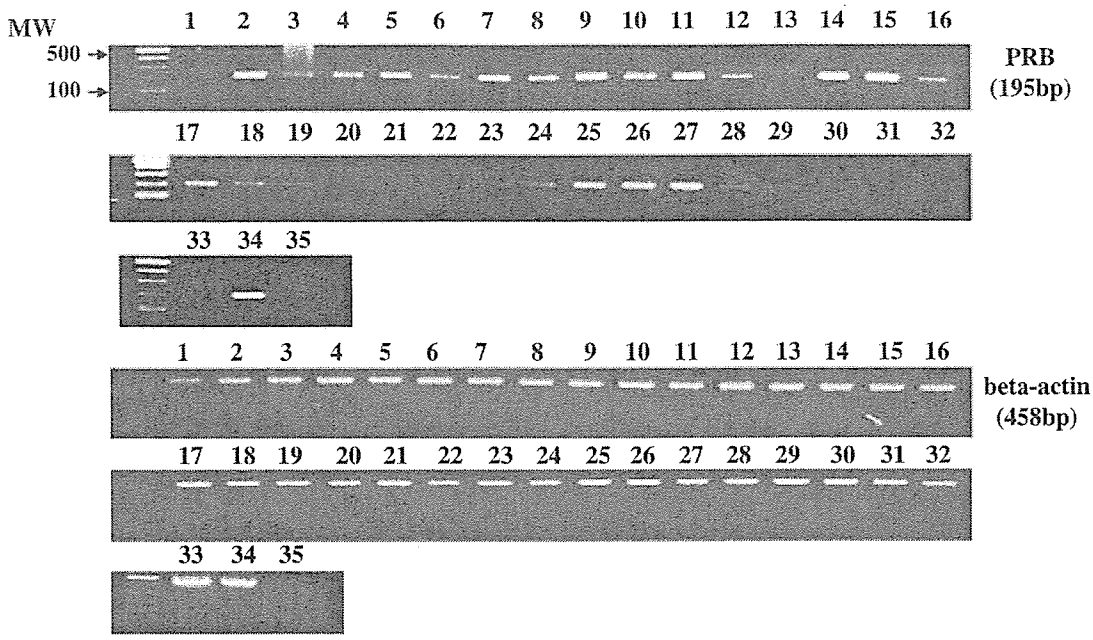

Fig. 2. Reverse transcription–polymerase chain reaction (RT-PCR) analysis of total RNA extracted from endometrioid endometrial carcinoma. Nos 4, 10, 13, 21, 29, 30, 31, 32 and 33 are progesterone receptor B (PRB)-negative cases, as determined by immunohistochemistry. No. 34 is a positive control. No. 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).

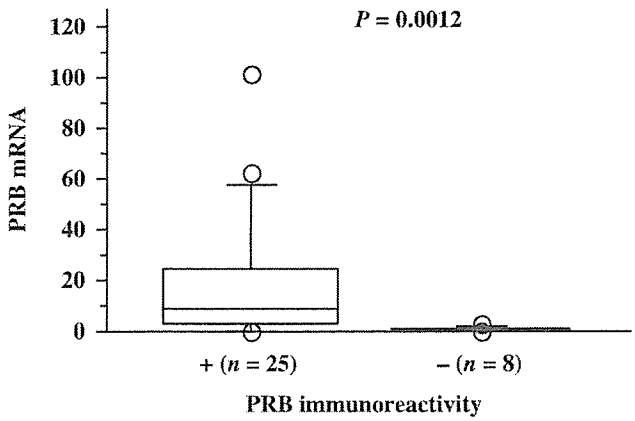

Fig. 3. Correlation between progesterone receptor B (PRB) immunoreactivity and its mRNA level determined by quantitative reverse transcriptin–polymerase chain reaction analyses in human endometrial carcinoma. There was a statistically significant positive correlation between PRB immunoreactivity and 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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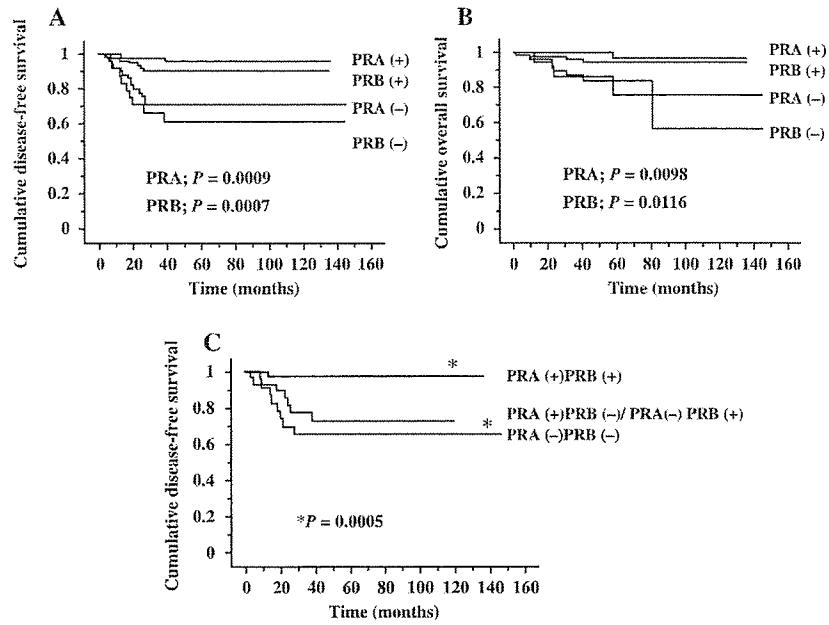

Fig. 4. Correlation between progesterone receptor A (PRA) or B (PRB) isoform immunoreactivity and (A) recurrence, and (B) survival for patients with endometrioid endometrial carcinoma. (C) Correlation between immunoreactivity for both isoforms and recurrence for patients with endometrioid endometrial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

References

1 Jemal A, Tiwari RC, Murray T *et al.* Cancer statistics, 2004. *CA Cancer J Clin* 2004; **54**: 8–29.

2 Graham JD, Clarke CL. Physiological action of progesterone in target tissues. *Endocr Rev* 1997; **18**: 502–19.

3 Hulka BS, Kaufman DG, Fowler WC, Grimson RC, Greenberg BG. Predominance of early endometrial cancers after long-term estrogen use. *JAMA* 1980; **244**: 2419–22.
4 Thomas DB. Do hormones cause cancer? *Cancer* 1984; **53**: 595–604.
5 Shumaker SA, Legault C, Rapp SR *et al.* Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. *JAMA* 2003; **289**: 2651–62.
6 Beresford SAA, Weiss NS, Voigt LF, McKnight B. Risk of endometrial cancer in relation to use of oestrogen combined with cyclic progestagen therapy in postmenopausal women. *Lancet* 1997; **349**: 458–61.
7 Wang CB, Wang CJ, Huang HJ *et al.* Fertility-preserving treatment in young patients with endometrial adenocarcinoma. *Cancer* 2002; **94**: 2192–8.
8 Kaku T, Yoshikawa H, Tsuda H *et al.* Conservative therapy for adenocarcinoma and atypical endometrial hyperplasia of the endometrium in young women: central pathologic review and treatment outcome. *Cancer Lett* 2001; **167**: 39–48.
9 Utsunomiya H, Suzuki T, Ito K *et al.* The correlation between the response to progestogen treatment and the expression of progesterone receptor B and 17β-hydroxysteroid dehydrogenase type 2 in human endometrial carcinoma. *Clin Endocrinol* 2003; **58**: 696–703.
10 Evans RM. The steroid and thyroid hormone receptor superfamily. *Science* 1988; **240**: 889–95.
11 Savouret JF, Rauch M, Redeuilh G *et al.* Interplay between estrogens, progestins, retinoic acid and AP-1 on a single regulatory site in the progesterone receptor gene. *J Biol Chem* 1994; **269**: 28 955–62.
12 Mote PA, Balleine RL, McGowan EM, Clarke CL. Colocalization of progesterone receptors A and B by dual immunofluorescent histochemistry in human endometrium during the menstrual cycle. *J Clin Endocrinol Metab* 1999; **84**: 2963–71.
13 Horwitz KB, Alexander PS. *In situ* photo-linked nuclear progesterone receptors of human breast cancer cells: submit molecular weights after transformation and translocation. *Endocrinology* 1983; **113**: 2195–201.
14 Kastner P, Krust A, Turcotte B *et al.* Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *EMBO J* 1990; **9**: 1603–14.
15 Vegeto E, Shahbaz MM, Wen DX, Goldman ME, O'Malley BW, McDonnell DP. Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. *Mol Endocrinol* 1993; **7**: 1244–55.
16 Giangrande PH, Kimbrel EA, Edwards DP, McDonnell DP. The opposing transcriptional activities of the two isoforms of the human progesterone receptor are due to differential cofactor binding. *Mol Cell Biol* 2000; **20**: 3102–15.
17 Richer JK, Jacobsen BM, Manning NG, Abel MG, Wolf DM, Horwitz KB. Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. *J Biol Chem* 2002; **277**: 5209–18.
18 Akahira J, Suzuki T, Ito K *et al.* Differential expression of progesterone receptor isoforms A and B in the normal ovary, and in benign, borderline, and malignant ovarian tumors. *Jpn J Cancer Res* 2002; **93**: 807–15.
19 Akahira J, Inoue T, Suzuki T *et al.* Progesterone receptor isoforms A and B in human epithelial ovarian carcinoma: immunohistochemical and RT-PCR studies. *Br J Cancer* 2000; **83**: 1488–94.
20 Hopp TA, Weiss HL, Hilsenbeck SG *et al.* Breast cancer patients with progesterone receptor PR-A-rich tumors have poorer disease-free survival rates. *Clin Cancer Res* 2004; **10**: 2751–60.
21 Arnett-Mansfield RL, DeFazio A, Wain GV *et al.* Relative expression of progesterone receptors A and B in endometrioid cancers of the endometrium. *Cancer Res* 2001; **61**: 4576–82.
22 De Vivo I, Huggins GS, Hankinson SE *et al.* A functional polymorphism in the promoter of the progesterone receptor gene associated with endometrial cancer risk. *Proc Natl Acad Sci USA* 2002; **99**: 12 263–8.
23 Sasaki M, Dharia A, Oh BR, Tanaka Y, Fujimoto S, Dahiya R. Progesterone receptor B gene inactivation and CpG hypermethylation in human uterine endometrial cancer. *Cancer Res* 2001; **61**: 97–102.
24 Fukuda K, Mori M. Prognostic significance of progesterone receptor immunohistochemistry in endometrial carcinoma. *Gynecol Oncol* 1998; **69**: 220–5.
25 Chambers JT, MacLusky N, Eisenfield A, Kohorn EI, Lawrence R, Schwartz PE. Estrogen and progestin receptor levels as prognosticators for survival in endometrial cancer. *Gynecol Oncol* 1988; **31**: 65–81.
26 Kleine W, Maier T, Geyer H, Pfleiderer A. Estrogen and progesterone receptors in endometrial cancer and their prognostic relevance. *Gynecol Oncol* 1990; **38**: 59–65.
27 Morris PC, Anderson JR, Anderson B, Buller RE. Steroid hormone receptor content and lymph node status in endometrial cancer. *Gynecol Oncol* 1995; **56**: 406–11.
28 Rose PG. Endometrial carcinoma. *N Engl J Med* 1996; **335**: 640–9.
29 Fujimoto J, Ichigo S, Hori M, Nishigaki M, Tamaya T. Expression of progesterone receptor form A and B mRNAs in gynecologic malignant tumors. *Tumour Biol* 1995; **16**: 254–60.
30 Fujimoto J, Ichigo S, Hirose R, Sakaguchi H, Tamaya T. Clinical implication of expression of progesterone receptor form A and B mRNAs in secondary spreading of gynecologic cancers. *J Steroid Biochem Mol Biol* 1997; **62**: 449–54.
31 Kumar NS, Richer J, Owen G, Litman E, Horwitz KB, Leslie KK. Selective down-regulation of progesterone receptor isoform B in poorly differentiated human endometrial cancer cells: implications for unopposed estrogen action. *Cancer Res* 1998; **58**: 1860–5.
32 Sakaguchi H, Fujimoto J, Hong BL, Nakagawa Y, Tamaya T. Drastic decrease of progesterone receptor form B but not A mRNA reflects poor patient prognosis in endometrial cancers. *Gynecol Oncol* 2004; **93**: 394–9.
33 Tavassoli FA, Devilee P. Pathology and genetics of tumours of the breast and female genital organs. In: *World Health Organization Classification of Tumours*. Lyon: World Health Organization, 2003; 113–45.
34 Creasman WT. Announcement FIGO, stages: 1988 revisions. *Gynecol Oncol* 1989; **35**: 125–7.
35 Clarke CL, Zaino RJ, Feil PD *et al.* Monoclonal antibodies to human progesterone receptor: characterization by biochemical and immunohistochemical techniques. *Endocrinology* 1987; **121**: 1123–32.
36 Mote PA, Balleine RL, McGowan EM, Clarke CL. Colocalization of progesterone receptors A and B by dual immunofluorescent histochemistry in human endometrium during the menstrual cycle. *J Clin Endocrinol Metab* 1999; **84**: 2963–71.
37 Gray GO, Satyaswaroop PG. Species crossreactivity of human progesterone receptor monoclonal antibodies: western blot analysis. *Biochem Biophys Res Commun* 1988; **157**: 1067–77.
38 Layfield LJ, Gupta D, Mooney EE. Assessment of tissue estrogen and progesterone receptor levels: a survey of current practice, techniques, and quantitation methods. *Breast J* 2000; **6**: 189–96.
39 Goldhirsch A, Wood WC, Gelber RD, Coates AS, Thurlimann B, Senn HJ. Meeting highlights: updated international expert consensus on the primary therapy of early breast cancer. *J Clin Oncol* 2003; **21**: 3357–65.
40 Wittwer CT, Ririe KM, Andrew RV, David DA, Gundry RA, Balis UJ. The LightCycler: a microVolume multisame fluorimeter with rapid temperature control. *Biotechniques* 1997; **22**: 176–81.
41 Read SJ. Recovery efficiencies on nucleic acid extraction kits as measured by quantitative LightCycler PCR. *Mol Pathol* 2001; **54**: 86–90.
42 Dumoulin FL, Nischalke HD, Leifeld L *et al.* Semi-quantification of human C-C chemokine mRNAs with reverse transcription/real-time PCR using multi-specific standards. *J Immunol Meth* 2000; **241**: 109–19.
43 Miyamoto T, Watanabe J, Hata H *et al.* Significance of progesterone receptor-A and -B expressions in endometrial adenocarcinoma. *J Steroid Biochem Mol Biol* 2004; **92**: 111–18.
44 Ariga N, Suzuki T, Moriya T *et al.* Progesterone receptor A and B isoforms in the human breast and its disorders. *Jpn J Cancer Res* 2001; **92**: 302–8.
45 Smid-Koopman E, Blok LJ, Kuhne LC *et al.* Distinct functional differences of human progesterone receptors A and B on gene expression and growth regulation in two endometrial carcinoma cell lines. *J Soc Gynecol Invest* 2003; **10**: 49–57.
46 Dai D, Wolf DM, Litman ES, White MJ, Leslie KK. Progesterone inhibits human endometrial cancer cell growth and invasiveness: down-regulation of cellular adhesion molecules through progesterone B receptors. *Cancer Res* 2002; **62**: 881–6.
47 Saito T, Mizumoto H, Tanaka R *et al.* Overexpressed progesterone receptor form B inhibits invasive activity suppressing matrix metalloproteinases in endometrial carcinoma cells. *Cancer Lett* 2004; **209**: 237–43.
48 Hanekamp EE, Gielen SC, De Ruiter PE *et al.* Differences in invasive capacity of endometrial cancer cell lines expressing different progesterone receptor isotypes: possible involvement of cadherins. *J Soc Gynecol Invest* 2005; **12**: 278–84.
49 Hanekamp EE, Gielen SC, Smid-Koopman E *et al.* Consequences of loss of progesterone receptor expression in development of invasive endometrial cancer. *Clin Cancer Res* 2003; **9**: 4190–9.
50 Dai D, Albitar L, Nguyen T, Laidler LL, Singh M, Leslie KK. A therapeutic model for advanced endometrial cancer: systemic progestin in combination with local adenoviral-mediated progesterone receptor expression. *Mol Cancer Ther* 2005; **4**: 169–75.

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