Progesterone receptor isoforms as a prognostic marker in human endometrial carcinoma

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The possible role of specific progesterone receptor (PR) isoforms (PRA and PRB) as predictive factors in endometrial carcinoma is unclear. The present study was undertaken to evaluate the clinical significance of intratumoral PR isoform status in patients with endometrioid endometrial carcinoma. We studied 103 cases of endometrioid endometrial carcinoma using immunohistochemistry. We correlated the findings with various clinicopathological parameters of the patients. PRA and PRB immunoreactivity was detected in 51/103 (48.5%) and 79/103 (76.7%) of carcinoma cases, respectively. A significant positive correlation was detected between the status of PRB immunoreactivity and the amount of PRB mRNA by real-time reverse transcription–polymerase chain reaction ($P = 0.012$). PR isoform expression was significantly lower in the cases with higher histological grade ($P = 0.0001$ and $P = 0.002$, for PRA and PRB, respectively). Cases that were negative for either one or both PR isoforms were significantly associated with shorter disease-free and overall survival of the patients. The absence of either one or both of these two PR isoforms was detected in all nine patients who died (100.0%), whereas the absence of these immunoreactivities was detected only in 43 of 94 (45.7%) patients who had lived during the same period. In addition, multivariate analysis demonstrated that an absence of PRA immunoreactivity was an independent risk factor in disease-free survival of the patients ($P = 0.0258$). The results of our study demonstrated that loss or absence of PR isoform expression determined by immunohistochemistry could become an important prognostic indicator in patients with endometrioid endometrial carcinoma. (*Cancer Sci* 2006; 97: 1308–1314)

Endometrial carcinoma is one of the most common malignancies of the female genital tract and its incidence, especially that of endometrioid endometrial carcinoma, has increased recently.[1] It is well known that uterine endometrial proliferation is under the control of both estrogen and progesterone. One of the physiological roles of progesterone in the regulation of glandular epithelium of the endometrium is to induce cellular differentiation and to antagonize estrogen-mediated cell proliferation.[2] Endometrial carcinogenesis is strongly associated with continued estrogen exposure without progesterone influence.[3,4] Progesterone has clinically been demonstrated to provide some protection against stimulatory effects of estrogenic agents. In addition, hormone replacement therapy using combinations of estrogens and progesterones yields a lower risk of endometrial carcinoma, despite increasing the incidence of breast carcinoma.[5,6] A number of the patients who wished to preserve their fertility were treated with progestin as a primary endocrine therapy for atypical hyperplasia and well-differentiated adenocarcinoma, although the effects of this treatment on the clinical outcome of patients have not always been satisfactory.[7–9]

Both estrogen and progesterone act through intranuclear receptors, estrogen receptors (ER) and progesterone receptors (PR), which belong to the superfamily of steroid hormone receptors.[10] The expression of ER and PR is generally considered to be coordinated because transcription of the PR gene is induced by estrogen and inhibited by progesterones in the great majority of estrogen-responsive cells.[11] In normal cycling human endometrium, PR is expressed abundantly in glandular epithelium during the proliferative phase of the cycle.[12] PR is present in two isoforms, termed PRA and PRB.[13] PRA is the truncated form of PRB, lacking 164 amino acids at the NH_2 terminus. These isoforms are translated from the same gene, but transcription is initiated from different promoters.[14] Studies addressing the individual effects of PR isoforms have been reported. Vegeto *et al*. reported that PRA could repress PRB activity in cells in which PRA was not transcriptionally active, and that PRA might be associated with a cell- and promotor-specific repressor of PRB.[15] Giangrande *et al*. also reported that differential cofactor binding resulted in the opposing transcriptional activities of PRA and PRB.[16] In addition, microarray analyses of human breast cancer cells expressing either PRA or PRB have confirmed that each PR isoform has a unique set of target genes, with little overlap.[17] These functional and transcriptional differences suggest that the development, invasiveness and metastatic potential of carcinoma cells can be influenced by the PR status of the tumor cells. We previously reported that loss of PRB was a significant prognostic factor in epithelial ovarian cancer.[18,19] In addition, breast carcinoma patients with PRA-rich tumors are in general associated with poorer disease-free survival rates.[20] In endometrial carcinoma, several studies demonstrated the PR isoform status of carcinoma cells.[21–23] Arnett-Mansfield *et al*. reported a reduced expression of either one or both of the PR isoforms in the great majority of endometrial tumors, compared with hyperplastic or normal endometrium.[21] De Vivo *et al*. demonstrated a polymorphism in the PRB promoter, which results in increased transcription of the PRB isotype. In a population-based study, this polymorphism was reported to be associated with increased risk for endometrial carcinoma.[22] In addition, hypermethylation of PRB alleles was detected in endometrial carcinoma.[23]

Results of previous studies demonstrated that high levels of ER and PR were directly correlated with a lower tumor grade, less myometrial invasion, and a lower incidence of lymph node metastases in the patients with endometrioid endometrial carcinoma.[24–27] In addition, the status of ER and PR in these carcinomas has been reported as an independent prognostic factor of the patients.[28] However, it is also true that there are many controversies regarding the possible roles of specific PR isoforms as predictive factors in endometrial carcinoma.[21,29–32] Fujimoto *et al*. reported that PRA could not be detected in advanced endometrial tumors.[29] In accordance with this, they later reported that PRB was expressed predominantly in distant metastases of endometrial carcinoma.[30] In contrast, Kumar *et al*. reported that downregulation of PRB may be associated with poorly differentiated endometrial carcinoma.[31] Sakaguchi *et al*.

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also proposed that the drastic decrement of PRB but not of PRA resulted in poor prognosis in endometrial carcinoma, although histological type was not described in their study.[32]

Therefore, in the present study, we carried out immunohistochemical analysis of 103 cases of endometrioid endometrial carcinoma, and correlated the findings with the clinicopathological features of the patients, including their clinical outcome, in order to study the possible roles and correlation between PR isoforms and prognosis of the patients.

Materials and Methods

Endometrial carcinoma patients and tissue preparation. One hundred and three endometrioid endometrial carcinomas (49 well differentiated, 32 moderately differentiated, 22 poorly differentiated; 66 stage I, 12 stage II, 22 stage III, 3 stage IV) were retrieved from the surgical pathology files of Tohoku University Hospital, Sendai, Japan. The protocol for this study was approved by the Ethics Committee at Tohoku University School of Medicine (Sendai, Japan). None of the patients examined had received irradiation, hormonal therapy or chemotherapy prior to surgery. The median follow-up time of the patients examined in this study was 60 months (range, 2–148 months). The disease-free and overall survival times of the patients were calculated from the time of initial surgery to recurrence or death, or the date of last contact. The survival times of patients still alive or lost to follow-up were censored in December 2004. The clinicopathological findings of the patients, including age, histology, stage, grade and preoperative therapy, were retrieved by extensive review of the charts. A standard primary treatment for endometrial carcinoma at Tohoku University Hospital was surgery consisting of total abdominal hysterectomy, salpingo-oopholectomy, pelvic and/or para-aortic lymphadenectomy and peritoneal washing cytology. Eighty-five out of 103 patients (83%) in this study underwent complete surgery as above. Six out of 85 patients had lymph node metastasis. The remaining 18 patients (17%) underwent total abdominal hysterectomy and salpingo-oopholectomy without lymphadenectomy because of obesity or their poor performance status. The lesions were classified according to the Histological Typing of Female Genital Tract Tumors by WHO and staged according to the International Federation of Gynecology and Obstetrics system.[33,34] Sixty-eight out of 103 patients received pelvic radiation therapy (50 Gy) or three to six courses of chemotherapy, consisting of the cisplatin-based combination regimen CAP (60–70 mg/m^2 cisplatin, 40 mg/m^2 doxorubicin and 500 mg/body cyclophosphamide) after operation. Patients who had early stage and low-grade disease (stage IA, G1, stage IA, G2 and stage IB, G1) and patients who were associated with poor performance status did not receive any adjuvant therapy. None of the patients received hormone therapy after operation. All specimens were processed routinely (i.e. 10% formalin fixed for 24–48 h, paraffin embedded, and thin sectioned [3 μM]).

Antibodies. Monoclonal antibodies for PRA (hPRa7) and PRB (hPRa2) were purchased from NeoMarkers (Fremont, CA, USA). The PRA (hPRa7) antibody used in this study recognized both PRA and PRB in immunoblot analysis.[35] However, Mote *et al*. reported that hPRa7 did not recognize PRB on immunohistochemistry in fixed tissues even after antigen retrieval, as evidenced by the absence of immunostaining by this antibody of the PRB-expressing MDA-MB-231/PRB cell line.[36] This was considered to be due to the inaccessibility of the epitope on PRB recognized by hPRa7 in 10% formalin-fixed and paraffin-embedded tissue specimens, possibly due to alteration of the conformation of the molecule in which the hPRa7 epitope is located in such a way to reduce its accessibility in immunohistochemistry. hPRa2 recognizes PRB exclusively.[35,37] Monoclonal antibodies for ERα, ERβ and Ki67 were purchased from Novocastra (Benton, NC, UK), Genetex (San Antonio, TX, USA) and DAKO Cytomation (Carpinteria, CA, USA), respectively.

Immunohistochemistry. Immunostaining was carried out by the streptavidin–biotin amplification method using a Histofine Kit (Nichirei, Tokyo, Japan). Antigen retrieval was carried out using an autoclave treatment for 5 min in citric acid buffer (2 mM citric acid and 9 mM trisodium citrate dehydrate, pH 6.0). The dilutions of the primary antibodies used in our study were as follows: PRA, 1/100; PRB, 1/100; ERα, 1/50; ERβ, 1/1500; and Ki67, 1/50. The antigen–antibody complex was visualized with 3,3′-diaminobenzidine (DAB) solution (1 mM DAB, 50 mM Tris-HCl buffer [pH 7.6] and 0.006% H_2O_2), and counterstained with hematoxylin. Proliferative-phase endometrial glands were used as positive controls for immunohistochemistry of PR isoforms[26] and breast cancers were used as positive controls for ERα and ERβ. As a negative immunostaining control, normal rabbit or mouse IgG was used instead of the primary antibodies. No specific immunoreactivity was detected in these tissue sections.

Scoring of immunoreactivity. Evaluation of PRA, PRB, ERα, ERβ and Ki-67 was carried out in high-power fields (×400) using a standard light microscope. Two of the authors (SS and KI) searched all of the tissue sections simultaneously and determined the most representative areas using a double-headed light microscope. In all of the cases examined, a total of more than 500 tumor cells from three different representative fields were counted independently by the two authors, and the percentage of immunoreactivity (i.e. the labeling index [LI]) was determined. After completely reviewing the immunostained sections of each lesion, two of the authors (SS and KI) independently divided the cases into the following two groups: +, >10% positive cells; and –, <10% positive cells. Layfield *et al*. proposed the separation of ER- and PR-positive cases using LI cut-off points of 10% in the immunohistochemical analysis of human breast cancer.[38] The eighth St Gallen meeting also recommended that approximately 10% positive staining of cells for either ER or PR might be considered as a reasonable threshold for definite endocrine responsiveness.[39] Therefore, in the present study, we used the same cut-off point of 10% between positive and negative PR isoforms, based on the results of the studies above. Cases with discordant results (interobserver differences of >5%) were reevaluated simultaneously the two authors above using a double-headed light microscope. Consequently, the interobserver differences were less than 5% in this study.

Reverse transcription–polymerase chain reaction. Thirty-three specimens of fresh frozen tissues of endometrial carcinoma (i.e. specimens frozen immediately in liquid nitrogen and stored at −80°C) were available for the present study. Total RNA was extracted by homogenizing frozen tissue samples in 1 mL TRIzol reagent (Life Technologies, Gaithersburg, Grand Island, NY, USA), followed by phenol–chloroform extraction and isopropanol precipitation. All RNA samples were quantified by spectrophotometry and stored at −80°C until processing for reverse transcription (RT). Total RNA (4 μg) was denatured at 70°C for 10 min and was reverse transcribed in the presence of 50 ng/μL Oligo (deoxythymidine) primer (Invitrogen, Carlsbad, CA, USA), 2.5 mmol/L $MgCl_2$, 0.5 mmol/L deoxy-NTPs, 10 mmol/L dithiothreitol and 10 IU ribonuclease H-reversed transcriptase (Superscript II RT, Invitrogen) for 60 min at 42°C and 15 min at 70°C on a PTC-200 Peltier Thermal Cycler DNA Engine (MJ Research, Watertown, MA, USA). RT–polymerase chain reaction (PCR) analysis was carried out in order to examine the presence or absence of genomic DNA contamination. The RT step was performed in the absence of Superscript II RNase H-reverse transcriptase, followed by PCR. RT-PCR products lacking reverse transcriptase in the initial RT step were run on an ethidium-bromide-stained 2% agarose gel. No bands were detected in these samples (data not shown). After an initial

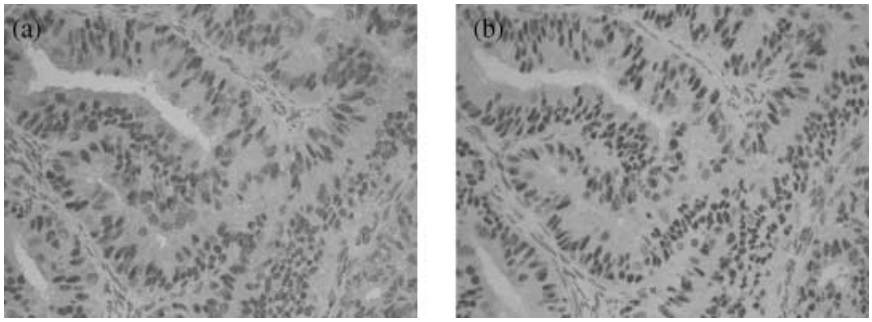

Fig. 1. Immunohistochemical staining for (a) progesterone receptor A (PRA) and (b) progesterone receptor B (PRB) in endometrioid endometrial carcinoma. PRA and PRB immunoreactive proteins were detected in the nuclei of carcinoma cells of G1 adenocarcinoma. Original magnification, ×400.

1 min denaturation step at 96°C, 35 cycles of PCR were carried out on thermal cycle under the following conditions: 45 s denaturation at 94°C, 30 s annealing at 58°C, and a 1.5 min extension at 72°C. In addition, cDNA was used as a template for real-time PCR. Real-time PCR was carried out with the Light Cycler System (Roche Diagnostics, Mannheim, Germany) using the DNA-binding dye SYBER Green I (Roche Diagnostics). The 20-μL reaction mixture contained 3 mM $MgCl_2$ for PRB and β-actin primer, 10 pmol/L of each primer and DNA-binding dye LightCycler-Fast Start DNA Master SYBR Green I. β-Actin expression was used to verify the integrity of RNA from each specimen. Human gene-specific primers used to amplify PRB and β-actin were as follows: PRB 5′ sense, ACACCTTGCCTGAAGTTTCG and PRB 3′ antisense, CTGTCCTTTTCTGGGGGACT (196 bp); β-actin 5′ sense, CCAACCGCGAGAAGATGAC and β-actin 3′ antisense, GGAAGGAAGGCTGGAAGAGT (459 bp). An initial denaturing step at 95°C for 10 min was followed by 35 cycles of 95°C for 15 s, 10 s annealing at 58°C (PRB) and 63°C (β-actin), and extension for 13 s at 72°C. The fluorescence intensity of the double-strand-specific SYBER Green I, which reflects the amount of specific PCR products formed, was read by the LightCycler at 85°C after the end of each extension step.[40] Using automated programs of the LightCycler software, the amount of PRB and β-actin template in each sample was calculated so as to dilute the standard cDNA equally. The actual values of PRB were corrected by the value of the β-actin template. Although conventional quantitative PCR requires the use of purified plasma cDNA in the construction of a standard curve, it was possible to semiquantify the PCR products with the LightCycler using purified cDNA of known concentrations.[41,42] In initial experiments, PCR products were purified and subjected to direct sequencing (ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit and ABI PRISM 310 Genetic Analyzer; Perkin-Elmer PE Applied Biosystems, Foster City, CA, USA) to verify amplification of the correct sequences. Frozen breast cancer tissue was used as a positive control. Negative control experiments did not contain cDNA substrate to study the presence of exogenous contamination of DNA. No amplified products were detected under these conditions.

Statistical analyses. Statistical analysis was carried out using SAS software (StatView, Version 5.0; SAS, Cary, NC, USA). The statistical significance of the association between PRA and PRB immunoreactivity and other parameters (grade, stage, age, ERα LI, ERβ LI and Ki-67 LI) was evaluated using the Mann–Whitney *U*-test and the χ^2-test. The statistical significance between PRA and PRB immunoreactivity was calculated using a correlation coefficient (r) and regression equation. The statistical significance between PRB immunoreactivity determined by immunohistochemistry and the status of mRNA determined by RT-PCR was evaluated using Fisher's exact probability test, and the statistical significance between PRB immunoreactivity and amounts of PRB mRNA determined by real time RT-PCR was evaluated using the Mann–Whitney *U*-test. The Kaplan–Meier method and statistical significance was calculated using a log-rank test. Univariate and multivariate analyses were evaluated using Cox's proportional hazards model. *P*-values less than 0.05 were considered significant.

Results

Immunohistochemistry and RT-PCR. Immunoreactivity for PRA and PRB was detected in the nuclei of carcinoma cells (Fig. 1). ERα, ERβ and Ki-67 were also confined exclusively to the nuclei of epithelial cells (data not shown). RT-PCR was carried out to confirm the expression of PRB using 33 cases in this study (Figs 2,3), because PRA has no specific sequence to distinguish it from PRB mRNA by RT-PCR. Twenty-five of these 33 cases were PRB positive and eight cases were PRB negative, as determined by immunohistochemistry. PRB mRNA was detected in 21 out of these 25 PR-positive cases (84%) and was not detected in five out of eight PRB-negative cases (Fig. 2). There was a statistically significant positive correlation between PRB immunoreactivity and mRNA expression examined by RT-PCR analysis ($P = 0.02$). In addition, amounts of PRB mRNA determined by real time RT-PCR were 8.89 (median values) in these PRB-positive and 0.41 (median values) in PRB-negative cases. A significant positive correlation was detected between PRB immunoreactivity and the amounts of PRB mRNA ($P = 0.012$) (Fig. 3). Eighty out of 103 cases (77.7%) demonstrated either or both PR isoforms in immunohistochemistry. Fifty-one out of 103 cases (48.5%) were PRA positive. Among these 51 PRA-positive cases only one case (1.9%) was PRA positive and PRB negative. However, PRB-positive cases were 76.7% (79/103), and 29 of these 79 PRB-positive cases (36.7%) were both PRB positive and PRA negative. The proportion of cases positive for both PRA and PRB was 48.5% (50/103), whereas the proportion of cases negative for both PRA and PRB was 22.3% (23/103). There was a significant positive correlation between PRA and PRB expression in endometrial carcinoma ($P = 0.004$). Results of the associations between clinicopathological parameters and immunoreactivity of PRA and PRB are summarized in Table 1. The status of PRA in G1, G2 and G3 endometrial carcinoma was 67.3% (33/49), 46.6% (15/32) and 13.6% (3/22), respectively, and the status of PRB was 87.8% (43/49), 78.1% (25/32) and 50.0% (11/22), respectively. PR immunoreactivity was significantly lower for carcinoma with higher histological grade ($P = 0.0001$ and $P = 0.002$, for PRA and PRB, respectively), whereas there were no correlation among the clinical stages of the cases. PRA and PRB expression was significantly positively correlated with ERα LI, and inversely with Ki-67 LI.

Relationship between PR isoform expression and prognosis. Progesterone receptor isoform status was evaluated as a prognostic variable in the patients with endometrioid endometrial carcinoma using univariate analysis. Results of univariate analysis are summarized in Table 2. The following variables were significantly associated with poorer disease-free survival and overall survival of the patients at the $P < 0.05$ levels: absence of PRA immunoreactivity; absence of PRB immunoreactivity;

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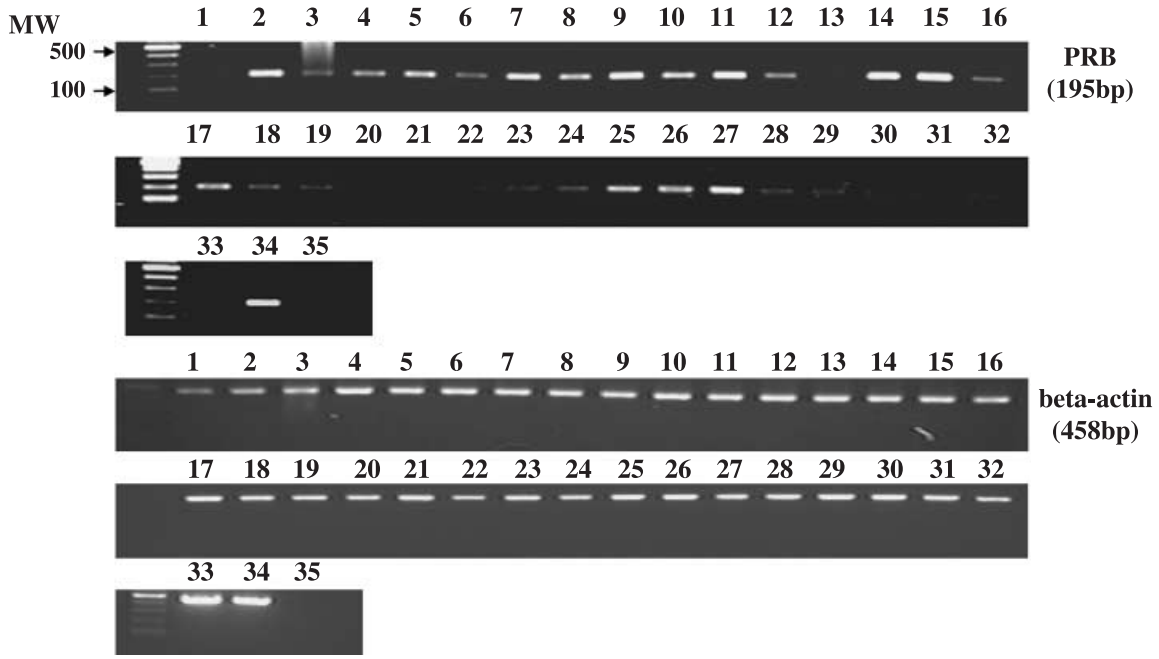

Fig. 2. Reverse transcription–polymerase chain reaction (RT-PCR) analysis of total RNA extracted from endometrioid endometrial carcinoma. Nos 4, 10, 13, 21, 29, 30, 31, 32 and 33 are progesterone receptor B (PRB)-negative cases, as determined by immunohistochemistry. No. 34 is a positive control. No. 35 is a negative control. PRB mRNA was detected in 21 out of these 25 PR-positive cases (84%) and not detected in five out of eight PR-negative cases. There was a statistically significant positive correlation between PRB immunoreactivity and mRNA expression examined by RT-PCR analysis ($P = 0.02$, Fisher's exact probability test).

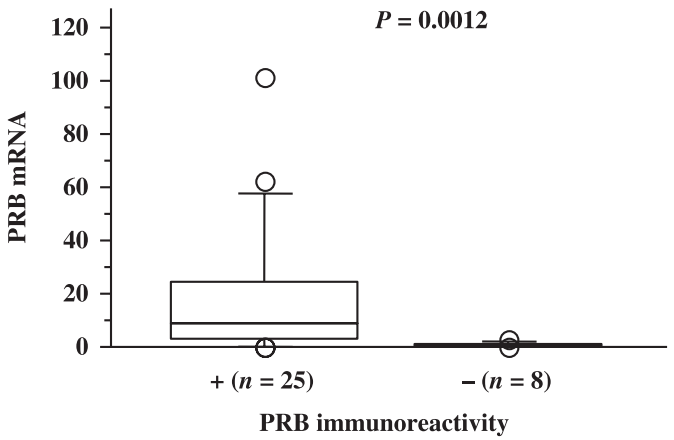

Fig. 3. Correlation between progesterone receptor B (PRB) immunoreactivity and its mRNA level determined by quantitative reverse transcriptin–polymerase chain reaction analyses in human endometrial carcinoma. There was a statistically significant positive correlation between PRB immunoreactivity and the amount of PRB mRNA ($P = 0.012$, Mann–Whitney *U*-test).

and histological grades. The disease-free and overall survival curves of the patients according to the Kaplan–Meier method are demonstrated in Fig. 2. The 5-year disease-free and overall survival rates were 95.6% and 96.4%, respectively, for PRA-positive cases and 71.1% and 84.3%, respectively, for PRA-negative cases. Patients with negative PRA in these carcinoma tissues were associated with a significantly poorer prognosis than those of PRA-positive cases at both disease-free ($P = 0.0009$) and overall survival ($P = 0.0098$) (Fig. 2A,B). Fig. 2 also demonstrates the greater disease-free and overall survival of the PRB-positive cases compared to PRB-negative cases ($P = 0.0007$ and $P = 0.0116$, respectively). The 5-year disease-free and overall survival times were 90.5% and 94.1%, respectively, for PRB-positive cases and 61.3% and 75.9%, respectively, for PRB-negative cases. In addition, the absence of either one or both of these two PR isoforms was associated with a significantly poorer prognosis at disease-free survival ($P = 0.0005$) (Fig. 2C). In addition, the absence of either one or both of these two PR isoforms was detected in all nine patients who died (100.0%), whereas the absence of these immunoreactivities was detected only in 43 of 94 (45.7%) patients who lived during the same period.

In order to determine whether the prognostic value of PRA or PRB expression was independent of other risk factors associated with clinical outcome of the patients with endometrioid endometrial carcinoma, we examined the results using multivariate analysis. The prognostic factors examined were the status of PRA or PRB, ER, stages and histological grades. As shown in Table 3, absence of PRA in carcinoma tissue was statistically significant as an independent risk factor only in disease-free survival of the patients ($P = 0.0258$), although PRB status was not a significant factor in disease-free or overall survival. Histological grade turned out to be an independent risk factor only in overall survival of the patients.

Discussion

This is the first study demonstrating that the absence of not only PRA but also PRB expression determined by immunohistochemistry is an important prognostic indicator of patients with endometrioid endometrial carcinoma. Progesterone is known to be one of the very important endocrine factors regulating cellular proliferation of the endometrium and its effects are mediated through PR.[10] PR has two isoforms, PRA and PRB, but the exact biological or clinical differences between the roles

Table 1. Correlation between progesterone receptor isoform A and B (PRA and PRB) immunoreactivity and clinicopathological parameters in endometrial carcinoma

Parameter	Total (n = 103)	PRA			PRB		
		+ (n = 51)	– (n = 52)	P-value	+ (n = 79)	– (n = 24)	P-value
Age (years)							
50	22	15	7		19	3	
>50	81	36	45	**0.048**	60	21	0.27
Grade							
1	49 (47.6%)	33	16		43	6	
2	32 (31.0%)	15	17		25	7	
3	22 (21.4%)	3	19	**0.0001**	11	11	**0.002**
Stage							
I, II	78 (75.7%)	40	38		63	15	
III, IV	25 (24.3%)	11	14	0.526	16	9	0.08
ERα LI (median)	23	34	11	**0.003**	34	4.5	**<0.0001**
ERβ LI (median)	5	5	8	0.3	11	2	0.089
Ki67 LI (median)	32	27	40	**0.003**	30	46	**0.002**

ER, estrogen receptor; LI, labeling index.

Table 2. Univariate analyses (P-values) of predictors of disease-free and overall survival for 103 patients with endometrial carcinoma

Variable	Disease-free survival	Overall survival
PRA (positive vs negative)	**0.0055**	**0.0354**
PRB (positive vs negative)	**0.0022**	**0.0225**
Age (≤50 years vs 50 years)	0.1159	0.0854
Stage (I/II vs III/IV)	0.2029	0.1163
Histological grade (1–3)	**0.0276**	**0.0067**
ERα (positive vs negative)	**0.0426**	0.2667
ERβ (positive vs negative)	0.4832	0.3965
Ki67 (positive vs negative)	0.4722	0.3487

ER, estrogen receptor; PR, progesterone receptor.

of these two PR isoforms in endometrial carcinoma remains largely unknown. The results of our present study demonstrated that PRB was more common than PRA in endometrioid endometrial carcinoma, which is consistent with a recent report by Miyamoto *et al*.[43] They reported PRB LI of 30.4%, whereas those of PRA were 11.3% in endometrial carcinoma. Sakaguchi *et al*. also reported that PRB expression was more common than PRA expression in endometrial carcinoma.[32] However, Arnett-Mansfield *et al*. reported that PRA, not PRB, was dominant in endometrial carcinoma.[21] This discrepancy of results may be explained by the number of cases examined, because Arnett-Mansfield *et al*. examined a relatively small number of cases (46 cases), whereas our present study as well as others examined PR expression in more than 100 patients with endometrial carcinoma. We demonstrated previously that PRB was expressed dominantly in all types of epithelial ovarian cancer.[18,19] In human breast cancer, however, PRA was dominant in invasive ductal carcinoma.[20,44] Therefore, the biological significance of PR isoforms may differ depending on tumors, even among human estrogen-dependent carcinomas.

Progesterone receptor and ER are known to be among the most extensively studied biological prognostic markers in endometrial carcinoma. However, the status of PR isoforms and their possible roles in conjunction with clinical outcome in patients with endometrial carcinoma have not been fully

Table 3. Multivariate analyses of predictors of disease-free and overall survival for 103 patients with endometrial carcinoma

Preditor	Disease-free survival		Overall survival	
	HR (95% CI)	P-value	HR (95% CI)	P-value
PRA (positive vs negative)	0.171 (0.036–0.808)	**0.0258**	0.196 (0.022–1.764)	0.1522
Histological grade (1–3)	1.333 (0.728–2.440)	0.3514	2.371 (0.948–5.931)	0.065
ERα (positive vs negative)	0.509 (0.186–1.394)	0.1888	0.748 (0.187–2.992)	0.6818
Stage (I–IV)	0.374 (0.231–2.287)	0.1053	1.451 (0.785–2.685)	0.2352
PRB (positive vs negative)	0.37 (0.121–1.125)	0.0798	0.445 (0.102–1.932)	0.2797
Histological grade (1–3)	1.569 (0.852–2.888)	0.1481	2.838 (1.116–7.217)	**0.0285**
ERα (positive vs negative)	0.557 (0.192–1.610)	0.2798	0.794 (0.188–3.360)	0.9184
Stage (I–IV)	0.2191 (0.837–2.171)	0.2192	1.387 (0.730–2.635)	0.3174

CI, confidence interval; ER, estrogen receptor; HR, hazard ratio; PR, progesterone receptor.

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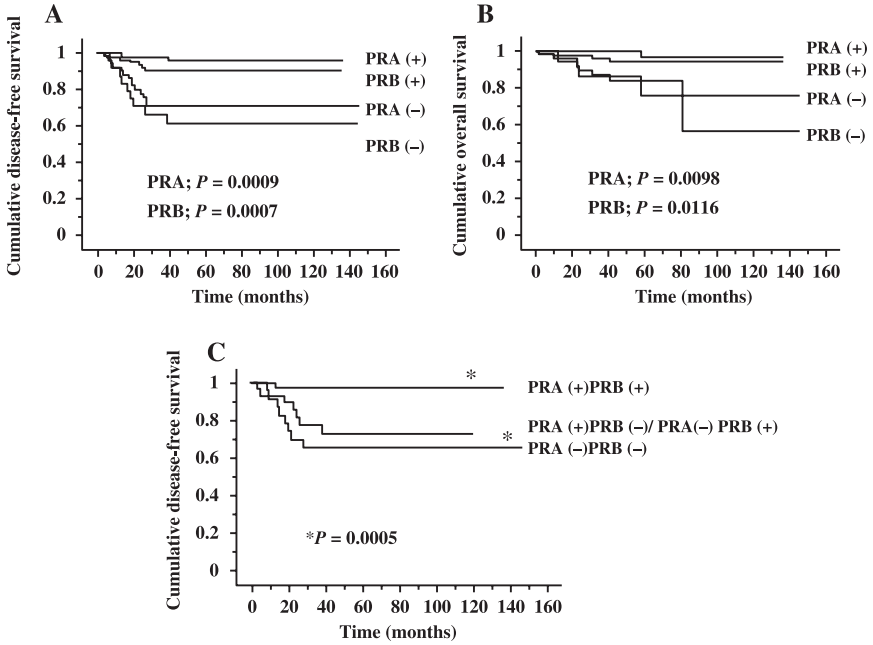

Fig. 4. Correlation between progesterone receptor A (PRA) or B (PRB) isoform immunoreactivity and (A) recurrence, and (B) survival for patients with endometrioid endometrial carcinoma. (C) Correlation between immunoreactivity for both isoforms and recurrence for patients with endometrioid endometrial carcinoma.

characterized. There have been some reports demonstrating the status of PR isoforms and clinical prognosis in endometrial carcinoma.[32,43] Miyamoto *et al*. carried out immunohistochemical analysis and demonstrated that PRB expression occurred significantly more frequently in grade 1 and was inversely correlated with poor prognosis on clinical outcome of patients, whereas PRA expression was also significantly higher in grade 1 and was inversely correlated with Ki-67 expression, but not with prognosis of the patients. They concluded that PRA and PRB expression was significantly correlated with biologically malignant potential.[43] Sakaguchi *et al*. examined mRNA levels of the PR isoforms and reported a significant positive correlation between PRA and PRB mRNA expression in endometrial carcinoma.[32] They quantified the mRNA levels of PRAB (PRA + PRB) using real-time RT-PCR, and they also calculated the mRNA levels of PRA from these data. There were no significant differences in the level of PRA mRNA between normal endometrium and each histological grade, although PRB expression was significantly higher in G1. In addition, PRB mRNA, but not PRA mRNA, status was significantly correlated with survival in endometrial carcinoma.[32] However, in these previous studies, the combined results for loss of expression of both of the PR isoforms and their prognostic correlations were not examined in endometrioid endometrial carcinoma. In the present study, both PRA and PRB were significantly lower for the higher histological-grade carcinoma cases, which is consistent with the results of previously reported studies.[30,32,43] Loss of both PRB and PRA expression in carcinoma tissue was significantly associated with an adverse clinical outcome in the patients. The absence of either one or both of these PR isoforms was associated with a significantly poorer prognosis at disease-free survival. In addition, multivariate analysis demonstrated that an absence of PRA immunoreactivity was an independent risk factor in disease-free survival of the patients (Table 3). Furthermore, only one case was PRB negative among these 51 PRA-positive cases. The number and disease-free survival curve was similar between the groups of $PRA^{-}PRB^{-}$ and $PRA^{+}PRB^{-}/PRA^{-}PRB^{+}$ (Fig. 4C). These results all indicate that the status of PRA in endometrial cancer is quite important in determining the postoperative course of the patients.

Each PR status is considered to strongly influence the abnormal proliferative, invasive and metastatic potential of endometrial carcinoma cells. Microarray analysis of human endometrial carcinoma cells expressing either PRA or PRB confirmed that each PR isoform has distinctly different target genes, with little overlap.[45] Several investigators demonstrated that progesterone acts principally through PRB to inhibit endometrial carcinoma cell invasiveness modulated by adhesion molecules, including integrin and matrix metalloproteinases.[46,47] However, Hanekamp *et al*. demonstrated recently that endometrial carcinoma cell lines, which expressed only PRA, expressed higher levels of cadherins and demonstrated a lower level of invasive properties compared to the cell lines that expressed PRB.[48] They also demonstrated that the loss of expression of both PR isoforms was associated with increased expression of CD44 and CSPG/versican, invasion-related proteins. They further suggested that these results may represent an early and possibly initializing event in the development of a more invasive phenotype in endometrial carcinoma.[49] Results of these studies in cell lines also suggest that a decrease or loss of PRA and/or PRB expression should become an important factor that contributes to invasive and metastatic potential and eventually poor prognosis in human endometrial carcinoma. Dai *et al*. studied the effectiveness of adenovirus-mediated PR gene transduction in combination with progestin therapy in mouse xenograft models, and demonstrated that the presence of both PRA and PRB provided a substantial benefit to animal survival compared with PRB alone.[50] Results of an inverse correlation between both PR isoforms and Ki-67 expression in our study also suggest the important roles of each PR isoforms for protecting against aggressive proliferation and development. In summary, the results of the present study indicate that the loss of PR isoform expression, especially PRA, in human endometrioid endometrial carcinoma may result in aggressive biological characteristics that play important roles in prognosis and recurrence.

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