Establishment and characterization of two different types of new human endometrial adenocarcinoma cell lines (HEC-251 and HEC-265)

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Summary

Objective: Endometrial carcinomas are grouped into two types of carcinogenic entities. These two different types of cell lines designated as HEC-251 and HEC-265 were established from human endometrial adenocarcinoma. We report their characteristics.

Materials: HEC-251 was derived from moderately differentiated endometrioid adenocarcinoma and HEC-265 from well-differentiated adenocarcinoma.

Results: These cell lines grow well and serial passages can be successively carried out more than 100 times. The monolayer cultured cells reveal neoplastic and pleomorphic features, and grow in multilayers. HEC-251 cells are immuno-cytochemically positive for p53 and HEC-265 cells for PgR. Both cell lines are transplantable to nude mice and reflect the original histopathologic characteristics.

Conclusion: The cell lines HEC-251 and HEC–265 will contribute to clarifying the characteristics of two different types of human endometrial carcinomas.

Key words: Endometrial adenocarcinoma; Uterine neoplasm; Cell line; p53; PgR.

Introduction

Endometrial adenocarcinoma of the uterus is one of the most common female genital carcinomas. An in vitro experimental study on endometrial carcinoma has helped to clarify details of the tumor, especially in humans [1]. It was suggested that there might be two different sequences of carcinogenesis in endometrial carcinoma. One develops carcinoma through endometrial hyperplasia [2]and mainly consists of well-differentiated cancer and coexists with endometrial hyperplasia [3]. The other is an estrogen-unrelated type that originates *de novo* from atrophic endometrium and develops poorly differentiated cancer without endometrial hyperplasia, and is associated with the gene mutation of p53 and c-erbB2/neu amplification [4]. The latter type of carcinoma occurs not infrequently in postmenopausal women and expresses aggressive behavior.

This paper describes the establishment of two different types of new cell lines derived from human endometrial adenocarcinomas, named HEC-251 and HEC-265. Characteristics of the two cell lines, inducing their morphology, growth characteristics, chromosome constitution, heterotransplantability and expression of specific substances are demonstrated.

Materials and Methods

Clinical history of Materials

HEC-251: The patient was a 53-year-old Japanese woman, gravida 2, para 2. With the clinical diagnosis of Stage II endometrial carcinoma, the patient underwent abdominal radical total hysterectomy, bilateral salpingo-oophorectomy (BSO), pelvic lymphadenectomy (PLN) and para-aortic lymphadenectomy (PAN). The histologic findings of the tumor revealed moderately differentiated endometrioid adenocarcinoma (Figure 1a).

HEC-265: The patient was a 59-year-old Japanese woman, gravida 3, para 1, who noted vaginal spotting. With the clinical diagnosis of Stage Ib endometrial carcinoma, the patient underwent abdominal simple total hysterectomy and BSO. The histologic findings of the tumor revealed well-differentiated endometrioid adenocarcinoma (Figure 1b).

Culture Methods

Eagle's minimum essential medium (MEM①, Nissui Seiyaku Co., Tokyo, Japan) supplemented with 12 mM sodium bicarbonate, 0.3 mg/ml L-glutamine, and 10% fetal bovine serum (FBS, ICN Biomedical Inc., Belgium) was utilized as the primary culture.

At primary culture, the tumor specimen was washed with phosphate-buffered saline (PBS) twice, and minced into fragments 2 to 3 mm in size. The tissue fragments were incubated either with 0.25% tripsin (HEC-251) or with dispase (1000 pU/ml, HEC-265) in PBS for 30 minutes at room temperature and centrifuged 800 rpm for five minutes after adding the same volume of the culture medium. The pellets were dispersed by adding the culture medium and the cells were seeded to 60 mm dishes and cultured in 5% CO_2 atmosphere at 37°C.

Microscopic observation

The cultured cells were observed with a phase-contrast microscope, and stained by Giemsa staining. The transplanted tumor tissues were fixed in 10% formalin solution, dehydrated through a graded series of ethanol, penetrated with xylene and embedded in paraffin. They were then sectioned at a thickness of 3 μm and stained with hematoxylin and eosin (HE).

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Growth characteristics

To evaluate the cell growth characteristics, 1×10^5 cells were seeded into 60 mm dishes and cultured for ten days. Medium exchange was carried out every other day and the cell number was calculated in triplicate drawing the growth curve of cells.

Chromosome analysis

The cells, 5×10^3, were cultured in a LAB-TEK chamber (No. 155379, Nalge Nunc International, Tokyo, JAPAN). After the cells had grown to 60-70% confluence in the chamber the medium was exchanged with the one containing colcemid, 5×10^{-7} M/l, and the cells were incubated for one to two hours to arrest the metaphase. Then, the specimen was treated by a hypotonic solution, being carefully placed into 0.7% sodium chloride solution, for one minute and then in 0.2% sodium chloride solution for 20 to 40 minutes. Subsequently, the cells were fixed by each of 1, 10, 100 percent Carnoy solutions (3:1 acetoalcohol), respectively, for one to two minutes and then for 15 minutes by the new absolute Carnoy solution. After air drying, the specimen was stained with 10% Giemsa solution (pH 6.8).

Immunohistochemical staining

The cells were seeded in LAB-TEK chambers (No. 177402, Nalge Nunc International, Tokyo, JAPAN) to 60% confluence. After the cells grew to a fullsheet, they were fixed in 95% ethanol and immunocytochemical stainings were performed by the labeled streptavidin-biotin method (LSAB method, LSAB-kit, DAKO, Kyoto, JAPAN) as described previously [5]. The formalin-fixed and paraffin-embedded tumor tissue was sliced 3 μm thick, and stained by the LSAB method as for the cultured cells.

Anti-p53 antibody (DO-7, 1:80 dilution, Novocastra, Newcastle, U.K.), Anti-ER (estrogen receptor, 6F11, 1:50, Novocastra), Anti-PgR (progesterone receptor, 1A6, 1:50, Novocastra), Anti-Ki-67 (rabbit polyclonal, 1:50, DAKO, Kyoto, Japan), Anti-bcl2 (clone 124, 1:200, Dako), Anti-p21 (EA10, 1:100, Oncogene, Tokyo, Japan), Anti-Cyclin A (6E6, 1:100, Novocastra), Anti-Cyclin D1 (DCS-6, 1:80, Oncogene), Anti-Cyclin E (13A3, 1:40, Novocastra), Anti-C-erbB-2 (CB11, 1:40, Novocastra) were used.

Less than 5% of positive cells was judged as minus (–), between 5 and 50% of positive rate as 1 plus (+), and greater than 50% as 2 plus (++).

Transplantation to nude mice

Immature female nude mice (BALB/c nu/nu), approximately nine weeks old, were used for the experiment. The cultured cells were dispersed into single cells by the trypsin-EDTA solution, washed with PBS and centrifuged twice. The cells were then mixed with Matrigel (Becton, Dickinson and Co.) with the final concentration of 1.7×10^7 cells/200 μl for HEC-251 and 1.1×10^7 cells /200 μl for HEC-265, and were injected subcutaneously in nude mice using a 23-gauge needle. One week after injection the developing tumors were harvested and were processed for H-E or immunohistochemical staining.

Results

Culture history

The primary cultures of the endometrial carcinomas from the operating theater were carried out on September 9, 1997 for HEC-251 and on June 5, 1998 for HEC-265.

The second passage could be performed after five days, respectively, and both HEC-251 and HEC-265 grew well in the dishes without interruption of growing. There was no mixed growth of fibroblasts. They have been maintained stably to the present without incident including infection.

Microscopic observation

HEC-251 cells show an epithelial shape and are arranged mainly in pavement-like and partly jigsaw-like patterns in a monolayer (Figure 2a). The nuclear shape is oval to round with marked anisonucleosis and prominent nucleoli (Figure 2b). Although they show piling-up growth, the cells do not form large clusters in this area, and not infrequently separate from clusters and float in the medium. Mitotic figures are frequent (Figure 2b).

HEC-265 cells, epithelial in shape, are arranged in typical jigsaw-like cellular patterns with monolayer growth and pile up forming large clusters showing a palisade or glandular structure (Figure 3a). The nuclear shape is oval to round with slight anisonucleosis and small nucleoli. A few mitotic figures are found (Figure 3b).

Growth characteristics

The population doubling times of HEC-251 at the third passage and that of HEC-265 at the seventh passage (Figure 4) were 36 and 72 hours, respectively.

Chromosome analysis

The chromosomes of HEC-251 and HEC-265 revealed human karyotype and were distributed in the diploid (2n) to tetraploid range examined at the third passage (Figure 5a) and in 2n at the seventh passage, respectively (Figure 5b). The typical karyotypes at the 2n range are shown in Figures 5a and 5b, showing the pseudo-diploid chromosome constitution, respectively.

Immunohistochemical analyses

Both HEC-251 cells and the original histologic specimen were positive for p53 (Figure 6a), whereas HEC-265 cells were negative and similar to the original tumor. In contrast, both HEC-265 cells (Figure 6b) and the original tumor were positive for PgR (progesterone receptor), whereas HEC-251 was negative. HEC-251 and HEC-265 cells were positive for p21, Cyclin D1 and Ki-67, and negative for estrogen receptor (ER) by immunohistochemical staining similar to the original pathologic specimens (Table 1). In contrast, HEC-265 cultured cells appeared positive

Table 1. — *The results of immunohisto- and cytochemical staining.*

	HEC-251		HEC-265	
	Original tumor	Cultured cell	Original tumor	Cultured cell
ER (Estrogen receptor)	–	–	–	–
PgR (Progesterone receptor)	–	–	++	+
p53	++	++	–	–
p21	+	+	++	+
Ki-67	++	++	+	+
Bcl-2	+	+	–	–
C-erbB2	++	++	–	+
Cyclin A	–	–	–	+
Cyclin D_1	+	++	+	+
Cyclin E	+	+	–	+

(–; Labelling Index < 5%, +; Labelling Index 5 to 50%, ++; Labelling Index > 50%).

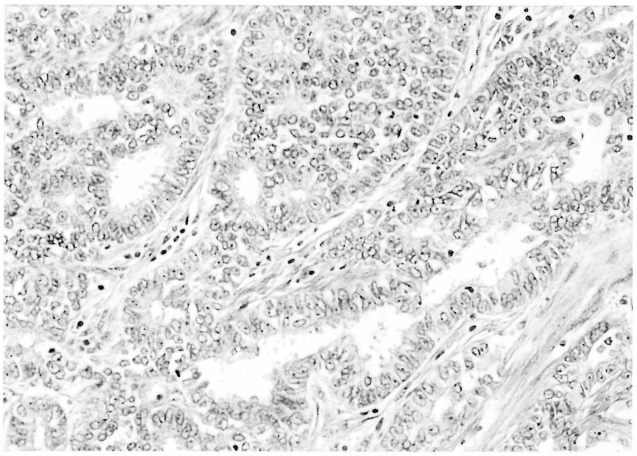


Fig. 1a

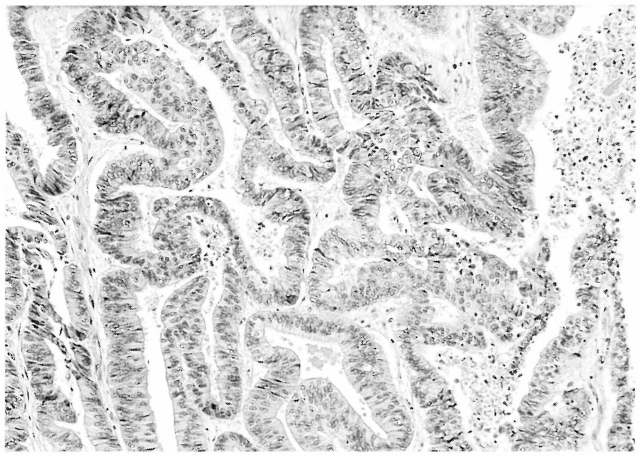


Fig. 1b

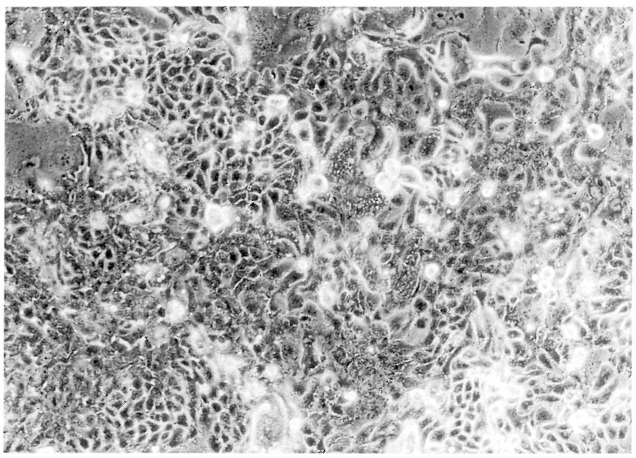


Fig. 2a

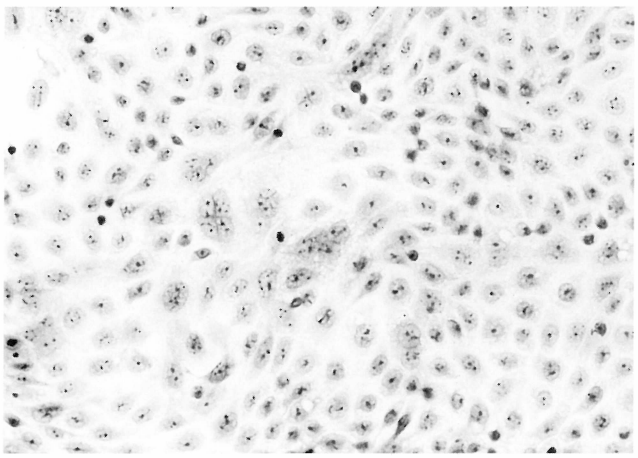


Fig. 2b

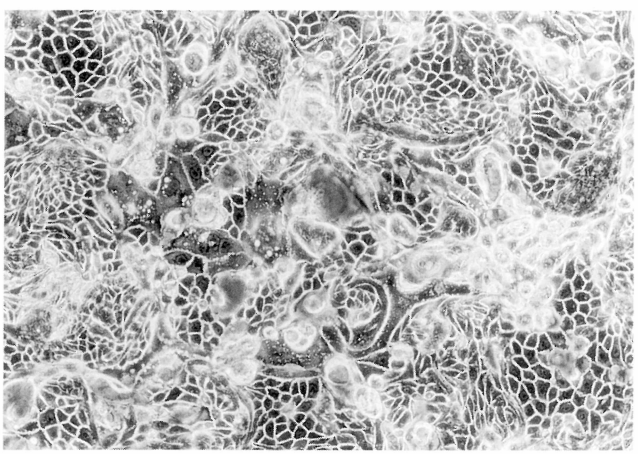


Fig. 3a

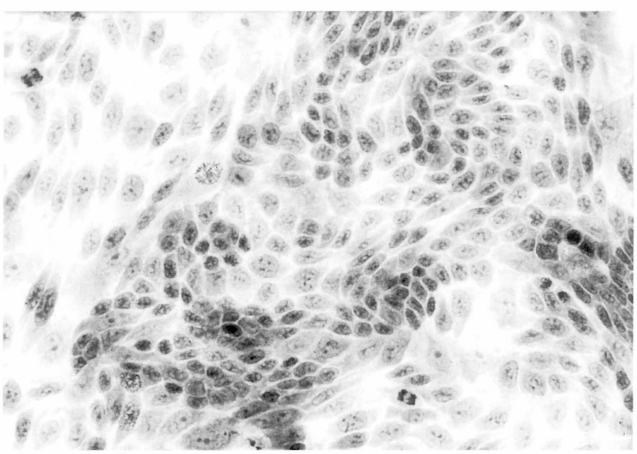


Fig. 3b

Figure 1. — The original tumors of HEC-251 (a) and HEC-265 (b) cells showing moderately and well-differentiated endometrioid adenocarcinomas, respectively (HE stain, original magnification × 20).
Figure 2. — Microscopic figures of HEC-251 showing pavement and incomplete jigsaw-puzzle-like arrangement with marked anisonucleosis, prominent nucleoli and frequent mitoses (a - phase-contrast microscope, b - Giemsa stain, original magnification × 20).
Figure 3. — Microscopic figures of HEC-265 showing marked jigsaw-puzzle-like arrangement with slight anisonucleosis and tiny nucleoli (a - phase-contrast microscopy, b - Giemsa stain, original magnification × 20).

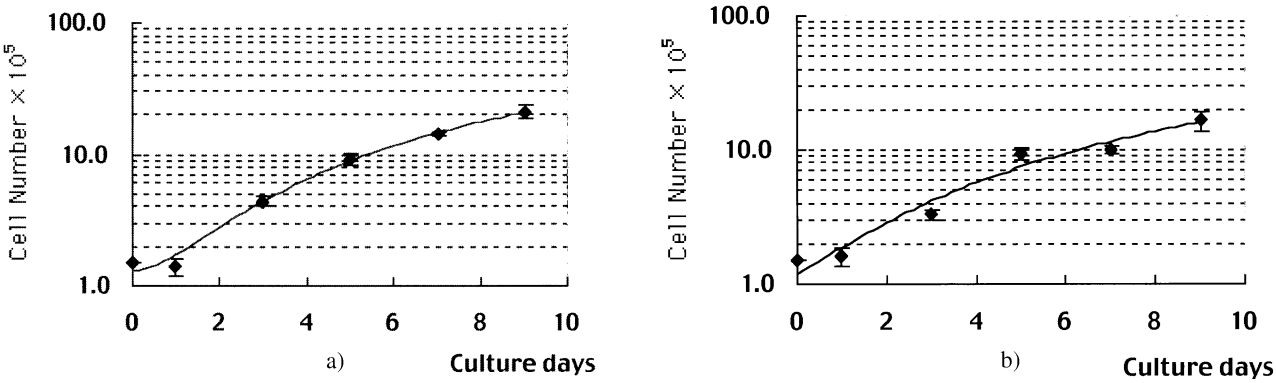


Figure 4. — Growth curve of HEC-251 cells (a) at third passage and HEC-265 cells (b) at seventh passage. Each point represents the mean values, and bars indicate ±SD of the triplicate determinations. The population-doubling time was 36 hours and 72 hours in the logarithmic growth phase, respectively.

Figure 5. — Karyotypes of HEC-251 (a) and HEC-265 (b) showing pseudodiploid constitution.

for Cyclin A, E and c-erbB-2, while these were negative on the original pathologic specimens (Figure 7).

Transplantation to nude mice

Both cell lines were transplantable subcutaneously in nude mice developing a solid tumor. The tumor tissues of both cell lines transplanted to nude mice reflected the features of their original tumor histopathogically. The tumor of HEC-251 cells was composed of a solid structure with a partial glandular structure (Figure 8a). The tumor from HEC-251 cells showed clearly a glandular structure (Figure 8b).

Discussion

In two different types of carcinogenesis in endometrial carcinoma [4, 6, 7], one is estrogen-dependent (type 1) and the other is estrogen-independent (type 2), mainly caused by p53 mutation. Type 1 develops in an unopposed estrogen environment leading to endometrial carcinoma via endometrial hyperplasia [2]. Kimura *et al.* [8] demonstrated that low PTEN expression was shown in type 1 G1 endometrial carcinoma, although p53 mutation was rarely detected. On the other hand, type 2 G3 endometrial carcinoma does not have PTEN mutation, but does have p53 mutation. p53 gene mutation in endometrial carcinoma relates to its progress and metastasis, and the prognostic influence of accumulated nuclear p53 protein originating from mutant p53 gene has been demonstrated in several studies [9-12]. HEC-251 cells derived from G2 endometrioid carcinoma are positive for p53 and negative for PgR. In contrast, HEC-265 cells derived from G1 carcinoma are inversely positive for PgR and negative for p53. By DNA sequence analysis, it was demonstrated that HEC-251 has the mutant p53 gene and HEC-265 has the wild type [13]. It is suggested that HEC-265 and HEC-251 are good comparative models for type 1 endometrial carcinoma and type 2 endometrial carcinoma, respectively.

Progestin has been reported to inhibit the growth of some endometrial carcinomas and its administration was established as progestin therapy [14, 15]. However, the detailed mechanism of action controlled by progestin is unclear. Quite a few endometrial carcinoma cell lines have been reported [16-20]. However, cell lines that preserve

ig. 6a

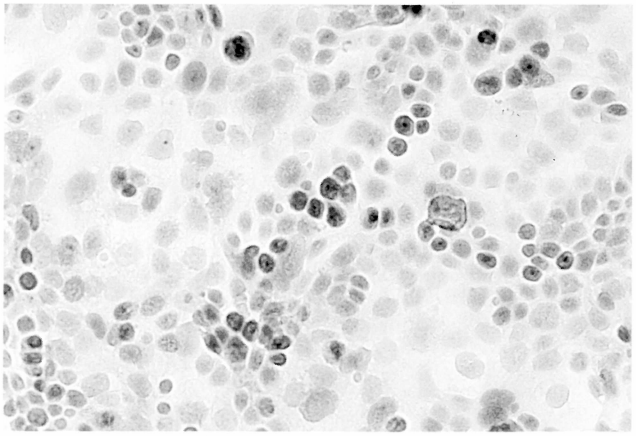
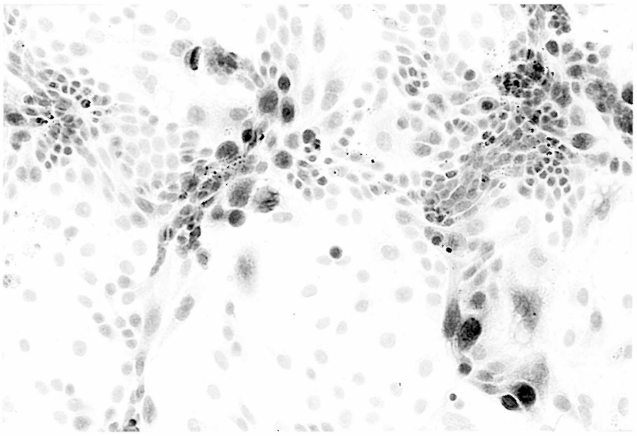


Fig. 6b



ig. 7a

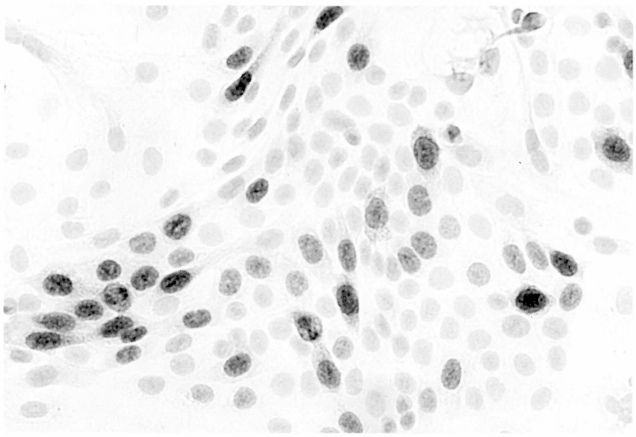
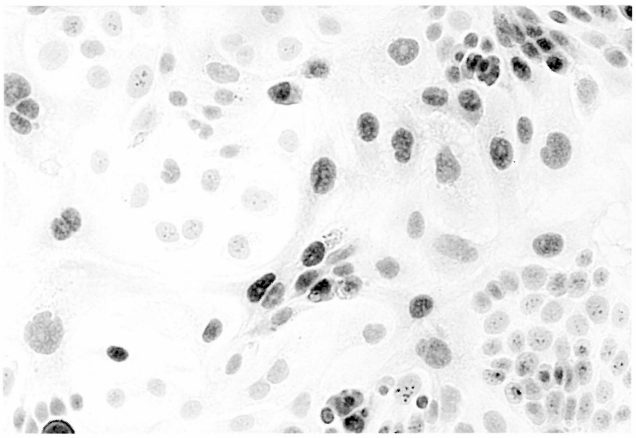


Fig. 7b



ig. 8a

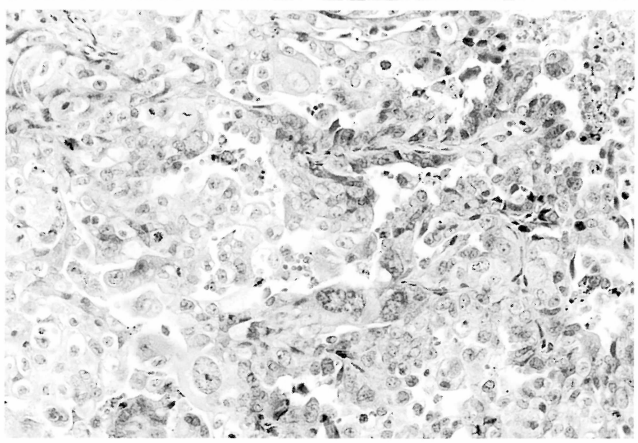


Fig. 8b

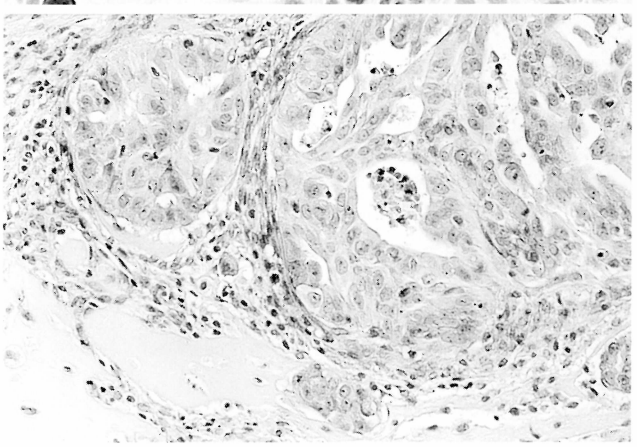


Figure 6. — Immunocytochemical stainings are positive for p53 in HEC-251 cells (a) and for PgR in HEC-265 cells (b) (original magnification × 20).
Figure 7. — Immunocytochemical stainings are positive for Cyclin A (a) and Cyclin E (b) in HEC-265 cells (original magnification × 20).
Figure 8. — Histologic findings of the tumors when HEC-251 (a) and HEC-265 (b) cells were transplanted subcutaneously into nude mice (original magnification × 20).

ER and PgR are rare. HEC-265 cells that are derived from G1 endometrioid adenocarcinoma and show immuno-reaction for PgR will be a useful model to perform research on hormonal reactions in endometrial carcinoma.

Conclusion

These cell lines HEC-251 and HEC–265 will help to clarify the characteristics of human endometrial carcinoma of two different types in carcinogenesis, since HEC-251 and HEC-265 may have originated from different carcinogenic entities; one with mutation of p53 and another related to hormones.

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