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Changes in Expression of Estrogen Receptors α and β in Relation to Progesterone Receptor and pS2 Status in Normal and Malignant Endometrium

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To clarify changes in estrogen receptor (ER) α and ERβ during endometrial tumorigenesis, 48 endometrial carcinomas (endometrioid type), as well as 40 samples of normal endometrial tissue, were investigated using a combination of reverse-transcription and polymerase chain reaction with Southern blot hybridization and western blot assays, and the results were compared with findings for progesterone receptor (PR) and pS2 mRNA status. In addition, 166 carcinomas were also examined for immunohistochemistry, along with 171 normal specimens. Relative amounts of ERα at both mRNA and protein levels were significantly greater than those for ERβ in normal and malignant endometrial lesions. ERα mRNA showed a stepwise decrease from normal or grade (G) 1 through to G3 tumor lesions, in line with changes in the protein levels, in contrast to ERβ mRNA or protein expression, which did not alter, suggesting a shift in the ratio of the two ER subtypes during endometrial tumorigenesis. PR mRNA expression was significantly correlated with ERα, but not ERβ mRNA status. Although significantly higher expression of pS2 mRNA or protein was observed in carcinomas than in the normal cases, there was no apparent association with the ER status. The findings suggest that alteration in estrogen signaling pathways may occur during endometrial tumorigenesis, and provide evidence that ERα expression may play an important role in the regulation of PR, but not pS2 expression in normal and malignant endometrium.

Key words: ERα — ERβ — PR — pS2 endometrial carcinoma

The steroid hormone estrogen plays an important role in the growth, differentiation, and function of many target tissues, in particular in the male and female reproductive systems. In the endometrium, hyperestrogenism is considered to the major risk factor for development of malignancy, with or without hyperplastic lesions.[1)]

Estrogen receptor (ER) α, a member of the superfamily of steroid and thyroid hormone, retinoic acid, and vitamin D receptors, is a ligand-induced transcriptional factor composed of different structural and functional domains.[2, 3)] The transactivation factor domains (TAF-1 and TAF-2) are located within the NH_2-terminal (A-B region) and the COOH-terminal (E) sites, whereas the DNA and ligand-binding domains reside in the C and E regions, respectively. Exon 5 deletion splicing of the ER transcript produces a truncated protein which lacks both the TAF-2 and ligand-binding regions, but retains its DNA binding and TAF-1 sequences.[4)] Previous studies demonstrated that overexpression of the variant is associated with an increase in estradiol-independent growth, resistance to tamoxifen, and enhanced progesterone receptor (PR) expression.[4, 5)]

Recently, a novel ERβ subtype has been discovered in human, rat, and mouse tissues, with a high degree of conservation of the DNA-binding domain (96%), and of the ligand-binding domain (58%), as compared to the amino acid sequence of the ERα protein.[6)] Although changes in the expression of two ER subtypes have been documented during breast and ovarian tumorigenesis,[7, 8)] little is known about the endometrial carcinoma case.

Several estrogen-inducible genes, including PR and pS2, have been described as markers of estrogen responsiveness in human hormone-dependent tumors.[4, 9)] In the present study, we investigated the possible contribution of changes in expression of ERα and ERβ at both mRNA and protein levels during endometrial tumorigenesis. The results were also compared with PR and pS2 status, and several clinicopathological factors.

MATERIALS AND METHODS

Cases A total of 48 endometrial carcinomas (endometrioid type), surgically resected at the Kitasato University Hospital during the period from 1995 to 1998, were investigated for mRNAs. Forty normal endometrial samples (17 cases with a regular menstrual cycle and 23 postmenopausal cases) obtained by total hysterectomy for non-malignant diseases were also examined. Tissues were snap-frozen immediately after surgical excision and stored at −80°C until use. Histological diagnosis was performed according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO). The carcinoma cases comprised 29 grade (G) 1, 13 G2, and 6 G3 lesions.

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Twenty-eight cases were classified as FIGO stage I and 20 as stages II/III/IV, while 22 showed upper myometrial invasion (upper half of myometrium) and 8 were positive for lymph node metastasis.

For immunohistochemical examination, specimens of 166 endometrial carcinomas (endometrioid type; 104 of G1, 35 of G2, and 27 of G3) were obtained by hysterectomy during 1988 to 1998, along with 42 samples of postmenopausal (atrophic) endometrium adjacent to carcinomas and 129 normal biopsy specimens (48 proliferative and 81 secretory stages). These tissues were routinely fixed in 10% formalin and processed for embedding in paraffin wax. Of cases available for investigation of the clinicopathological factors, 110 were categorized in stage I and 20 in stages II/III/IV. In addition, 90 demonstrated upper myometrial invasion and 21 featured metastasis to lymph nodes.

RT-PCR assay Total cellular RNAs were extracted using Isogen (Nippon Gene Co., Tokyo) and cDNAs were synthesized from 5 μg of total RNA using RAV-2 reverse transcriptase (TaKaRa, Shiga) in the presence of random primers (TaKaRa) and a ribonuclease inhibitor (TaKaRa) in a 20 μl reaction volume at 42°C for 60 min.

One microliter of cDNA solution was amplified by Taq polymerase (TaKaRa) in a volume of 10 μl. For mRNA detection of *ERα*, *PR*, and *pS2* genes, the PCR procedure was performed with 35 cycles of denaturation at 94°C for 0.5 min, annealing at 55°C for 0.5 min and extension at 72°C for 0.5 min, with a pre-denaturing time of 2 min and a final extension time of 5 min. The primer sequences were: ERα, 5′-CAGGGGTGAAGTGGGGTCTGCTG-3′ and 5′-ATGCGGAACCGAGATGATGTAGC-3′[10]; PR, 5′-GCTCCCGCAGCTCGGCTACC-3′ and 5′-ACAGCCTGATGCTTCATCCC-3′[11]; pS2, 5′-ACCATGGAGAACAAGGTGAT-3′ and 5′-AAATTCACACTCCTCTTCTG-3′. For ERβ mRNA analysis, nested PCR was performed according to the methods described by Vladusic *et al.*[12] The primers for the first-round PCR were: 5′-TCACTTCTGCGCTGTCTGCAGCG-3′ and 5′-CCTGGGTCGCTTGACCAGA-3′ and those for the second-round PCR were: 5′-GGCCAAGAGAAGTGGCGGCCACG-3′ and 5′-AAACCTTGAAGTAGTTGCCAGGAG-3′.

Competitive RT-PCR assays for the two ER subtypes were also conducted according to the methods described by Pujol *et al.*,[13] using a common forward primer (5′-AAGAGCTGCCAGGCCTGCC-3′) for both genes and different reverse primers for ERα (5′-TTGGCAGCTCTCATGTCTCC-3′) and for ERβ (5′-GCGCACTGGGGCGGCTGATCA-3′).

As a negative control, water was used instead of template cDNA for each examination. To examine the quality and quantity of the synthesized cDNA, the *β-actin* gene was also amplified as previously described.[14]

Southern blot hybridization A 10 μl aliquot of each PCR reaction mixture was electrophoresed in a 3% agarose gel and transferred to a Hybond N nylon membrane (Amersham, Tokyo) with 10× SSC solution overnight. Filters were hybridized overnight with digoxigenin-labeled exon-specific probes using DIG Easy Hyb solution (Boehringer Mannheim, Tokyo). The sequences of the oligonucleotide probes were: probe ERα-1 (5′-AAACGCTCTAAGAAGAACAGCCTGGCCTTG-3′), probe ERβ (5′-TGAGCCCCGAGCAGCTAGTGCTCACCCTCC-3′), probe PR (5′-CCGCAGGTCTACCCGCCCTATCTCAACTAC-3′), and probe pS2 (5′-GAAAGACAGAATTGTGGTTTTCCTGGTGTC-3′). For detection of competitive PCR products, probe ERα-2 (5′-CTCCGCAAATGCTACGAAGTGGGAATGATG-3′) and probe ERβ were also applied. Hybridization signals were detected with a DIG Luminescent Detection kit (Boehringer Mannheim). The conditions used for hybridization, washing, and detection were in line with the manufacturer's recommendations. Hybridization for *β-actin* gene transcripts was also performed as previously described.[14]

Quantitation of hybridization signals was achieved by densitometric analysis using NIH Image version 1.58 software, according to the method described by Dotzlaw *et al.*[15] Briefly, to control for variations between experiments, a value of 100% was assigned to the case exhibiting the highest signal measured, and all signals were expressed as a percentage of this signal. The relative amounts were calculated by normalization to the hybridization signals for β-actin in each case: the value for mRNA signals of the former was divided by that for the latter.

Immunohistochemistry Immunohistochemistry was performed using a combination of microwave-oven heating and the standard streptavidin-biotin-peroxidase complex (LSAB kit; Dako, Copenhagen, Denmark) methods. For detection of ERβ immunoreactivity, biotinylated horse anti-goat IgG (Vector Lab., Burlingame, CA) was used as the secondary antibody. The antibodies used were anti-ERα mouse monoclonal antibody (×80 dilution, Novocastra Lab. Ltd., Newcastle, UK), anti-ERβ (N-19) goat polyclonal antibody (×200 dilution, Santa Cruz Biotechnology, Santa Cruz, CA), anti-PR mouse monoclonal antibody (×80 dilution, Novocastra Lab. Ltd.), and anti-pS2 protein rabbit polyclonal antibody (×1000 dilution, Novocastra Lab. Ltd.).

Scoring of the immunohistochemistry results was carried out on the basis of both the distribution of immunopositive cells and the immunointensity, as previously described.[16, 17] Immunoreactivity scores were generated by multiplication of the values for the two parameters.

Western blot assay Tissue samples were homogenized in 0.01 *M* phosphate-buffered saline (PBS) and the supernatants were used for western blot assays. Twenty-microgram aliquots of proteins were separated by 10% sodium

dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and then electroblotted onto Immobilon-P (Millipore, Tokyo). After blocking, the membranes were incubated overnight at 4°C with optimum dilutions of primary anti-ERα mouse monoclonal antibody (×500 dilution, Novocastra Lab. Ltd.). Binding was visualized using the Western blot Chemiluminescence Reagent ("NEN" Life Science Products, Boston, MA).

Statistics Comparative data were analyzed using the Mann-Whitney *U* test, the Pearson's correlation coefficient, and the χ^2 test. The cut-off for statistical significance was set as $P<0.05$.

RESULTS

ERα, ERβ, PR, and pS2 mRNA expression Two different sizes of RT-PCR products for ERα transcripts were observed in normal and tumor samples. The larger band (483 bp) and smaller fragment (344 bp) corresponded to the expected sizes for wild type (Wt) and exon 5-deleted (del 5) variant forms, respectively. The latter was detected only in the presence of the former. Wt ERα transcripts with del 5-variants were detected in 41 (85.4%) of 48 tumors and in all normal tissues. By nested PCR assay, amplicons for ERβ transcripts were also generated as two different fragments, the Wt (429 bp) and del 5-variant (290 bp) forms. In the informative cases, Wt ERβ expression with or without the variant form was observed in 22 (64.7%) of 34 tumors and in 25 (64.1%) of 39 normal cases. The primer sets for *PR* or *pS2* genes produced bands with molecular weights of 174 bp and 246 bp, respectively, positive in 47 (97.9%) and 43 (89.6%) of 48 tumors, and all and 25 (64.1%) of 39 normal samples (Fig. 1).

The relative Wt ERα mRNA values were significantly lower in G3 carcinomas than G1 tumors, while there was no association between Wt ERβ transcripts and histological malignancy. The relative PR mRNA amounts showed a stepwise decrease from G1 through to G3 carcinomas, in contrast to pS2 mRNA values which were significantly higher in G1 or G2 tumors than normal tissue, but without any differences between tumor grades (Fig. 2). No differences in the relative mRNA levels for all transcripts investigated were noted among atrophic, proliferative, and secretory stages in normal endometrium.

Positive correlations between the relative amounts of del 5-variant and Wt forms in both ERs were apparent for both normal and tumor cases (Table I), while no differences in the ratios were noted between the two (data not shown). PR mRNA expression was significantly associated with that of Wt ERα, but not Wt ERβ transcripts, whereas the level of pS2 mRNA was not directly related to any of the hormone receptors (Table I).

By competitive PCR assay, ERα products with a molecular weight of 168 bp were detected in all of the samples investigated, while a 267 bp specific fragment for *ERβ*

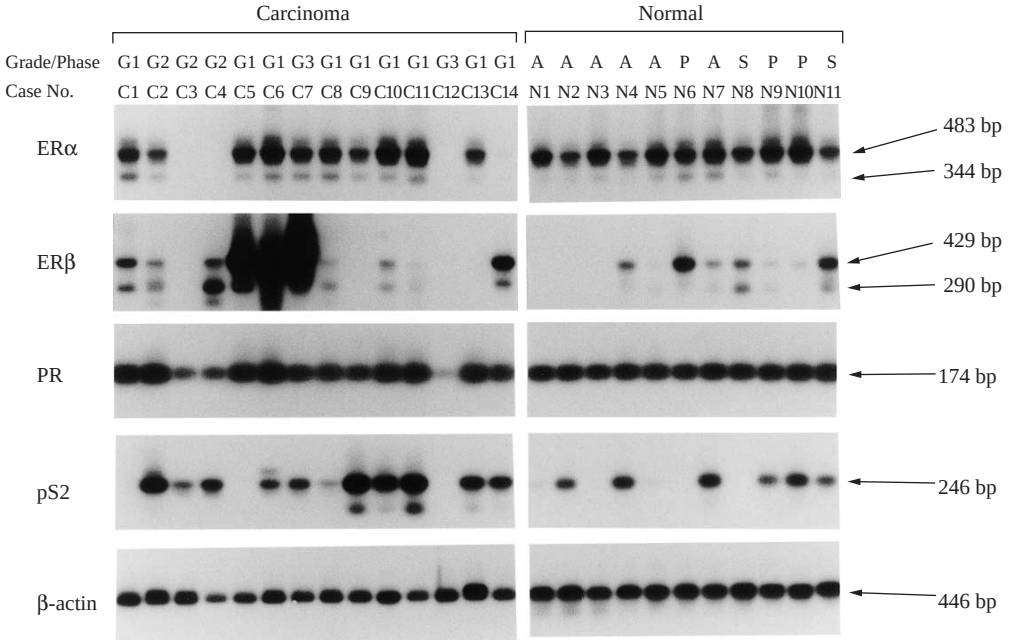

Fig. 1. mRNA analysis by a combination of RT-PCR and Southern blot hybridization using exon-specific oligonucleotide probes. G, grade; A, atrophic endometrium; P, proliferative phase; S, secretory phase; ER, estrogen receptor; PR, progesterone receptor.

gene was coamplified with 7 (14.6%) of 48 tumors and 3 (8.6%) of 35 normal tissues (Fig. 3).

The relative mRNA amounts of two Wt ERs, PR, and pS2, and ratios of del 5-ER/Wt ERα or β were not related to any of the clinicopathological factors investigated, including FIGO stage, degree of myometrial invasion, or lymph node status, with the exception of an association between high PR mRNA expression and upper myometrial invasion ($P<0.05$).

ERα, ERβ, PR, and pS2 protein expression In normal tissue with the regular menstrual cycle or atrophic endometrium, strong ERα and PR immunoreactivity was detected in glandular, stromal, and myometrial cells, with distinct nuclear staining. Variability in immunointensity and heterogeneous distribution of cells immunopositive for ERα and PR were also noted in carcinoma cases. In contrast, weak ERβ immunoreactivity was sporadically observed in a few cases of normal and tumor epithelial cells. Weak to moderate cytoplasmic pS2 immunoreactivity was sporadically observed in normal or atrophic glandular epithelial cells, although stromal components completely

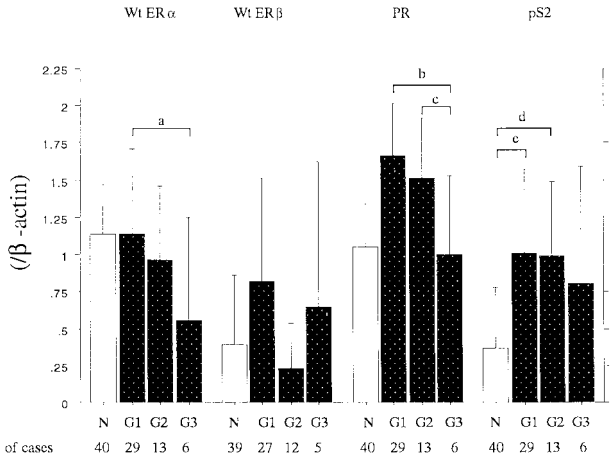

Fig. 2. Relative amounts of mRNA expression in normal and malignant endometrium. Wt, wild type; ER, estrogen receptor; PR, progesterone receptor; N, normal; G, grade. The data are mean±SD values. a, $P=0.0049$; b, $P=0.0018$; c, $P=0.0035$; d, $P=0.0002$; e, $P<0.0001$.

Table I. Correlation of mRNA Amounts among ERα, ERβ, PR, and pS2 in Normal and Malignant Endometrium

	Wt ERα versus				Wt ERβ versus			PR versus
	del 5-ERα *r* (*P*)	Wt ERβ *r* (*P*)	PR *r* (*P*)	pS2 *r* (*P*)	del 5-ERβ *r* (*P*)	PR *r* (*P*)	pS2 *r* (*P*)	pS2 *r* (*P*)
Em Ca	0.86 (<0.0001) *n*=50	0.11 (0.46) *n*=46	0.66 (<0.0001) *n*=50	0.24 (0.09) *n*=50	0.81 (<0.0001) *n*=46	0.08 (0.58) *n*=46	0.25 (0.9) *n*=46	0.3 (0.04) *n*=50
Normal	0.67 (<0.0001) *n*=40	0.08 (0.62) *n*=39	0.45 (0.004) *n*=40	0.2 (0.22) *n*=40	0.65 (<0.0001) *n*=39	0.2 (0.21) *n*=39	0.32 (0.05) *n*=39	0.15 (0.34) *n*=40

ER, estrogen receptor; PR, progesterone receptor; Wt; wild type; del 5, exon 5 deleted variant; *r*, Pearson's correlation coefficient; Em Ca, endometrial carcinoma; *n*, number of cases.

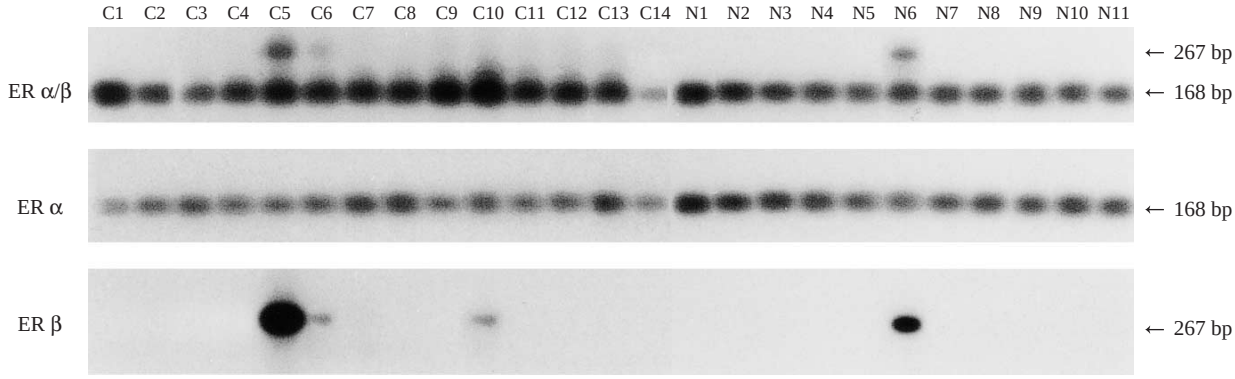

Fig. 3. Competitive PCR assay for ERα and ERβ in endometrial carcinoma cases (lanes, C1 to C14) and normal endometrial tissues (lanes, N1 to N11). Note weak signals for ERβ in C6 and C10 when hybridized with probe ERβ only, but not a combination of probes ERα-2 and ERβ. The short fragment (less than 267 bp) in C5 may be non-specific. Between each hybridization the filter was stripped before being rehybridized with another probe.

lacked any reaction. Moderate to strong immunoreactivity for pS2 protein was observed in some carcinomas, with a variation in immunopositive cell distribution (Fig. 4).

In premenopausal endometrium, average ERα and PR score values showed a significant decrease with transition from proliferative to secretory phases, while no apparent changes in average ERβ and pS2 score values were noted. The values in atrophic endometrium were similar to those of proliferative rather than secretory stages. Average ERα score values significantly decreased in the sequence lead-

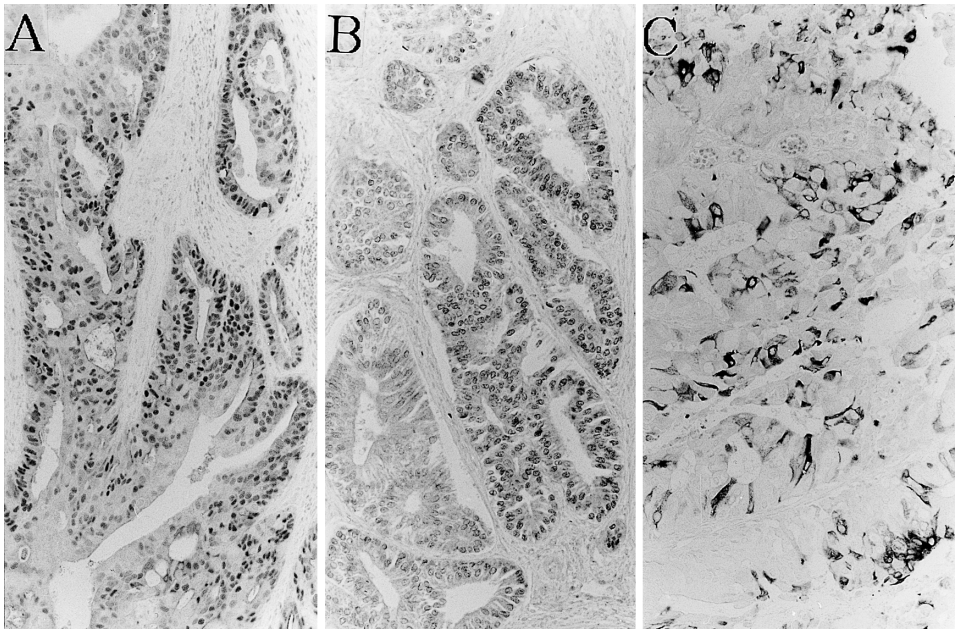

Fig. 4. Immunohistochemistry for ERα (A), ERβ (B), and pS2 (C) in endometrial carcinomas. ERα and ERβ show distinct nuclear immunostaining, while cytoplasmic pS2 immunoreactivity is evident. Original magnification, ×200.

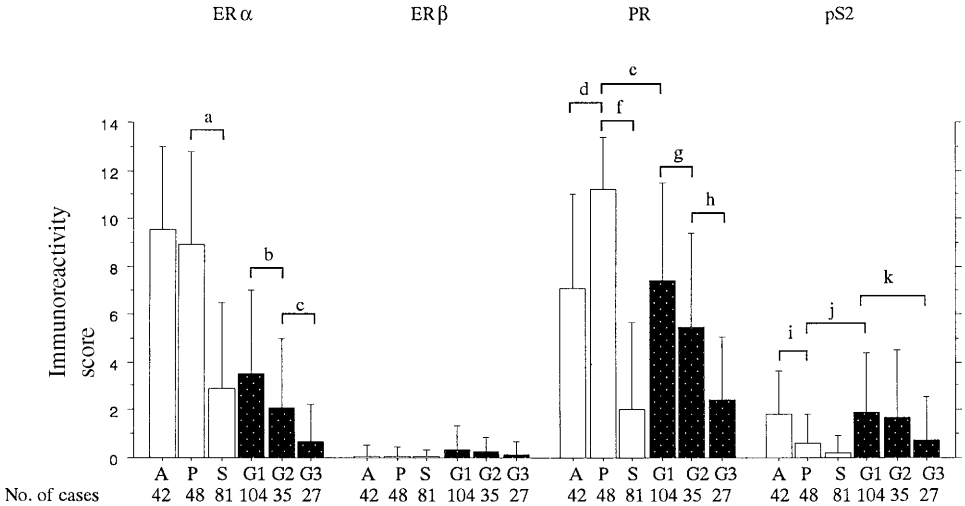

Fig. 5. Immunoreactivity scores for the two ER subtypes, PR, and pS2 proteins. A, atrophic endometrium; P, proliferative phase; S, secretory phase; G, grade. The data are mean±SD values. a, $P<0.0001$; b, $P<0.0001$; c, $P=0.0268$; d, $P<0.0001$; e, $P<0.0001$; f, $P<0.0001$; g, $P=0.0128$; h, $P=0.0022$; i, $P=0.0028$; j, $P<0.0001$; k, $P=0.0212$.

ing from atrophic or proliferative endometrium to G3 carcinomas, while there were no changes in ERβ values among these. PR and pS2 scores also showed a stepwise decrease from G1 through to G3 tumors, although the latter for G1 tumors was significantly higher than for premenopausal endometrial specimens (Fig. 5).

As shown in Table II, ERα but not ERβ values were positively correlated with PR scores in both normal and malignant categories, while pS2 scores did not show any association with the two ERs or the PR status.

The average PR score values were significantly associated with upper myometrial invasion (P=0.0046) and negative lymph node metastasis (P=0.0039), while the ERs and pS2 scores were not related to any of the clinicopathological factors investigated.

Correlation between mRNA and protein expression for ERα, ERβ, PR, and pS2 Samples were placed in one of three categories, negative, low-, and high-level groups, on the basis of average immunoreactivity scores for each molecule (ERα, 5.4±4.8; ERβ, 0.2±0.7; PR, 6.1±4.4; pS2, 1.6±1.9, mean±SD) in normal and malignant endometrial samples for which mRNA analysis was possible.

High ERα protein scores were related to high levels of Wt ERα mRNA expression and lower values for Wt ERβ, while there was no association between levels of ERβ mRNAs and the proteins. Significant differences in relative amounts of PR or pS2 mRNAs were observed between negative and high-level protein score categories (Fig. 6).

Western blot assay Wt ERα proteins were detected as bands with a molecular weight of 65 kDa in both normal and malignant tissues, but 42 kDa bands corresponding to the del 5-ERα variant were not found in any of the samples investigated (Fig. 7A).

ERα positivity in western blot assays was significantly associated with the immunoreactivity scores in all categories (Fig. 7B), with a significant decrease from normal through to G3 tumors (Table III).

Table II. Correlation of Immunoreactivity Scores among ERα, PR, and pS2 in Normal and Malignant Endometrium

	n	ERα versus		PR versus
		PR r (P)	pS2 r (P)	pS2 r (P)
Em Ca	166	0.48 (<0.0001)	0.06 (0.46)	0.15 (0.06)
Normal	171	0.76 (<0.0001)	0.2 (0.007)	0.16 (0.03)

ER, estrogen receptor; PR, progesterone receptor; n, number of cases; r, Pearson's correlation coefficient; Em Ca, endometrial carcinoma.

DISCUSSION

The present study clearly demonstrated the amounts of ERα at both mRNA and protein levels to be significantly greater than those for ERβ in normal and malignant endometrial tissue. Differences in ERβ mRNA positivity between nested and competitive PCR assays may be simply due to their sensitivities. With regard to the absence of differences in mRNA levels among various stages in normal endometrium, in contrast to the immunohistochemical

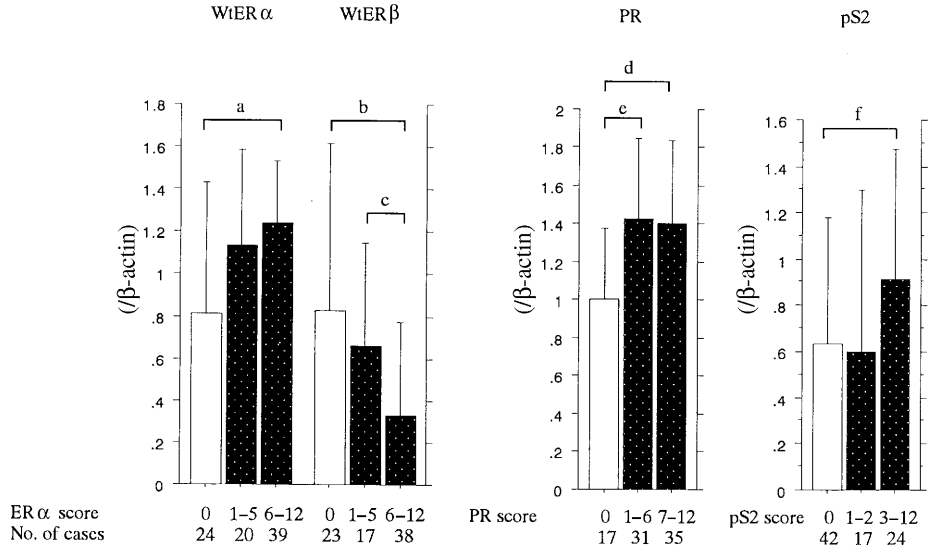

Fig. 6. Correlations among relative mRNA amounts and immunoreactivity scores. The data are mean±SD values. a, P=0.015; b, P=0.007; c, P=0.02; d, P=0.003; e, P=0.002; f, P=0.04.

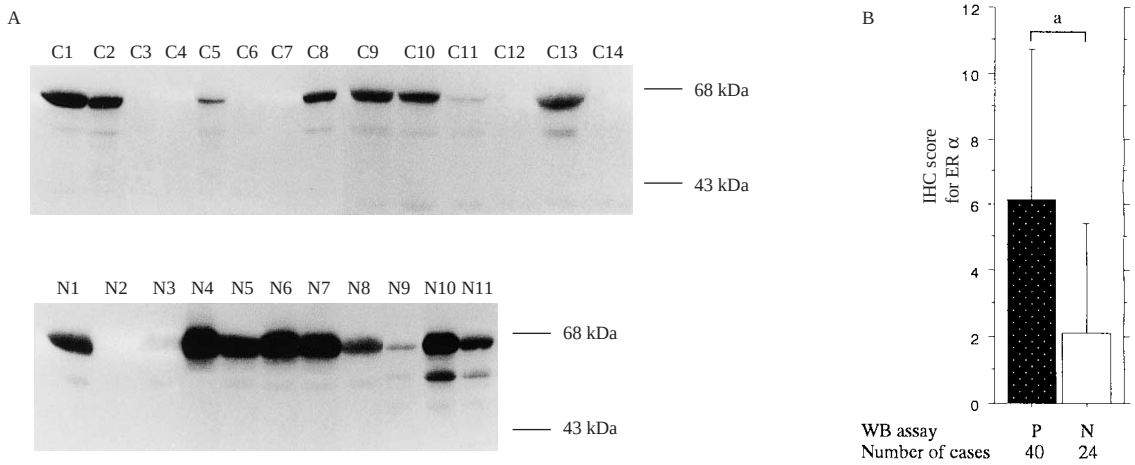

Fig. 7. A) Western blot assay for ERα protein in endometrial carcinomas (lanes, C1 to C14) and normal endometrial tissues (lanes, N1 to N14). Although an approximately 50 kDa band is also observed in some cases, its significance is unclear. B) Relation between western blot (WB) assay and immunoreactivity (IHC) score for ERα. P, positive; N, negative. a, $P<0.0006$.

Table III. ERα Positivity in Normal and Malignant Endometrium by Western Blot Assay

	Normal endometrium *n* (%)	Endometrial carcinoma			
		Grade 1 *n* (%)	Grade 2 *n* (%)	Grade 3 *n* (%)	
Positive	19 (76%)	11 (45.8%)	9 (69.2%)	1 (16.7%)	$P=0.021$
Negative	6 (24%)	13 (54.2%)	4 (30.8%)	5 (83.3%)	

n, number of cases.

findings, the possible reasons include the following: 1) the relatively small number of cases investigated for mRNA analysis; 2) the presence of stromal cells expressing ERα or PR.

There are three potential estrogen signaling pathways, involving ERα/ERα and ERβ/ERβ homodimers, as well as ERα/ERβ heterodimers.[18] Brandenberger *et al.*[19] have recently demonstrated that endometrial stromal cells isolated from normal endometrium and endometriosis predominantly express ERα mRNA, but ratios of ERα:ERβ mRNAs were significantly higher in the former than the latter, suggesting a difference in the roles of the two ERs in growth responses in normal and ectopic endometrium. In addition, equal or increased ERα mRNA expression in conjunction with a decreased ERβ mRNA expression indicates differential roles for ERα- and ERβ-driven pathways during breast and ovarian tumorigenesis.[7, 8]

In our results, Wt ERα mRNA showed a stepwise decrease from normal or G1 through to G3 tumors, in line with changes in the protein expression determined by both immunohistochemistry and western blot assay, in contrast to Wt ERβ mRNA or protein expression, which did not alter. It is therefore suggested that changes in the ratio of two ER subtypes may cause alteration in estrogen signaling pathways during endometrial tumorigenesis. One reason for the strong ERα mRNA signals in 2 of 6 G3 tumors as compared to the protein levels may be the relatively high sensitivity of PCR assay. In addition, the anomalous results between mRNA and western blot assays for ERα, in particular G1 tumors, may be due to the protein degradation during sample preparation and processing or the heterogeneous distribution of tumor cells expressing the protein.

A dominant positive function of del 5-ERα variants has been demonstrated in a yeast transfection assay,[5] and these variants may be responsible for estrogen-independent growth and resistance to antiestrogen therapy in breast carcinomas.[4, 20] However, not all studies support this hypothesis, and very low transcriptional activity and dominant negative activity when the variant is expressed together with Wt ERα have been reported.[21, 22] Ohlsson *et al.*[23] recently found that although the del 5-ERα variant

mRNA is more stable than Wt ERα mRNA, the stability of the variant protein may be significantly lower than that of the Wt form, casting doubt on any biological significance in breast carcinomas. In the present study, although del 5-ERα variant mRNA was frequently coexpressed with the Wt form in both normal and malignant tissues, as well as ERβ mRNA, truncated proteins could not be detected in any of the samples. In addition, the ratios of del 5-variant:Wt ERα mRNA was not associated with any of the prognostic factors investigated. These results suggest that the variant is not of great importance in the development and progression of endometrial carcinomas.

In PR-positive ovarian carcinoma xenografts, reduction of circulating levels of estrogen by ovariectomy has been found to reduce the PR concentration, while exogenous administration causes an increase, supporting the hypothesis that PR levels are regulated by estrogen.[24] In the present case, PR mRNA expression appeared to be paralleled by protein levels, significant correlations for ERα but not ERβ mRNA expression being evident in both normal and tumor cases. Given the induction of PR after 17β-estradiol treatment in the ER-positive PE04 ovarian carcinoma cell line,[25] our data suggest that ERα may play a central role in the regulation of PR expression in the endometrium.

We previously demonstrated that prolonged progesterone administration can suppress cell proliferation in endometrial carcinomas without altering apoptosis, indicating a possible contribution to the regulation of tumor growth.[26] In general, a moderate to high level of PR expression is linked with a good outcome in patients with malignant tumors of the female reproductive system.[24, 27, 28] The significant association between PR expression at mRNA or protein levels and favorable prognostic factors found here may be in line with this.

pS2 protein expression is considered to be an indicator of ER functional activity, since estrogen can promote its transcription, acting via an estrogen response element (ERE) region.[29, 30] In addition to estradiol, epidermal growth factor (EGF), c-H-ras, and c-jun have been reported to regulate *pS2* gene expression.[31] Previous studies have demonstrated that it is significantly related to ER protein expression in breast and endometrial carcinomas, so that it may be an additional useful prognostic factor,[32, 33] in contrast to the large bowel case where expression is independent of the ER status.[34] In this study, although higher values for pS2 mRNA and protein were observed in endometrial carcinomas than in normal tissues, no association with the ER status or prognostic factors was noted. Although the reason for the discrepancy is unclear, factors other than activated ER may predominantly influence pS2 expression in endometrial tissues. This conclusion is in line with our finding of no cyclic pS2 expression during the normal menstrual cycle.

In conclusion, the present study demonstrated changes in ERα and ERβ mRNA and protein expression between normal and malignant endometrium, and provided evidence that ERα may play an important role in the regulation of PR, but not pS2 expression.

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