	codon	mutation	amino acid
HEC-155	8	288	GAA-AAA	Glu-Lys
HEC-180	6	195	ATC-AAC	Ile-Asn

Discussion

UPSC is a distinct histologic type of endometrial cancer with a high rate of relapse and poor prognosis. Unlike UEC, extrauterine metastasis of UPSC is not correlated with histological grade, or depth of myometrial invasion [7]. To make a comparison between UPSC and UEC, 266 women with Stage I to IV endometrial carcinoma were investigated [8]. In the literature, the most prominent characteristics of UPSC were older age, more frequent DNA aneuploidy, and higher positive rate for p53 than in UEC. Overexpression of p53 was also detected in the putative precursor, endometrial intraepithelial carcinoma, as well as the progressive stage of UPSC [9, 10]. Both cell lines and their original tumor tissues were characterized by the overexpression and mutation of p53. In contrast to p53, however, the inhibitor of apoptosis bcl-2 [11] was little expressed in the two cell lines, though it was expressed in the original tumor tissues. Since apoptosis is regulated not only by bcl-2 but also by p53, it would be of interest whether there is an inverse relationship between the expression of bcl-2 and p53 in the cell lines vs their original tumor tissues [12].

p27 was originally cloned from cell-cell contact and transforming growth factor-ß (TGF-ß)-mediated G1-arrested cells [13]. p27 is also regarded as a cyclin-dependent kinase inhibitor of the G1 to S phase of the cell cycle, and functions as a suppressor of cell cycle promotion. Bamberger *et al.* demonstrated that p27 expression was decreased in endometrial cancer cells compared to normal endometrial cells [15]. However p27 staining in endometrial cancers indicated no association with clinical stage, age, histlogical type or prognosis [16]. On the other hand, Watanabe *et al.* reported that p27 was paradoxically overexpressed in high grade endometrial adenocarcinomas and was a predictive factor for a poor prognosis [17]. Recently, a high incidence of p27 abnormalities was described in UPSC [18]. In the present study, we confirmed that the expression of p27 was more prominent in the two cell lines than their original tumors. The two cell lines were not able to form a tumor in either nude mice or SCID mice, although cell cycle parameters, including Ki-67, were overexpressed.

Conclusion

The basis for the aggressiveness and high mortality rate associated with UPSC is still unknown, and optimal adjuvant therapy for UPSC is yet to be established. Therefore, we certainly believe that these newly established cell lines will be useful for investigating the characteristics of UPSC.

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Address reprint requests to:
T. IIDA, M.D.
Department of Obstetrics and Gynecology
St. Marianna University
School of Medicine
2-16-1 Sugao, Miyamae-Ku
Kawasaki 216-8511 (Japan)