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Culture characters, genetic background, estrogen/progesterone receptor expression, and tumorigenic activities of frequently used sixteen endometrial cancer cell lines

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ABSTRACT

Background: This study aimed to determine the *in vitro* and *in vivo* properties of sixteen frequently used endometrial cancer (EC) cell lines, including the cell proliferation rate, morphology, hormone receptor expression patterns, PTEN, hMLH1 expression, p53 mutation, karyotype, and tumorigenicity in mouse xenograt model.

Methods: Twelve type I (AN3, ECC-1, EN, EN-1, EN-11, HEC-1A, HEC−1B, Ishikawa, KLE, MFE-280, MFE-296, MFE-319) and four type II (ARK1, ARK2, HEC-155/180, SPEC-2) endometrial cancer cell lines were studied. Cell proliferation and morphology were determined using cell growth curves and light microscopy, respectively. Real-time PCR was performed to measure the mRNA levels of target genes. Denaturing High Performance Chromatography (DHPLC) screening and PCR/sequencing were performed to identify p53 mutations. G-banding was applied for karyotyping. Tumorigenicity was evaluated using mouse xenograft.

Results: The population doubling time of the cell lines ranged between 19 and 41 h. Ishikawa, ECC-1, and MFE-280 have high while AN3 and EN1 have low expression of ER-α and ER-β. Expression of total PR and PR-B uniformly decreased in all type II cell lines and several type I cell lines (AN3, HEC-1A, HEC−1B, KLE, EN-1). Regression analyses revealed significant correlations between PR-B and total PR ($p < .001$), between isoforms ER-α and ER-β ($p < .001$), and between total PR and ER ($p < .001$), mRNA levels in type I cell lines. p53 mutations were detected in exons 5–8 of seven out of twelve type I and one out of four type II cell lines. PTEN expression was more uniformly suppressed in type II than type I cells, while hMLH1 did not show this pattern. All the five cell lines tested contained severe karyotype abnormalities. Mouse xenograft results indicated that HEC-1A, HEC-1B and EN-1 type I as well as ARK1 and ARK2 type II cell lines had potent tumorigenic activities. Low PR-B and ER-α expression in type I cell lines were associated with high tumorigenic activity.

Conclusions: This study provides resource information on EC cell lines commonly used in laboratories, which could be used for choosing cell lines suitable for specific research purposes. The results of karyotype analysis and p53 mutation together with hormone receptor expression pattern and morphology comparison strongly suggested an independent nature of these cell lines, excluding the possibility of cross-contamination between cell lines.

Additionally, this information suggests potential directions for future studies on the pathogenic mechanisms of endometrial cancer.

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1. Introduction

Cancer cell lines provide a useful tool for studying human cancers in both *in vitro* and *in vivo* systems. This experimental tool is frequently applied for studying endometrial cancer (EC), a common gynecologic malignancy. Cell lines such as Ishikawa, AN3, KLE, HEC-1A, and HEC-1B have been applied to investigate the biological behaviors, hormonal regulation, pathological mechanisms, and drug sensitivity/resistance of endometrial cancer. Knowledge on their basic biological characteristics is required for the best application of these cell lines in laboratory studies.

Endometrial cancer is the fourth most frequent malignancy in females. According to NIH statistical analyses, the incidence rate of EC is rising in most countries worldwide [1]. Endometrial cancer patients are classified into two subtypes. Type I (endometrioid histology) and type II (serous or clear cell histology) differ dramatically in their clinico-pathologic characteristics. Type I EC is often associated with excessive exposure to endogenous or exogenous estrogens [2]. Estrogen receptor negativity has been correlated with the clinical stage, histological grade and patient survival, and has been found to be an independent predictor of poor prognosis [3–5]. Two isoforms of ER have been identified. ER-α mediates the responsiveness of the endometrium to estrogen but not ER-β [6]. Loss of ER-α expression is related to poor survival of EC patients, while ER-β status has not been correlated with clinico-pathological characteristics [3]. Progesterone is used to treat early stage and PR-expressing endometrial cancers in women wishing to preserve fertility. However, advanced or recurrent endometrial cancers respond poorly to progesterone. PR status is an important prognostic indicator that correlates well with treatment responses [7]. The isoform PR-B may play a more critical role in mediating the inhibitory effects of progesterone on cell growth [8]. Hormone receptor status, therefore, represents a crucial aspect of endometrial cancers .

PTEN, hMLH-1 and p53 are tumor suppressor genes (TSG) implicated in EC pathogenesis. PTEN is mutated in 30–50% of EC cases, making it the best known genetic alteration associated with EC. This tumor suppressor gene encodes a phosphatase that negatively regulates the PI3-kinase/Akt pathway that mediates cell-cycle arrest and apoptosis. Germline mutations in PTEN cause Cowden syndrome, which is characterized by hamartomas and increased susceptibility to breast, thyroid, and endometrial cancers [9–11]. In many EC cases, PTEN expression is suppressed or completely silenced by DNA hypermethylation [7]. Germline mutations of DNA mismatch repair genes have been identified as the cause of hereditary nonpolyposis colorectal cancer (HNPCC) as well as the familial endometrial cancers [12, 13]. Genetic or epigenetic inactivation of a member of this family, hMLH1, is considered an early event in endometrial carcinogenesis [7]. In contrast to PTEN and hMLH1, p53 mutation is found to be the most frequent genetic alteration in type II EC, presents in about 90% of serous carcinomas of the endotrium [14], in addition to its dominant role in cancers from many other organs.

Cell morphology, karyotype, population doubling time and tumorigenic potential in mouse xenograft are basic *in vitro* and *in vivo* characteristics of established cell lines. Additionally, information on p53 mutation and expression levels of ER, PR, PTEN and hMLH1 are required when choosing a suitable cell line model for EC study. Systematic characterization of these cell lines will also lead to a better understanding of EC pathogenesis.

2. Materials and methods

2.1. Cell lines and reagents

Human endometrial cancer cell lines AN3, KLE and HEC-1A, HEC-1B were purchased from American Type Culture Collection (ATCC, Rockville, MD). The Ishikawa cell line was generously provided by Dr. Masato Nishida (Kasumigaura National Hospital, Japan). All other cell lines were generously provided by Dr. Gottfried E. Konecny (Mayo Clinic, Rochester, MN). The cells were grown in the specific media listed in Table 1, containing 10% or 20% fetal bovine serum (Bio-Whittaker, Walkersville, MD), 100 μg/ml streptomycin, 100 units/ml penicillin, and 2 mM L-glutamine. Cells were maintained at 37 °C in an atmosphere containing 5% CO_2 and 100% humidity.

2.2. Morphology and population doubling time

The cells were grown to approximately 50% confluence. Representative pictures were taken with light microscopy under 20× magnification. Cells were plated in 6-well plates to achieve 10–20% confluence on day 0. Cell number in each plate was counted every 24 h to generate the growth curve. The growth curve was linearized by log 2

Table 1
Cell Line summary.

Cell line	Medium	PDT (hours)	ER-α	ER-β	PR PR-B	hMLH-1	PTEN	P53 Mutation	Tumorigenic Potential
Type I									
AN3	DMEM F12	31.4	Low	Low	Low	Low	High	+	Strong
ECC1	RPMI	22.3	High	High	High	Low	Low	+	Weak
EN	DMEM	18.9	Low	Low	High	High	High	+	Moderate
EN1	DMEM	22.4	Low	Low	Low	High	Low	−	Strong
EN11	DMEM 20% FBS	26.6	High	Low	*	Low	Low	+Intronic	Weak
HEC1A	DMEM F12	29.2	Low	Low	Low	High	High	−	Strong
HEC1B	DMEM F12	26.8	Low	Low	Low	High	High	−	Strong
Ishikawa	MEM	28.8	High	High	High	Low	Low	+	Weak
KLE	DMEM F12	34.3	Low	Low	Low	High	High	−	Strong
MFE280	DMEM 20% FBS	35.6	High	Low	High	High	High	−	Moderate
MFE296	DMEM	25.1	Low	Low	High	Low	High	+	Strong
MFE319	DMEM 20% FBS	41.4	High	Low	High	Low	High	+	Moderate
Type II									
ARK1	RPMI	20.0	High	Low	Low	Low	High	−	Strong
ARK2	RPMI	20.7	Low	High	Low	Low	Low	−	Strong
HEC155	DMEM 20% FBS	25.8	Low	High	Low	High	High	−	Weak
SPEC2	RPMI	31.8	High	Low	Low	High	High	+ Deletion	Strong

The population doubling time (PDT) for each cell line was listed. The ER-α, ER-β, PR, PR-B, and PTEN mRNA levels were classified into low and high groups. hMLH1 levels were equally divided between low and high. The details of P53 mutation and tumorigenic potential were further detailed in Tables 2 and 3, respectively. * In EN11, PR-B transcription level is low, but total PR level is high.

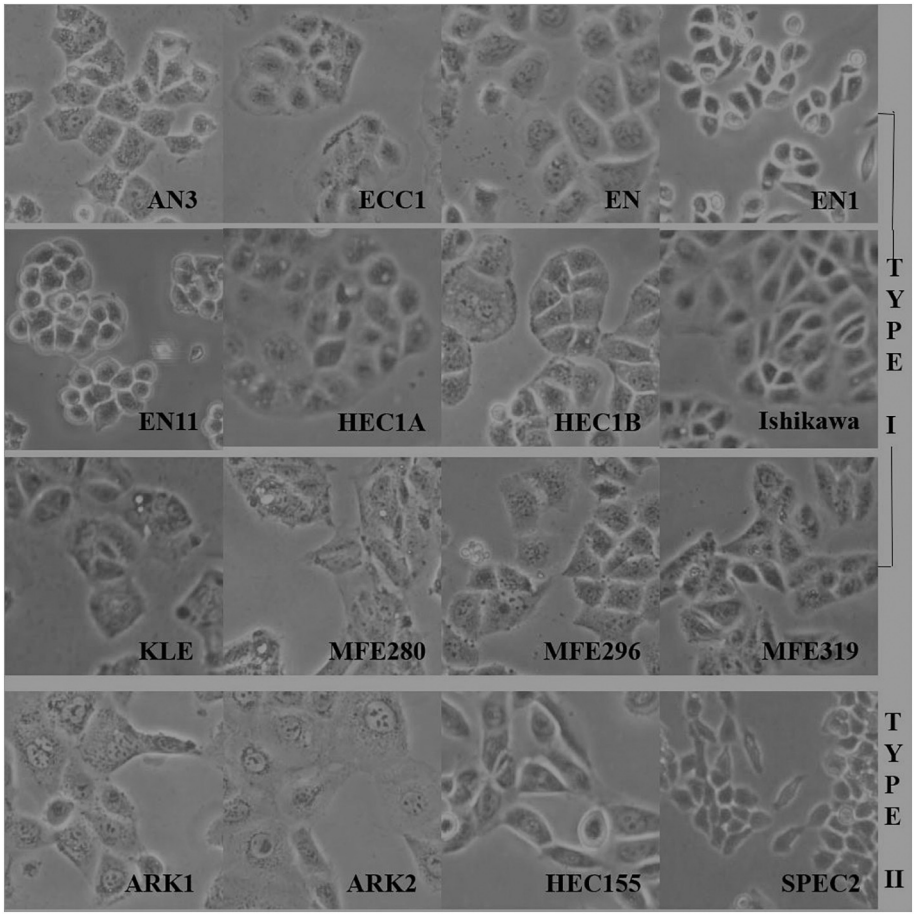

Fig. 1. Tissue culture morphology. Light microscopy pictures of cells under 20 x magnification. The top three rows included the type I endometrial cancer cell lines, and the last row included the type II cell lines.

(ratio day X to day 0). The population doubling time (PDT) was calculated from the slope of this linear regression line.

2.3. Karyotype analyses

Cells were grown on coverslips in small Petri dishes in the appropriate media. To produce metaphase spreads, cells were incubated with standard medium containing ethidium bromide (10 microgram/ml) for 20 min. This was followed by the addition of colcemid at 60 ng/ml to prevent spindle fiber formation and to arrest nuclear division at metaphase. After 20 min in colcemid, the cells were placed in a hypotonic solution (75 mM KCl) and incubated for 30 min at room temperature to achieve expansion of the cells. Several drops of Carnoy fixative (methanol:acetic acid, 3:1) were added to each Petri dish to begin the fixation process. After 5 min, the cells were gently washed in fixative solution three times. The coverslips were then dried in a warm, humid chamber to allow optimal chromosome spreading. The chromosomes were analyzed following G banding.

2.4. RNA isolation, cDNA synthesis, and quantitative real-time PCR

Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA). cDNA was synthesized with 1 μg RNA using the SuperScript™ kit (Invitrogen, Carlsbad, CA). The 20 μl reverse transcription products were diluted to 100 μl, and 2 μl was used for each real-time PCR. Real-time PCR was performed using the Cyber Green Master Mix (Invitrogen, Carlsbad, CA). The sequences of primers and the sizes of the corresponding amplicons are: PR-B-forward: 5′-gactgagagcttcacagtat, PR-B-reverse: 5′-tctcctaactcggggagttct (187 bp); ER-alpha-forward: 5′-atgagagctgccaacctttg, ER-alpha-reverse: 5′-ggttggtcagtaagcccatc (190 bp); ER-beta-forward: 5′-tggagtctggtcgtgtgaag, ER-beta-reverse: 5′-acttcaccattcccacttcg (167 bp); PR-forward: 5′-atgagccggtccgggtgcaag, PR-reverse: 5′-gccacccagagcccgaggttt (243 bp); GAPDH-forward: 5′-gaaggtgaaggtcggagtc, GAPDH-reverse: 5′-gaagatggtgatgggatttc (225 bp). To verify the PCR specificity, an aliquot of final PCR products was resolved by agarose gel electrophoresis, and a single DNA band with the predicted size indicated specific amplification. The PCR conditions are: pre-denaturing at 94 °C for 10 min, followed by 40 cycles of amplification of denaturing at 94 °C for 15 s, annealing at 56 °C for 30 s, and extension at 72 °C for 30 s. Internal control reactions on the GAPDH (reference gene) mRNA were performed side by side for each sample.

2.5. P53 mutation screening

The four pairs of primers used for mutation screen from exons 5–8 of the p53 gene are as follows:

Exon 5: 5'- GCC GTC TTC CAG TTG CTT and 5'- CAA CCA GCC CTG TCG TCT CT -3';

Exon 6: 5'- GGG GCT GGA GAG ACG ACA and 5'- TCC TCC CAG AGA CCC CAG TT;

Exon 7: 5'- CTT GCC ACA GGT CTC CCC AA and 5'- AGG GGT CAG AGG CAA GCA GA;

Exon 8: 5'- AAA TGG GAC AGG TAG GAC and 5'- AAG TGA ATC TGA GGC ATA AC;Samples used for mutation screening and sequencing were amplified in 12.5 μl reaction volumes containing 10 ng of genomic DNA, 25 pmoles of sense and antisense primers for each exon, dNTPs (Perkin-Elmer, Foster City, CA), 0.1 μl of *Taq* polymerase (AmpliTaq Gold: Perkin-Elmer), 1 × concentration of the buffer provided by the

manufacturer, and 2.0 mM of $MgCl_2$. PCR amplification was performed for 35 cycles: 94 °C for 30 s, the optimized annealing temperature for 45 s, and 72 °C for 45 s. Final extension was performed at 72 °C for 10 min, with initial denaturation at 95 °C for 9 min. The PCR products were analyzed with denaturing high performance liquid chromatography (DHPLC), and samples with abnormall DHPLC profile were sequenced to identify the mutation.

2.6. Mouse xenograft model

Six-week-old female immunodeficient athymic, NCR Nu-Nu mice were purchased from NCI/NIH and maintained under pathogen-free conditions with irradiated chow. 10 million cells in 0.2 ml PBS were injected subcutaneously into the right flank of each mouse. Five mice were injected with each cell line. Thirty days after the inoculation, animals were sacrificed by carbon dioxide asphyxiation. Tumors were carefully dissected from the mice and weighed.

2.7. Data analyses

The threshold cycle number (CT) measured by real-time PCR was used to calculate relative PR, PR-B, ER-α and ER-β, hMLH-1 and PTEN mRNA levels. The CT reading of each target gene (CT_{gene}) was normalized against that of the reference gene GAPDH using the formula $\Delta CT = CT_{gene} - CT_{GAPDH}$. The difference between the gene of interest and GAPDH was further converted to relative fold ($F = 2^{\Delta CT}$). Relative mRNA levels and standard error were calculated and presented. Gene expression data for the 16 cell lines were subject to univariate linear regression analysis to identify potential correlations. Certain variables were converted from continuous to nominal to explore the potential correlations further. Due to the small sample sizes, Fisher's exact test was used to determine statistical significance. $p < .05$ was used as the criteria for significant difference.

3. Results

3.1. Tissue culture morphology

In general, the cells displayed typical features of transformed cells including the pleomorphism, hyperchromatic nuclei, loss of normal polarity, disorganized cell-cell alignment, an increased nuclear to cytoplasmic ratio, and active mitosis. As can be seen in representative photographs in Fig. 1, 13 of the 16 cell lines were characterized by large, polygonal cells, with large, round nuclei, and growth into a flat sheet. The exceptions were EN1, EN11, and SPEC2. These cell lines were characterized by smaller, round or polygonal cells with small nuclei, scant cytoplasm, and growth in clusters. This growth pattern may be related to the poor attachment of cells to the plate.

3.2. Population doubling time

Cell growth was measured in the linear phase. The population doubling time (PDT) varied between 18.9 h for EN to 35.6 h for MFE280 (Table 1). In univariate analysis, the PDT of the cell lines was not significantly associated with the histologic type, p53 mutation, tumorigenic activity, or mRNA levels of PR, PR-B, ER-α, ER-β, hMLH-1, or PTEN.

3.3. mRNA levels of PR, PR-B, ER-α, ER-β, hMLH-1, and PTEN

Relative mRNA levels were plotted in Fig. 2. The mRNA levels of PR and PR-B were uniformly low in type II endometrial cancer cell lines, while in type I cells, the PR and PR-B levels displayed dramatic variability. The mRNA level of PR-B was linearly correlated with that of PR ($p < .001$). The ratios of PR-B to PR mRNA ranged from 0.22 (AN3) to 1.68 (ARK1). There was no obvious difference in ER-α and ER-β mRNA levels among type I and II cells. The mRNA levels of ER-β was linearly correlated with that of ER-αin type I ($p < .001$) but not in type II cells ($p = .58$). In addition, expression levels of total PR mRNA was linearly correlated with that of ER-α + ER-β($p < .001$) among type I cell lines.

It is noteworthy that ECC1 was remarkable for expressing a high hormone receptor level. Total PR, PR-B, ER-α, and ER-β receptor levels were 100-fold, 500-fold, 10 to 20 fold, and 20 to 60 fold higher than the two cell lines with the next highest receptor levels (MFE280 and MFE319).

hMLH-1 mRNA levels were the lowest in AN3, EN11, MFE296, MFE319, and ARK2 cell lines. hMLH-1 mRNA level was not significantly correlated with the hormone receptor or PTEN levels. PTEN levels were the lowest in ECC1, EN1, EN11, Ishikawa, and ARK2. PTEN levels were not significantly correlated with those of PR, PR-B or ER-α levels. PTEN expression was more uniformly suppressed in type II than type I cells, while hMLH1 did not show this pattern. For the purposes of this investigation, the five cell lines with the lowest PTEN mRNA levels were classified as “low PTEN,” and the four cell lines with the highest ER-β levels were classified as “high ER-β.” High ER-βlevels appeared to be potentially associated with lower PTEN levels, but the association did not reach statistical significance ($p = .0632$).

As summarized in Table 1, ER-α, ER-β, PR, PR-B, and PTEN mRNA levels were classified into low and high levels. Since PR and PR-B mRNA levels were closely associated (with the exception of EN11 in which PR level was high while PR-B level was low), results were reported in the same column.

3.4. Karyotype analysis

Among the five endometrial cancer cell lines examined, all were found to have severely abnormal karyotypes (Fig. 3, Table 2). These cell lines have various aneuploidy and displayed multiple and clonal, anomalies in chromosomal structure, including the addition or deletion of large segments, and the presence of marker chromosomes. Overall, Ishikawa cells have relatively fewer abnormalities, while HEC1B and KLE contain more alterations. The chromosomal abnormalities tend to be diversified, and no apparently common change could be recognized.

3.5. p53 mutation analysis

95% of the p53 point mutations in cancers occur in the central DNA binding domain [15]. These hotspot mutations in exons 5–8 were detected in 8 out of 16 cell lines. The details of the p53 mutations were listed in Table 3. Two cell lines were found to have two mutations (MFE296 and MFE319). Of the type II cell lines, only SPEC2 demonstrated a p53 mutation (deletion of codon 218). EN1 harbored what has been reported to be a natural polymorphism in codon 213. EN11 demonstrated an intronic mutation of unclear significance. Presence or absence of p53 mutations were not correlated with any of the other markers studied.

3.6. Tumorigenicity in vivo

Two parameters were considered in evaluating the tumorigenic potential of each cell line: mean tumor mass > 0.1 g, and presence of tumor weighing > 0.01 g in more than two of five mice. The tumorigenic potential was defined as strong when both criteria were met, moderate when one was met, and weak when neither was met (Tables 1, 4 and Fig. 4).

Correlation between tumorigenic potential and other variables was determined by dividing cell lines in to group with “strong” tumorigenic potential and “other.” Low PR-B was associated with strong tumorigenic potential in type I and II cells ($p = .035$ by 2-tail Fisher's exact test). In type I cell lines, low ER-α was also associated with strong tumorigenic potential ($p = .0152$ by 2-tail Fisher's exact test), but no such association was found in type II cell lines. Other variables including PR,

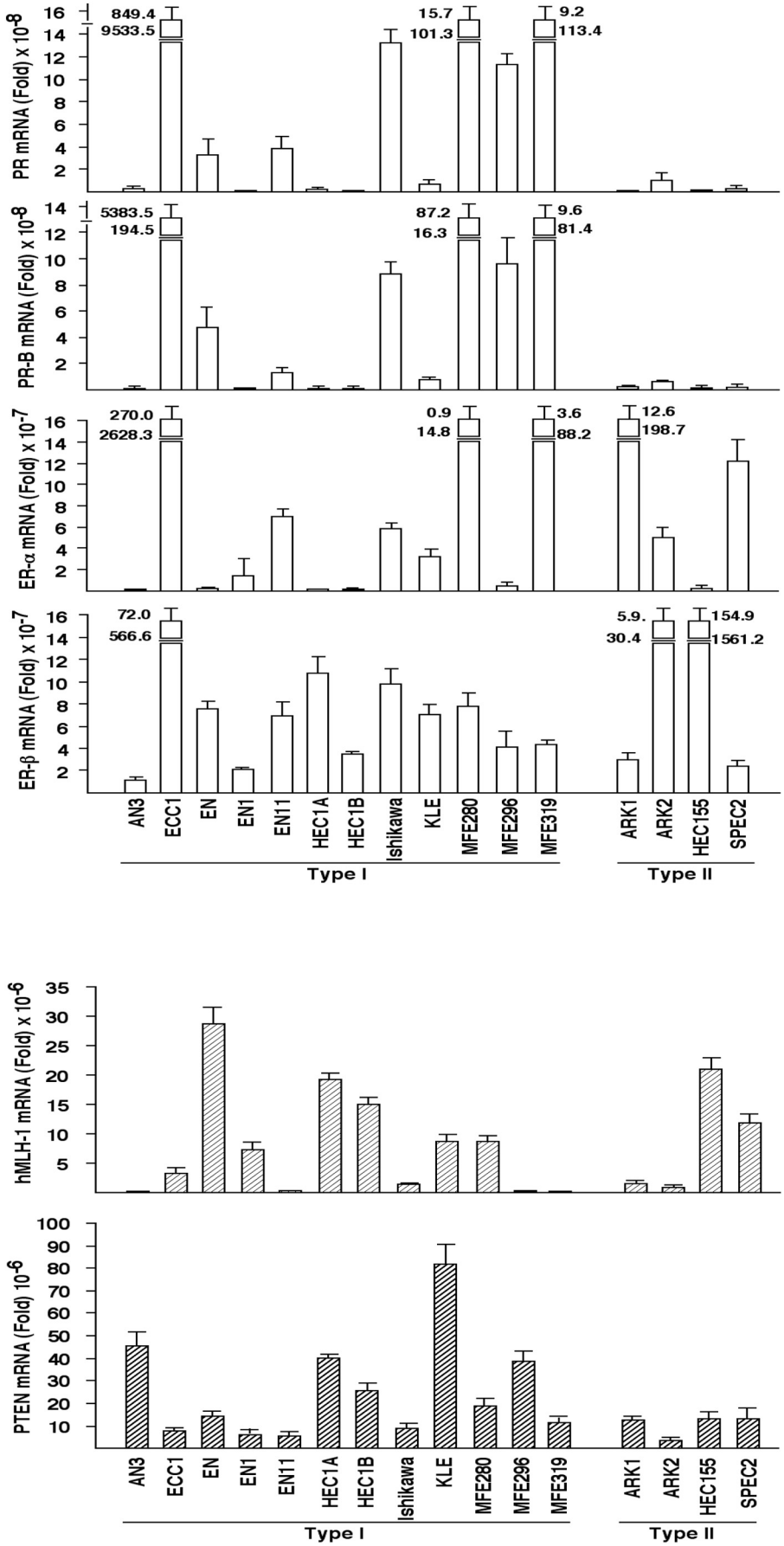

Fig. 2. mRNA analysis of PR, PR-B, ER-α, ER-β, hMLH-1, PTEN. Relative mRNA level of the target genes was measured with real-time PCR, using GAPDH as reference gene. The values that are off the scale are marked with the mean and standard error.

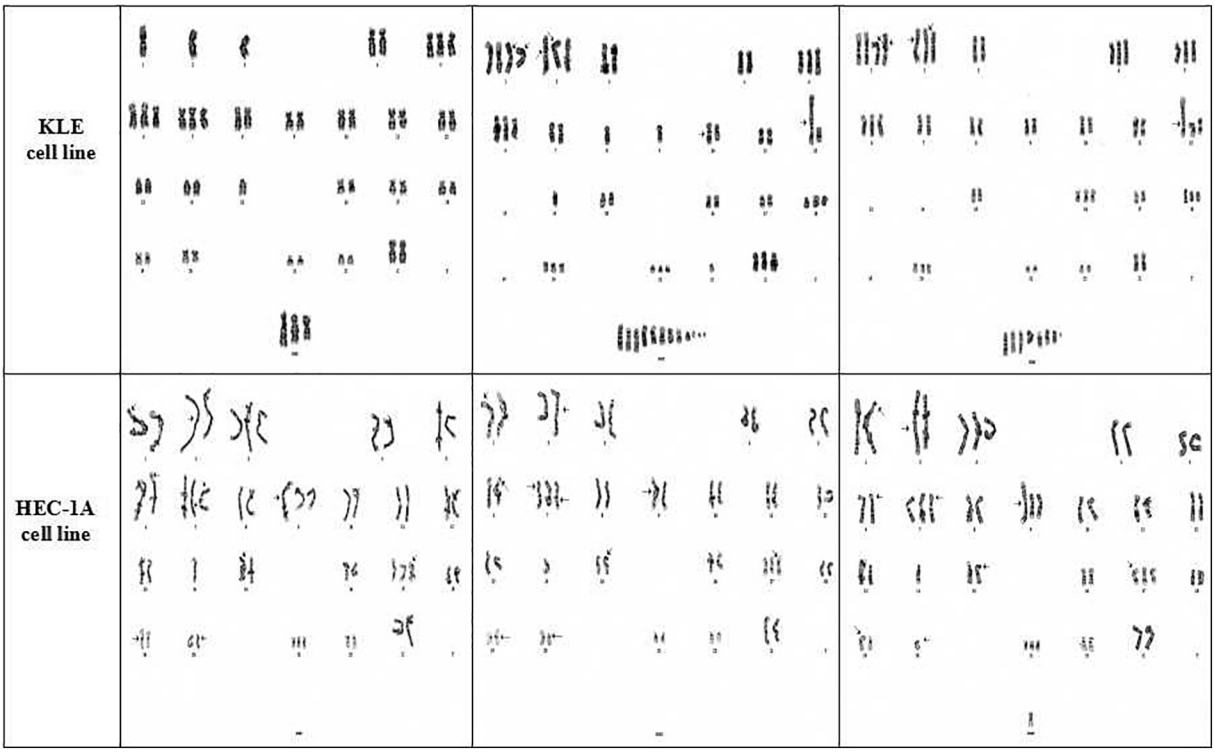

Fig. 3. Mean tumor mass in mouse xenograft. Mean tumor mass and standard error were shown for each from each cell line were plotted.

ER-β, ERα + β, hMLH1, PTEN levels, PDT, and p53 mutations were not correlated with tumorigenic potential.

Since ER-α, and PR-B expression appeared to be differentially associated with tumorigenic potential, the combined effect of PR-B and ER-α was examined by creating a variable of PR-B_ER-α. Low PR-B and ER-α was found to be associated with strong tumorigenic potential in type I ($p = .0152$) but not in type II cells.

4. Discussion

Analyses of ER-α and ER-βlevels showed that ER-α is the predominant isoform of ER. It accounted for approximately 80% of the total ER. The mRNA levels of the two ER isoforms were correlated in type I cell lines. Previous studies have evaluated hormone receptor levels in endometrial cancer tissues by immunohistochemical staining and found ER-αdomination [3, 16, 17] as well as the dependent expression of the two isoforms [16]. In this study, we found that PR and PR-B mRNA levels were low in 30–40% of type I cells and 100% of type II cells. Among the type I cell lines that had low PR-B levels, AN3, HEC1A, HEC1B, and KLE have been found to contain high DNA methyltransferase 3B (DNMT3B) levels [18]. In KLE and HEC1B, treatment with epigenetic modulators reversed the PR-B hypermethylation and increased the PR-B mRNA expression [19]. In endometrial cancer tissues (predominantly type I tumors), 87% of the samples with loss of PR-B expression showed biallelic PR-B hypermethylation [20]. These studies suggest that epigenetic silencing may be responsible for the low PR-B expression levels in type I endometrial cancers as well as cell lines. The correlation between total ER and PR in these cell lines suggests that a common mechanism, *e.g.*, upregulation of DNA methyltransferase and epigenetic silencing, may commonly exist for steroid receptor inactivation among some EC cell lines.

ECC-1 cell line preserves its steroid hormone receptor expression and hormone sensitivity, much like the Ishikawa cell line [19]. The real-time PCR result showed striking high levels of all hormone receptor levels in ECC-1 compared to the other cell lines. MEF280 and MFE319 also contain relatively higher PR, PR-B, and ER-α levels, but their ER-β levels were comparatively lower. This cell line could be a suitable model for studying the hormonal responses in cell culture systems.

PTEN is mutated in 30–50% of endometrial cancer cases, making it the most common genetic alteration associated with this disease. Epigenetic modifications represents an additional mechanism of PTEN inactivation in approximately 20% of endometrial cancers [7]. This study identified five cell lines with low PTEN mRNA. Our data also suggests that high ER-β level tend to be associated with low PTEN

Table 2
Karyotype of five EC cell lines.

Cell Line	Karyotype	Conditions
AN3	48–50,XX	dic(X;11)(q22;p11.2) [2],add(3)(p25)[28],+5[29],+6[28],+7[25],+8[16],+11[3],+18[2],-21[7], +1-4mar[cp30]
HEC-1A	46–50,X,-X	add(1)(p36.1),ins(2;?),(q21;?),+3,-5,del(6)(p23),+add(7)(p15),add(7)(q22),+9,der(9;14)(q10; q10),15 ph+,-16,+17,add(17)(p11.1),i(19)(q10),-20,add(20)(p11.2),+21,+1-3mar[cp30]
KLE	55–60,XX,-X,-X,+X	+add(1)(p32),+der(1;14)(q10;q10),+add(2)(p13) × 2,+4,+5,+6,-8,-9,add(10)(q22),add(12)(p13), −13,-13,-14,-15,+16,+16,+18,-19,-19,-20,+20,+21,-22,+8-11mar,+1-3r[cp18]/48,XX,-1,-2,-3, +5, +6,+7,-15,+3mar [1]
HEC-1B	80–82.XX,-X,-X	add(1)(p36.1) × 2,-2,ins(2;?)(q21;?),+3,i(3)(q10),-4,-4,?add(4)(q31.3),-5,-6,del(6)(q23),der(6) del(6)(p23)t(3;6)(p21;p23) × 2,-8,-8,-9,add(9)(q32),-10,-11,-12,-13,-14,-15,15 ph+,-16,-17, add(17)(p11.2),i(17)(q10),-18,-19,i(19)(q10),-20,-20,-21,-22,-22,add(22)(p11.2),+4-7mar, +1r[cp29]
Ishikawa	44–47,X,-X	der(3)add(3)(p21)add(3)(q25),-4,add(4)(q31.3),add(5)(p15.3),add(7)(q32),der(9)t(9;14)(p22; q11.2),-10,-11,-14,-15,-16,-17,add(19)(p13.3),-20,-21,+3-8mar

Table 3
p53 mutation analysis.

	Exon 6		Exon 7		Exon 8	
Cell Line	DHPLC	Sequence	DHPLC	Sequence	DHPLC	Sequence
Type I Cell Lines						
AN3	+	G638A; Arg213Gln				
ECC1			+	A736G; Met246Val		
EN					+	A818G; His273Arg
EN1	+	A639G; polymorph intron + 2 T– > C				
EN11						
HEC1A						
HEC1B						
Ishikawa			+	A736G; Met246Val		
KLE						
MFE280						
MFE296	+	A659G; Tyr220Cys			+	C916T; Arg306Stop
MFE319					+	C817T; His273Cys A818G; His273Arg
Type II Cell Lines						
ARK1						
ARK2						
HEC155						
SPEC2	+	652del GTG; Val218 deletion				

DHPLC screen in Exon 5 for all cell lines was negative. The sequencing results for DHPLC + cell lines showed the nucleotide change on the left; and amino acid (codon) change on the right.

Table 4
Mouse xenograft activity.

Cell Line	Mean Tumor Mass	Standard Error of the Mean	# of Mice out of total of 5 Tumor Size > 5 mm or Weight > 0.01 g	Tumorigenic Potential
Type I				
AN3	3.24	2.26	3	strong
ECC1	0.04	0	1	weak
EN	0.03	0.006	3	moderate
EN1	0.56	0.10	5	strong
EN11	0.01	0	1	weak
HEC1A	0.88	0.25	5	strong
HEC1B	0.3	0.037	5	strong
Ishikawa	0.02	0.006	2	weak
KLE	0.22	0.087	5	strong
MFE280	0.075	0.037	4	moderate
MFE296	0.23	0.097	5	strong
MFE319	0.02	0.006	3	moderate
Type II				
ARK1	0.91	0.22	4	strong
ARK2	0.57	0.18	4	strong
HEC155	0.0083	0.006	1	weak
SPEC2	0.29	0.13	4	strong

The tumorigenic potential of each cell line is determined by two criteria: whether the mean tumor mass is > 0.1 g, and whether more than half of the five mice yield tumors weighing > 0.01 g. The tumorigenic potential is strong when both criteria are met, moderate when one is met, and weak when neither is met.

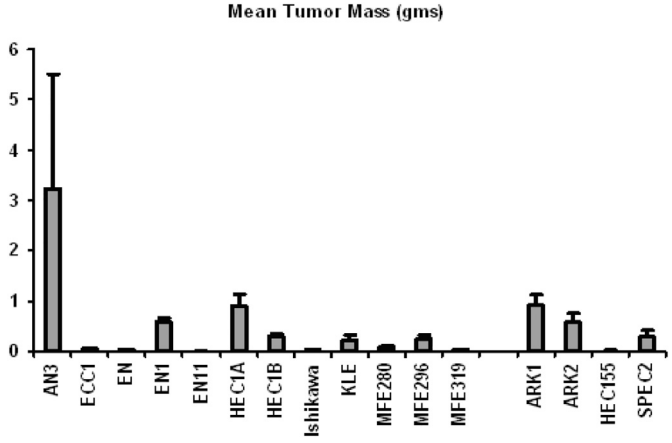

Fig. 4. Representative karyotype of KLE and HEC-1A cell lines. Note the different karyotypes of different clones of the same cell line.

levels, even though the *p*-value did not reach a statistical significance possibly due to the small sample size. Interestingly, other investigators have reported that estradiol down-regulates PTEN activity [21] and increases Akt phosphorylation in Ishikawa cells [22]. Estrogen can also stimulate the phosphatidylinositol 3-kinase (PI3K) pathway which involves PTEN and Akt acitivties [23]. These endometrial cancer cell lines can be powerful tools in elucidating the mechanism of estrogen regulation of the PTEN pathway. A few questions can be answered using appropriate cell lines: which ER isoform is responsible for estrogen-dependent PTEN/Akt regulation; what are the mechanisms of PTEN inactivation by estrogen (*e.g.*, protein phosphorylation, somatic mutation, or epigenetic inactivation); what are the downstream effects on apoptosis and tumorigenesis if the estrogen regulation of PTEN pathway is disrupted.

The results of p53 mutation screening were somewhat surprising. It is known that the p53 mutation is a frequent genetic alteration in type II cancers, but very rare in type I cancers [24]. However, in our study, screening using the DHPLC method identified only SPEC2 contains a deletion in p53, but not in other type II cell lines. The in-frame deletion of codon 218 in exon 6 has been reported in breast carcinoma and been associated with poor prognosis [25]. The sensitivity of DHPLC detection of p53 mutation is fairly high, reaching 95–100% [26]. The low rate of p53 mutation suggested that other pathways such as Her2/neu amplification, or p16 inactivation might be implicated in these cell lines. The results of karyotype analysis and p53 mutation together with hormone receptor expression pattern and morphology comparison strongly suggested an independent nature of these cell lines, excluding the possibility of cross-contamination between cell lines.

The criteria used to classify tumorigenic potential ensured that cell lines designated for having strong tumorigenicity would generate measurable tumors within a reasonable period of time for *in vivo* study purposes. Recent studies suggest that PR-B plays an important role in mediating the inhibitory effects of progesterone on cell growth [8]. The observed association between low PR-B and strong tumorigenic

potential in these cells lines is consistent with the thought on the function of PR-B in EC. Most of the type I cell lines with low PR-B expression and strong tumorigenic potential will be good candidates for studying the effect of PR-B status on tumor response to progesterone treatment.

Previous experiments have demonstrated that in the ER-α and ER-β double knockout mice, the endometrium became insensitive to estrogen. Estrogen responsiveness of the endometrium is preserved in ER-β knockout [6]. When HEC1B cells are transfected with ER-α antisense oligodeoxyribonucleotides (ODN), the cells lose the ability to proliferate in response to estradiol, but not when the ER-β antisense ODN is transfected [27]. With these results in mind, it was intriguing to see that in our study low ER-α levels were associated with strong tumorigenic potential in type I cell lines. While the association that we identified suggests implications of ER-αin EC development, its exact pathologic role requires further investigation to clarify. It should be pointed out that, while this study covers almost all the endometrial cancer cell line commonly used for laboratory studies, the investigation is limited to only a few aspects of biological properties and genes. Comprehensive approach such as those of genomics, transcriptomics, and proteomics can be done in future to obtain more useful information.

In summary, this study systematically characterized sixteen commonly used endometrial cancer cell lines from *in vitro* and *in vivo* standpoints. The information on the tumorigenic potentials, the expression levels of hormone receptors, hMLH1, and PTEN provide references for investigators when choosing the appropriate cell lines to study the relevant pathways in endometrial cancer. The significant correlations identified among the hormone receptor levels, tumor suppressor genes, and tumorigenic potential provide circumstantial evidence in support of the involvement of ER and PR in the tumorigenesis of endometrial cancer.

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