OPEN

Immunolocalization of Corticotropin-Releasing Hormone (CRH) and Its Receptors (CRHR1 and CRHR2) in Human Endometrial Carcinoma

CRHR1 as a Potent Prognostic Factor

Naoko Sato, PhD, Kiyoshi Takagi, PhD, Takashi Suzuki, MD, PhD, Yasuhiro Miki, PhD, Sota Tanaka, MD, PhD, Satoru Nagase, MD, PhD, Hitoshi Warita, MD, PhD, Shin Fukudo, MD, PhD, Fumiko Sato, PhD, Hironobu Sasano, MD, PhD, and Kiyoshi Ito, MD, PhD

Objective: Corticotropin-releasing hormone (CRH), a major regulator of the stress response, regulates various biological functions through its interaction with CRH receptors 1 (CRHR1) and 2 (CRHR2). CRH, CRHR1, and CRHR2 have recently been reported in several types of carcinoma, but the significance of these proteins has remained largely unknown in human endometrial carcinoma.

Materials and Methods: A total of 87 endometrial carcinoma specimens were obtained from Japanese female patients who underwent surgical treatment, fixed in 10% formalin, and embedded in paraffin wax. Immunohistochemistry for CRH, CRHR1, and CRHR2 was performed, and clinical data were obtained from the medical records.

Results: Immunopositivity of CRH, CRHR1, and CRHR2 in the specimens was 26%, 15%, and 10%, respectively. Univariate analysis revealed that immunohistochemical CRH status was positively associated with CRHR1 and CRHR2 status and that CRHR1 status was significantly associated with the risk of recurrence and poorer clinical outcome, whereas CRHR2 status was marginally associated with better prognosis for overall survival. Multivariate analysis demonstrated CRHR1 status as an independent prognostic factor for both disease-free and overall survival.

Conclusions: These results suggest that intratumoral CRH-CRHR1 signaling plays an important role in the progression of endometrial carcinoma and that CRHR1 is a potent prognostic factor in patients with this disease.

Key Words: Corticotropin-releasing hormone, Corticotropin-releasing hormone receptor 1, Endometrial cancer, Immunohistochemistry, Prognosis

Received February 3, 2014, and in revised form August 12, 2014.
Accepted for publication August 12, 2014.

(*Int J Gynecol Cancer* 2014;24: 1549–1557)

Tohoku University Graduate School of Medicine, Sendai, Japan.
Address correspondence and reprint requests to Kiyoshi Ito, MD, Obstetrics and Gynecology, Tohoku University Graduate School of Medicine, Sendai, 980–8557 Japan.
E-mail: kito@med.tohoku.ac.jp.

This research was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare, Japan.

ISSN: 1048-891X
DOI: 10.1097/IGC.0000000000000269

ThinkSCIENCE K.K. (info@thinkscience.co.jp) provided editing services, which were funded by the grants.

Supplemental digital content is available for this article. Direct URL citation appears in the printed text and is provided in the HTML and PDF versions of this article on the journal's Web site (www.ijgc.net).

The authors declare no conflicts of interest.

Endometrial carcinoma is the most common malignancy found in the female genital tract, and its incidence, particularly that of the most common histological type, endometrioid endometrial carcinoma, has increased recently.[1,2] The estimated 5-year overall survival for early-stage endometrial carcinoma is 82% but decreases remarkably to 67% for regional disease and 16% for distant disease.[3] Although the majority of patients (approximately 83%) are diagnosed as having stage I or II disease, those with advanced-stage endometrial carcinoma or unfavorable pathological characteristics have poor prognosis.[2] Therefore, it is important to evaluate the clinical and biological markers in patients with endometrial carcinoma to predict recurrence after surgery and to evaluate the indications of additional therapies appropriately.

Corticotropin-releasing hormone (CRH) is a major regulator of the stress response to internal and external factors.[4] The stress response is characterized by an activation of the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis.[5] Corticotropin-releasing hormone is secreted by the paraventricular nucleus of the hypothalamus in response to stress and stimulates the anterior lobe of the pituitary gland to release adrenocorticotropic hormone through its interaction with CRH receptors 1 (CRHR1) and 2 (CRHR2).[6] Adrenocorticotropic hormone in turn stimulates the adrenocortex to produce and secrete cortisol.[6,7] The actions of CRH and its receptors are thought to play important roles in various stress-related disorders.[8]

Recent studies have demonstrated that CRH is present not only in the central nervous system but also in various peripheral organs.[9] Corticotropin-releasing hormone mediates endocrine responses to stress by activating the HPA axis as well as via direct actions in the periphery.[10,11] Of note, the expression of CRH and its receptors has also been reported in several types of carcinoma.[12,13] Specifically, Miceli[14] reported CRH, CRHR1, and CRHR2 immunoreactivity in endometrial carcinoma. Although stress is known to be a promoter of tumor growth,[15] it remains unclear whether CRH, CRHR1, or CRHR2 immunoreactivity in endometrial carcinoma is a risk factor for poor prognosis. In this study, we tested the hypothesis that the expression of CRH, CRHR1, and CRHR2 in endometrial carcinoma is associated with poor prognosis.

MATERIALS AND METHODS

Ethics Statement

All subjects provided written informed consent for histopathological examination of their resected tissues. The research protocol for this study was approved by the Ethics Committee of Tohoku University Graduate School of Medicine.

Patients and Tissues

A total of 87 women with endometrioid endometrial adenocarcinoma participated in this study. All patients underwent surgical treatment from 1993 to 2003 at the Department of Obstetrics and Gynecology, Tohoku University Hospital, Japan, at a mean age of 56 years (range, 30–79 years). The standard primary treatment for endometrial carcinoma at Tohoku University Hospital is surgery consisting of total abdominal hysterectomy, salpingo-oopholectomy, pelvic and/or para-aortic lymphadenectomy, and peritoneal washing cytology. Of the 87 patients, 74 underwent lymphadenectomy. None of the patients examined had received irradiation, hormonal therapy, or chemotherapy before surgery. The patients were initially staged according to the International Federation of Gynecology and Obstetrics 1988 classification,[16] which was recorded in their medical records, but for this study, we reevaluated the stage according to the International Federation of Gynecology and Obstetrics 2009 staging guidelines.[17] After surgery, 50 patients received pelvic radiation therapy or chemotherapy.

Clinical outcome was evaluated based on disease-free and overall survival, which were calculated from the time of initial surgery to recurrence and/or death or to the date of last contact. The mean follow-up time was 94 months (range, 8–224 months), and all patients were managed at the Tohoku University Hospital or its affiliated hospitals during the study period.

Immunohistochemistry

Specimens were fixed in 10% formalin and embedded in paraffin wax. Goat polyclonal antibodies against CRH (C-20), CRHR1 (V-14), and CRHR2 (N-20) were purchased from Santa Cruz Biotechnology (Dallas, TX). Mouse monoclonal antibodies against the estrogen receptor (ER; ER1D5), progesterone receptor (PR; MAB429), and Ki-67 (MIB1) were purchased from Immunotech (Marseille, France), Chemicon (Temecula, CA), and DAKO (Carpinteria, CA), respectively.

A Histofine Kit (Nichirei Biosciences, Tokyo, Japan), which uses the streptavidin-biotin amplification method, was used for antibody staining. Antigen retrieval was performed by heating the slides in a microwave for 20 minutes in citric acid buffer (2-mM citric acid and 9-mM trisodium citrate dehydrate; pH 6.0) for staining with anti-CRH and CRHR1 antibodies and by heating the slides in an autoclave at 120°C for 5 minutes in citric acid buffer for ER, PR, and Ki-67 immunostaining. No antigen retrieval was performed for CRHR2 immunostaining. The dilutions of the primary antibodies were as follows: CRH, 1/50; CRHR1, 1/50; CRHR2, 1/50; ER, 1/50; PR, 1/40; and Ki-67, 1/50.[18] Antigen-antibody complexes were 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. Human placental tissue was used as a positive control for CRH, CRHR1, and CRHR2 immunostaining.[14] As a negative control, normal goat or mouse immunoglobulin G was used instead of the primary antibody.

When CRH, CRHR1, and CRHR2 immunoreactivity was detected in the cytoplasm of the endometrial carcinoma cells, cases with more than 10% positive carcinoma cells were considered positive for CRH, CRHR1, and CRHR2 because 10% is frequently used as the cutoff for the evaluation of cytoplasmic immunostaining.[19] Estrogen receptor, PR, and Ki-67 immunoreactivity was detected in the nucleus and evaluated in more than 1000 carcinoma cells in each case, and the percentage of immunoreactivity (labeling index [LI]) was determined. Cases with LI of 10% or greater were considered positive for ER and PR.[20]

Statistical Analysis

The associations between CRH, CRHR1, and CRHR2 immunohistochemical status and clinicopathological factors were evaluated using Student *t* test or cross-tabulation using the χ^2 test. Disease-free and overall survival curves were generated according to the Kaplan-Meier method, and statistical significance was calculated using Cox proportional hazard model. Univariate and multivariate analyses were performed according to Cox model. $P < 0.05$ was considered significant, and $P \geq 0.05$ but $P < 0.10$ was considered borderline significant, and these values were subjected to multivariate analysis.[21] Statistical analysis was performed using JMP Pro version 9.02 (SAS Institute, Inc, Cary, NC).

RESULTS

Immunolocalization of CRH, CRHR1, and CRHR2 in Endometrial Carcinoma

Corticotropin-releasing hormone, CRHR1, and CRHR2 immunoreactivity was detected in the cytoplasm of endometrial carcinoma cells (Fig. 1A–C). Corticotropin-releasing hormone, CRHR1, and CRHR2 were weakly positive in the nonneoplastic endometrial glands and negative in the stroma. Of the 87 cases of endometrial carcinoma, 23 (26%), 13 (15%), and 9 (10%) were positive for CRH, CRHR1, and CRHR2, respectively.

The association between immunohistochemical CRH status and the various clinicopathological parameters of the patients are summarized in Table 1. Corticotropin-releasing hormone immunoreactivity was significantly associated with CRHR1 status ($P = 0.004$) and CRHR2 status ($P = 0.001$). However, no significant association was detected between CRH status and other factors such as patient age, stage, status of adjuvant therapy after surgery, lymph node metastasis, myometrial invasion, histological grade, lymphovascular invasion, ER status, PR status, and Ki-67 LI. Furthermore, neither CRHR1 status (Table 2) nor CRHR2 status (Table 3) was significantly associated with any of the factors examined.

Association Between CRH, CRHR1, and CRHR2 Status and the Clinical Outcomes of Patients With Endometrial Cancer

Corticotropin-releasing hormone status was not significantly associated with disease-free ($P = 0.17$, Fig. 2A) or overall ($P = 0.23$, Fig. 2B) survival in any of the patients. In contrast, CRHR1 status was significantly associated with an increased incidence of recurrence ($P = 0.023$, Fig. 2C) and worse prognosis ($P = 0.009$, Fig. 2D). Corticotropin-releasing hormone receptor 2 status was not significantly associated with the incidence of recurrence ($P = 0.61$, Fig. 2E) but was marginally associated with better clinical outcome ($P = 0.093$, Fig. 2F). Similar tendencies were also detected when the cases were classified into 3 groups according to immunointensity (ie, negative, weakly positive, and strongly positive[22]) (disease-free survival: $P = 0.15$ for CRH; $P = 0.019$ for CRHR1; and $P = 0.76$ for CRHR2) (Supplemental Digital Content Figure S1 [A–C], http://links.lww.com/IGC/A232) and overall survival ($P = 0.010$ for CRH; $P = 0.003$ for CRHR1; and $P = 0.24$ for CRHR2; data not shown).

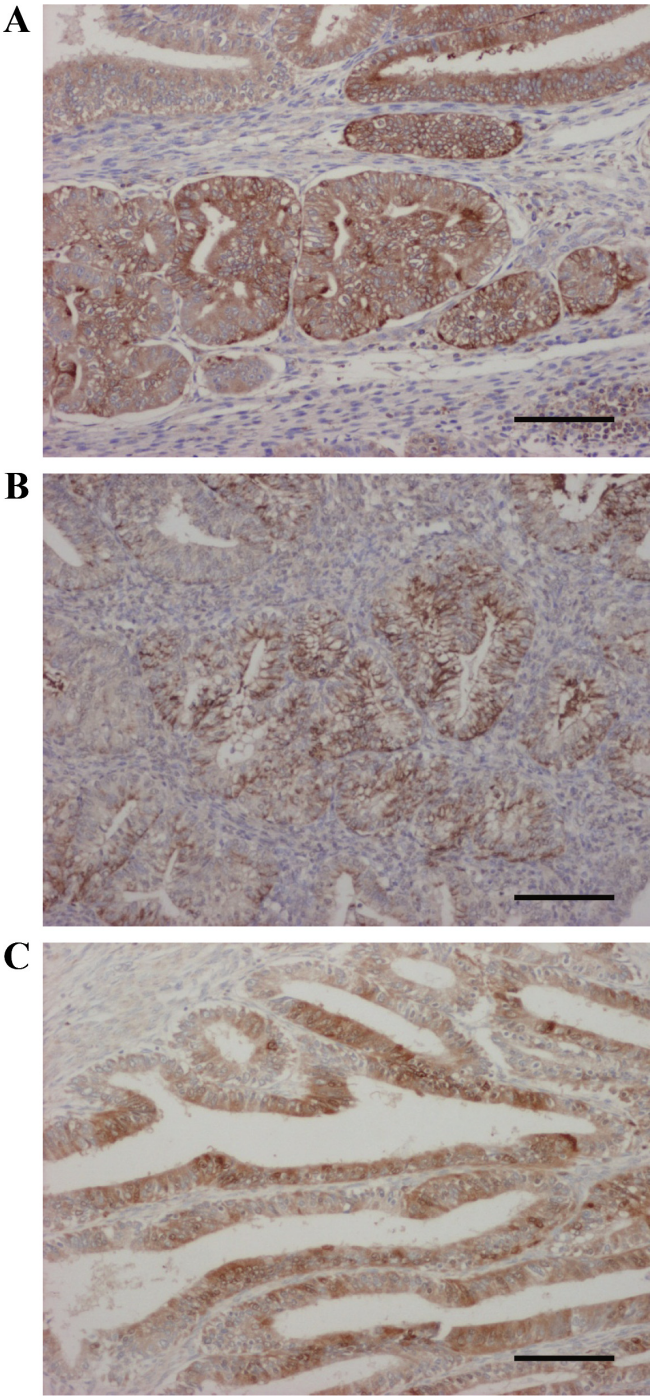

FIGURE 1. Immunohistochemistry for CRH (A), CRHR1 (B), and CRHR2 (C) in endometrial carcinoma specimens. Corticotropin-releasing hormone, CRHR1, and CRHR2 are immunolocalized in the carcinoma cells. Bar, 100 μm.

The results of univariate analysis of disease-free survival by Cox model indicated PR status ($P = 0.001$) and

TABLE 1. Association between immunohistochemical CRH status and clinicopathological parameters in 87 endometrial carcinomas

	CRH Status		
	Positive (n = 23)	**Negative (n = 64)**	***P***
Age, y*	57 (13)	55 (10)	0.47
Stage			0.21
I	17 (25%)	52 (75%)	
II	0 (0%)	3 (100%)	
III	6 (46%)	7 (54%)	
IV	0 (0%)	2 (100%)	
Adjuvant therapy after surgery			0.70
Received	14 (28%)	36 (72%)	
Not received	9 (24%)	28 (76%)	
Lymph node metastasis (n = 74)			0.76
Present	1 (20%)	4 (80%)	
Absent	18 (26%)	51 (74%)	
Myometrial invasion			0.79
>50%	9 (28%)	23 (72%)	
≤50%	14 (25%)	41 (75%)	
Histological grade			0.62
I	13 (31%)	29 (69%)	
II	6 (21%)	23 (79%)	
III	4 (25%)	12 (75%)	
Lymphovascular invasion			0.72
Present	7 (29%)	17 (71%)	
Absent	16 (25%)	47 (75%)	
ER status			1.0
Positive	15 (26%)	43 (74%)	
Negative	8 (28%)	21 (72%)	
PR status			1.0
Positive	15 (26%)	42 (74%)	
Negative	8 (27%)	22 (73%)	
Ki-67 LI, %*	30 (29)	40 (32)	0.20
CRHR1 status			
Positive	8 (62%)	5 (38%)	**0.004**
Negative	15 (20%)	59 (80%)	
CRHR2 status			
Positive	7 (78%)	2 (22%)	**0.001**
Negative	16 (21%)	62 (79%)	

$P < 0.05$ is considered significant and is indicated in boldface.
*Data are presented as mean (SD). All other values represent the number of cases and their percentage of positive and negative cases.

CRHR1 status ($P = 0.023$) as significant prognostic factors for disease-free survival and stage ($P = 0.057$) and lymph node metastasis ($P = 0.096$) as marginally significant prognostic factors (Table 4). Multivariate analysis demonstrated PR ($P = 0.010$) and CRHR1 ($P = 0.027$) as independent prognostic factors with relative risks greater than 1.0. In the univariate analysis for overall survival, PR status ($P = 0.015$) and CRHR1 status ($P = 0.009$) were significant

TABLE 2. Association between immunohistochemical CRHR1 status and clinicopathological parameters in 87 endometrial carcinomas

	CRHR1 Status		
	Positive (n =13)	**Negative (n = 74)**	***P***
Age, y*	56 (12)	56 (11)	0.98
Stage			0.63
I	12 (17%)	57 (83%)	
II	0 (0%)	3 (100%)	
III	1 (8%)	12 (92%)	
IV	0 (0%)	2 (100%)	
Adjuvant therapy after surgery			0.77
Received	7 (14%)	43 (86%)	
Not received	6 (16%)	31 (84%)	
Lymph node metastasis (n = 74)			0.31
Present	0 (0%)	5 (100%)	
Absent	12 (17%)	57 (83%)	
Myometrial invasion			0.63
>50%	4 (13%)	28 (87%)	
≤50%	9 (16%)	46 (84%)	
Histological grade			0.41
I	6 (14%)	36 (86%)	
II	3 (10%)	26 (90%)	
III	4 (25%)	12 (75%)	
Lymphovascular invasion			0.69
Present	3 (13%)	21 (87%)	
Absent	10 (16%)	53 (84%)	
ER status			1.0
Positive	9 (16%)	49 (84%)	
Negative	4 (14%)	25 (86%)	
PR status			0.36
Positive	7 (12%)	50 (88%)	
Negative	6 (20%)	24 (80%)	
Ki-67 LI, %*	30 (25)	39 (32)	0.37
CRHR2 status			
Positive	3 (33%)	6 (67%)	0.13
Negative	10 (13%)	68 (87%)	

*Data are presented as mean (SD). All other values represent the number of cases and their percentage of positive and negative cases.

TABLE 3. Association between immunohistochemical CRHR2 status and clinicopathological parameters in 87 endometrial carcinomas

	CRHR2 Status		
	Positive (n = 9)	Negative (n = 78)	*P*
Age, y*	54 (14)	56 (11)	0.50
Stage			0.82
I	7 (10%)	62 (90%)	
II	0 (0%)	3 (100%)	
III	2 (15%)	11 (85%)	
IV	0 (0%)	2 (100%)	
Adjuvant therapy after surgery			0.12
Received	3 (6%)	47 (94%)	
Not received	6 (16%)	31 (84%)	
Lymph node metastasis (n = 74)			0.49
Present	0 (0%)	5 (100%)	
Absent	6 (9%)	63 (91%)	
Myometrial invasion			0.34
>50%	2 (6%)	30 (94%)	
≤50%	7 (13%)	48 (87%)	
Histological grade			0.76
I	5 (12%)	37 (88%)	
II	2 (7%)	27 (93%)	
III	2 (13%)	14 (88%)	
Lymphovascular invasion			0.24
Present	1 (4%)	23 (96%)	
Absent	8 (13%)	55 (87%)	
ER status			1.0
Positive	6 (10%)	52 (90%)	
Negative	3 (10%)	26 (90%)	
PR status			1.0
Positive	6 (11%)	51 (89%)	
Negative	3 (10%)	27 (90%)	
Ki-67 LI (%)*	21 (16)	39 (32)	0.10

*Data are presented as mean (SD). All other values represent the number of cases and their percentage of positive and negative cases.

prognostic factors, and CRHR2 status ($P = 0.093$) was a borderline significant factor. Subsequent multivariate analysis revealed all 3 of these variables as independent prognostic factors ($P = 0.018$, $P = 0.006$, and $P = 0.019$, respectively) (Table 5).

DISCUSSION

To our knowledge, this is the first study to demonstrate a significant association between CRHR1 immunoreactivity and poor prognosis in patients with carcinoma. In this study, CRHR1 immunoreactivity was detected in 15% of endometrial carcinoma cases and was positively associated with CRH immunoreactivity, whereas immunohistochemical CRHR1 status was significantly associated with increased recurrence and poor clinical outcomes. The results of multivariate analysis demonstrated CRHR1 status as an independent prognostic factor for both disease-free and overall survival in patients with endometrial carcinoma.

In previous studies, CRHR1 immunoreactivity was detected in several human carcinomas such as adrenal cortical (23%),[22] breast (31%),[23] ovarian (64%),[24] and endometrial (92%)[14] carcinoma. Corticotropin-releasing hormone binds to both CRHR1 and CRHR2, but its affinity is approximately 10-fold higher for CRHR1 than for CRHR2.[25] Both the molecular evidence for CRHR1 and the poor prognosis of carcinoma are increasing. Minas et al[24] reported that CRH increases the expression of the Fas ligand in ovarian carcinoma cells through CRHR1, thereby potentiating their ability to induce apoptosis of activated peripheral blood lymphocytes. In contrast, Jo et al[26] demonstrated that stimulation with CRH enhanced the in vitro migration and invasiveness of endometrial carcinoma cells (Ishikawa cells) through increased levels of matrix metalloprotease 2 and matrix metalloprotease 9 proteins. Moreover, CRH inhibits apoptosis[27] and induces cell proliferation by activating transforming growth factor β/Smad2 signaling[9] in breast carcinoma cells, although it has also been reported that CRH inhibits the growth of Ishikawa cells through CRHR1.[28] Therefore, CRH-CRHR1 signaling is suggested to regulate various biological functions and may play important roles in the aggressiveness of endometrial carcinoma. Miceli[14] did not find any significant association between CRHR1 immunoreactivity and clinical outcome in patients with endometrial cancer, which might be partly caused by the comparatively small sample set (n = 37) and short follow-up period (8–82 months) in their study.

In this study, both PR status and CRHR1 status were independent prognostic factors. Recently, Huvila et al[29] demonstrated that PR status was a more significant predictor of relapse in 182 patients with early-stage endometrioid endometrial adenocarcinoma than the commonly accepted variables such as histological grade, lymphovascular invasion, and myometrial invasion, which is consistent with the present results. In addition, we could not detect any significant associations between stage and histological grade or prognosis, which may be partly caused by the relatively limited number of patients examined (n = 87). Corticotropin-releasing hormone receptor 2 immunoreactivity was detected in 10% of endometrial carcinoma cases and was positively associated with CRH immunoreactivity. Interestingly, in contrast to CRHR1, CRHR2 status turned out to be a better independent prognostic factor for overall survival in multivariate analysis. In other studies, CRHR2 immunoreactivity has been detected in several human malignancies including adrenocortical,[22] ovarian,[24] endometrial,[14] and breast[23] carcinoma, with immunopositivity ranging from 18% to 64%. Kaprara et al[23] reported distinct distributions of CRHR1 and CRHR2 in breast carcinoma and suggested different biological functions for each receptor. Corticotropin-releasing hormone receptor 2 plays almost an antagonistic

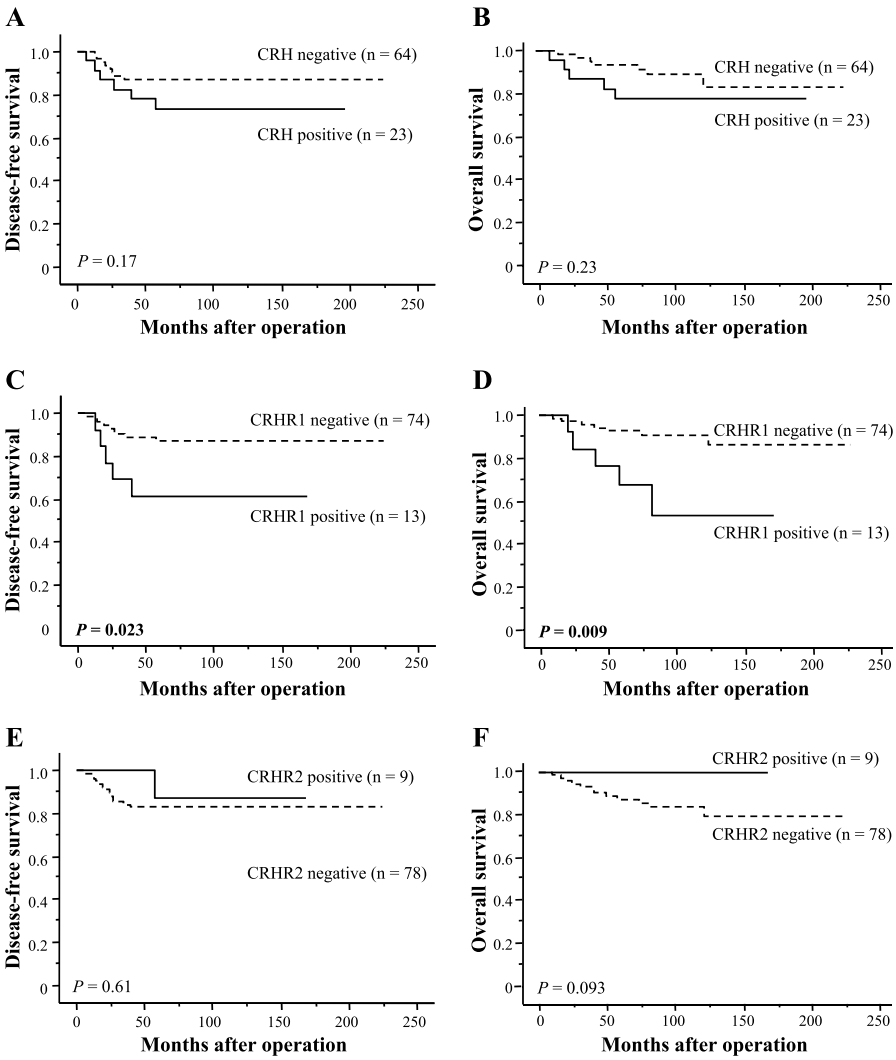

FIGURE 2. Disease-free (A, C, and E) and overall (B, D, and F) survival of the 87 patients with endometrial cancer according to CRH (A and B), CRHR1 (C and D), and CRHR2 (E and F) status determined by the Kaplan-Meier method. *P* values were evaluated using Cox model.

role to CRHR1[6]; activation of CRHR1 causes anxiety, a proinflammatory response, and pronociceptive effects of visceral pain, whereas stimulation of CRHR2 provokes anxiolysis, anti-inflammatory effects, and an antinociceptive response.[8] Moreover, CRHR2 has a higher affinity to urocortin than to CRH.[25,30] Urocortin inhibits proliferation of melanoma cells,[31] and Florio et al[32] suggested that decreased urocortin expression causes the progression of endometrial carcinoma. In addition, CRHR2 is a tonic suppressor of vascularization, and the mechanism of this action has been postulated to modulate angiogenesis in cancer.[33] Taken together, CRHR2 can be considered to play a role in the characterization of less aggressive phenotypes of endometrial carcinoma, a role different from that of CRHR1.

The impact of stress on the development of cancer has been widely proposed.[15,34,35] During chronic stress and depression, persistent activation of the HPA axis based on excessive release of CRH is responsible for an impaired immune response, contributing to the development and progression of several types of cancer.[15] Corticotropin-releasing hormone in the periphery originates from the peripheral tissue and/or partially from peripheral blood, which contains CRH spilled over from the central nervous system.[36] Corticotropin-releasing hormone in the periphery stimulates CRH receptors in the whole body including the brain, uterus, and ovarium.[36] Because negative emotional processes are mediated by either central or peripheral CRH,[6,37] aberrant expression of CRH in endometrial carcinoma might affect the negative emotional state of the patients. Arranz et al[9] demonstrated that chronic stress augments tumor growth in breast tumor-bearing mice, which was promoted by peripheral CRH and not by the HPA axis. Taken together, intratumoral CRH-CRHR1 signaling likely plays an important role in the progression of endometrial carcinoma and may become an important therapeutic target for

TABLE 4. Univariate and multivariate analysis of disease-free survival of 87 patients with endometrial cancer

	Univariate	Multivariate	
Variable	***P***	***P***	**Relative Risk (95% Cl)**
Age (≥50/<49), y	0.51	ND	ND
Stage (III, IV/I, II)	0.057*	0.79	1.4 (0.07–9.2)
Adjuvant therapy after surgery (received/not received)	0.28	ND	ND
Lymph node metastasis (present/absent)	0.096*	0.25	3.9 (0.4–83)
Myometrial invasion (>50%/≤50%)	0.95	ND	ND
Histological grade (III/I, II)	0.30	ND	ND
Lymphovascular invasion (present/absent)	0.43	ND	ND
ER status (negative/positive)	0.45	ND	ND
PR status (negative/positive)	**0.001***	**0.010**	5.1 (1.5–24)
Ki-67 LI (≥10%/<10%)	0.98	ND	ND
CRH status (positive/negative)	0.17	ND	ND
CRHR1 status (positive/negative)	0.023*	0.027	5.2 (1.2–22)
CRHR2 status (negative/positive)	0.61	ND	ND

Data considered significant ($P < 0.05$) are in boldface.
Relative risks are presented as mean (95% CI).
*Significant ($P < 0.05$) and borderline-significant ($0.05 \leq P < 0.10$) values were examined in the multivariate analyses in this study.
CI, confidence interval; ND, not determined.

improving the clinical outcome of patients with endometrial carcinoma.

There are several limitations to this study. First, this study is descriptive and lacks in vitro experiments or the use of models. Therefore, further investigations are required to clarify the molecular functions of CRH-CRHR1 signaling associated with the poor clinical outcomes of patients with endometrial cancer. Second, we do not know the determinants

TABLE 5. Univariate and multivariate analysis of overall survival of 87 patients with endometrial cancer

	Univariate	Multivariate	
Variable	***P***	***P***	**Relative Risk (95% Cl)**
Age (≥50/<49), y	0.31	ND	ND
Stage (III, IV/I, II)	0.46	ND	ND
Adjuvant therapy after surgery (received/not received)	0.23	ND	ND
Lymph node metastasis (present/absent)	0.47	ND	ND
Myometrial invasion (>50%/≤50%)	0.70	ND	ND
Histological grade (III/I, II)	0.38	ND	ND
Lymphovascular invasion (present/absent)	0.23	ND	ND
ER status (positive/negative)	0.87	ND	ND
PR status (positive/negative)	**0.015***	**0.018**	4.2 (1.3–16)
Ki-67 LI (≥10%/<10%)	0.81	ND	ND
CRH status (positive/negative)	0.23	ND	ND
CRHR1 status (positive/negative)	**0.009***	**0.006**	6.3 (1.8–22)
CRHR2 status (negative/positive)	0.093*	**0.019**	ND† (1.6–12)

Data considered significant ($P < 0.05$) are in boldface.
Relative risks are presented as mean (95% Cl).
*Significant ($P < 0.05$) and borderline-significant ($0.05 \leq P < 0.10$) values were examined in the multivariate analyses in this study.
†Relative risk was not estimable because no patients died in the CRHR2-positive group.
CI, confidence interval; ND, not determined.

of CRH, CRHR1, and CRHR2 expression in endometrial carcinoma cells; how endometrial carcinoma cells mediate CRH signaling remains to be elucidated. Third, this is a retrospective study, and the positive findings are based on only 13 (15%) of 87 patients with positive CRHR1. Cohort studies with a larger sample population and a long-term follow-up are the next step in confirming the roles of the CRH signaling system in cancer progression.

In conclusion, our results suggest that intratumoral CRH-CRHR1 signaling plays an important role in the progression of endometrial carcinoma. Further study is warranted to confirm CRHR1 as a potent prognostic factor in endometrial carcinoma in humans.

ACKNOWLEDGMENTS

The authors appreciate the technical assistance and suggestions provided by Dr Masashi Aoki and Ms Risa Ando (Department of Neurology, Tohoku University Graduate School of Medicine, Sendai, Japan), Mr Hiroki Utsunomiya (Department of Gynecology and Obstetrics, Tohoku University Graduate School of Medicine, Sendai, Japan), and Dr Nobuyoshi Ozawa (Ozawa Ladies Total Clinic, Sendai, Japan).

REFERENCES

1. Jemal A, Tiwari RC, Murray T, et al. Cancer statistics, 2004. *CA Cancer J Clin*. 2004;54:8–29.
2. Alhilli MM, Podratz KC, Dowdy SC, et al. Risk-scoring system for the individualized prediction of lymphatic dissemination in patients with endometrioid endometrial cancer. *Gynecol Oncol*. 2013;131:103–108.
3. Siegel R, DeSantis C, Virgo K, et al. Cancer treatment and survivorship statistics, 2012. *CA Cancer J Clin*. 2012;62: 220–241.
4. Tsatsanis C, Dermitzaki E, Venihaki M, et al. The corticotropin-releasing factor (CRF) family of peptides as local modulators of adrenal function. *Cell Mol Life Sci*. 2007;64: 1638–1655.
5. McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev.* 2007;87: 873–904.
6. Fukudo S, Saito K, Sagami Y, Kanazawa M. Can modulating corticotropin releasing hormone receptors alter visceral sensitivity? *Gut*. 2006;55:146–148.
7. Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science*. 1981;213: 1394–1397.
8. Fukudo S. Role of corticotropin-releasing hormone in irritable bowel syndrome and intestinal inflammation. *J Gastroenterol*. 2007;42:48–51.
9. Arranz A, Venihaki M, Mol B, et al. The impact of stress on tumor growth: peripheral CRF mediates tumor-promoting effects of stress. *Mol Cancer.* 2010;9:261.
10. Tache Y, Perdue MH. Role of peripheral CRF signalling pathways in stress-related alterations of gut motility and mucosal function. *Neurogastroenterol Motil*. 2004;16:137–142.
11. Anton PM, Gay J, Mykoniatis A, et al. Corticotropin-releasing hormone (CRH) requirement in *Clostridium difficile* toxin A–mediated intestinal inflammation. *Proc Natl Acad Sci U S A*. 2004;101:8503–8508.
12. Reubi JC, Waser B, Vale W, Rivier J. Expression of CRF1 and CRF2 receptors in human cancers. *J Clin Endocrinol Metab.* 2003;88:3312–3320.
13. Kaprara A, Pazaitou-Panayiotou K, Kortsaris A, et al. The corticotropin releasing factor system in cancer: expression and pathophysiological implications. *Cell Mol Life Sci*. 2010;67: 1293–1306.
14. Miceli F. Expression and subcellular localization of CRH and its receptors in human endometrial cancer. *Mol Cell Endocrinol*. 2009;305:6–11.
15. Reiche EM, Nunes SO, Morimoto HK. Stress, depression, the immune system, and cancer. *Lancet Oncol*. 2004;5:617–625.
16. Tavassoli FADP. Pathology and genetics of tumours of the breast and female genital organs. *World Health Organization Classification of Tumors*. Lyon, France: International Agnecy for Research on Cancer (IARC): 2003:113–145.
17. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. *Int J Gynecol Obstet*. 2009;105:103–104; Erratum in *Int J Gynaecol Obstet*. 2010; 108:176.
18. Ota K, Ito K, Suzuki T, et al. Peroxisome proliferator-activated receptor gamma and growth inhibition by its ligands in uterine endometrial carcinoma. *Clin Cancer Res*. 2006;12: 4200–4208.
19. Takagi K, Moriya T, Kurosumi M, et al. Intratumoral estrogen concentration and expression of estrogen-induced genes in male breast carcinoma: comparison with female breast carcinoma. *Horm Cancer.* 2013;4:1–11.
20. Saito S, Ito K, Nagase S, et al. Progesterone receptor isoforms as a prognostic marker in human endometrial carcinoma. *Cancer Sci*. 2006;97:1308–1314.
21. Sato-Tadano A, Suzuki T, Amari M, et al. Hexokinase II in breast carcinoma: a potent prognostic factor associated with hypoxia-inducible factor-1alpha and Ki-67. *Cancer Sci*. 2013;104:1380–1388.
22. Fukuda T, Takahashi K, Suzuki T, et al. Urocortin 1, urocortin 3/stresscopin, and corticotropin releasing factor receptors in human adrenal and its disorders. *J Clin Endocrinol Metab.* 2005;90:4671–4678.
23. Kaprara A, Pazaitou-Panayiotou K, Chemonidou MC, et al. Distinct distribution of corticotropin releasing factor receptors in human breast cancer. *Neuropeptides*. 2010;44:355–361.
24. Minas V, Rolaki A, Kalantaridou SN, et al. Intratumoral CRH modulates immuno-escape of ovarian cancer cells through FasL regulation. *Br J Cancer.* 2007;97:637–645.
25. Vaughan J, Donaldson C, Bittencourt J, et al. Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. *Nature*. 1995;378:287–292.
26. Jo YH, Choi YJ, Kim HO, et al. Corticotropin-releasing hormone enhances the invasiveness and migration of Ishikawa cells, possibly by increasing matrix metalloproteinase-2 and matrix metalloproteinase-9. *J Int Med Res*. 2011;39:2067–2075.
27. Androulidaki A, Dermitzaki E, Venihaki M, et al. Corticotropin releasing factor promotes breast cancer cell motility and invasiveness. *Mol Cancer.* 2009;8:30.
28. Graziani G, Tentori L, Muzi A, et al. Evidence that corticotropin-releasing hormone inhibits cell growth of human breast cancer cells via the activation of CRH-R1 receptor subtype. *Mol Cell Endocrinol*. 2007;264:44–49.
29. Huvila J, Talve L, Carpen O, et al. Progesterone receptor negativity is an independent risk factor for relapse in patients with early stage endometrioid endometrial adenocarcinoma. *Gynecol Oncol*. 2013;130:463–469.

30. Hsu SY, Hsueh AJ. Human stresscopin and stresscopin-related peptide are selective ligands for the type 2 corticotropin-releasing hormone receptor. *Nat Med*. 2001;7: 605–611.
31. Carlson KW, Nawy SS, Wei ET, et al. Inhibition of mouse melanoma cell proliferation by corticotropin-releasing hormone and its analogs. *Anticancer Res*. 2001;21:1173–1179.
32. Florio P, De Falco G, Leucci E, et al. Urocortin expression is downregulated in human endometrial carcinoma. *J Endocrinol*. 2006;190:99–105.
33. Bale TL, Giordano FJ, Hickey RP, et al. Corticotropin-releasing factor receptor 2 is a tonic suppressor of vascularization. *Proc Natl Acad Sci U S A*. 2002;99:7734–7739.
34. Hasegawa H, Saiki I. Psychosocial stress augments tumor development through beta-adrenergic activation in mice. *Jpn J Cancer Res*. 2002;93:729–735.
35. Saez MD, Barriga C, Garcia JJ, et al. Exercise-induced stress enhances mammary tumor growth in rats: beneficial effect of the hormone melatonin. *Mol Cell Biochem*. 2007;294:19–24.
36. Fukudo S. Hypothalamic-pituitary-adrenal axis in gastrointestinal physiology. Chapter 28, Section II: Neurogastroenterology. Wood JD Section Editor. In: Johnson L ed. *Physiology of the Gastrointestinal Tract*. 5th ed. Oxford, United Kingdom: Elsevier; 2012:795–815.
37. Steckler T, Holsboer F. Corticotropin-releasing hormone receptor subtypes and emotion. *Biol Psychiatry.* 1999;46: 1480–1508.

