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CRP 1846C>T Genetic Polymorphism Is Associated with Lymph Node Metastasis and/or Severe Lymphatic Invasion in Endometrial Cancer

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Endometrial cancer (EC) rates are rising in Japan. Lymph node (LN) metastasis is an important prognostic factor in EC, and its risk is increased with higher tumor grade, deep myometrial invasion, larger tumor size, and lymphovascular space invasion (LVSI). Current methodologies to assess these factors are unreliable. We previously showed the association between C-reactive protein (CRP) 1846C>T (rs1205) polymorphism and LN metastasis in esophageal, non-small cell lung, and breast cancers. The CRP gene is located on chromosome 1q21-q23, and the polymorphism in the noncoding region (1846C>T) of this gene decreases serum CRP levels. We investigated the relationship between CRP 1846C>T genetic polymorphism and LN metastasis or LVSI in 130 EC patients using polymerase chain reaction-restriction fragment length polymorphism. The CRP 1846C/T genotype was C/C in 11 patients, C/T in 58 patients and T/T in 61 patients. The patients were divided into two groups based on their CRP 1846 genotypes: "C/C" and "C/T + T/T". Nine (7%) and 18 (13%) patients, all with the polymorphism, had LN metastasis and moderate or prominent lymphatic invasion, respectively. LN metastasis and/or severe lymphatic invasion were observed in the C/T + T/T group, while patients with the C/C genotype had no LN metastases or severe lymphatic invasion. Univariate and multivariate logistic regression models revealed that the C/T + T/T patients had a significant likelihood of developing LN metastasis and/or severe lymphatic invasion. Our results suggest that CRP genetic polymorphism is a novel risk predictor of LN metastasis and/or lymphatic invasion in EC.

Keywords: 1846C>T genetic polymorphism; C-reactive protein; endometrial cancer; lymph node metastasis; lymphovascular invasion
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Introduction

The incidence of endometrial cancer (EC) is increasing in Japan. Approximately 10,000 Japanese women were diagnosed with EC in 2008 (Matsuda et al. 2014). The majority of EC patients who are treated in the early stage of the disease have a favorable outcome; however, approximately 20% of EC patients are diagnosed with International Federation of Gynecology and Obstetrics (FIGO) stage III or IV disease and have relatively poor prognosis (Yamagami and Aoki 2015). Such patients are at risk of extrauterine lesions, i.e., invasion to the serosa or other organs, vaginal and/or parametrial involvement, lymph node (LN) metastases, and distant metastases.

LN involvement is an important prognostic factor in EC, as it is in most cancers (Barrena Medel et al. 2011; Milam et al. 2012). In the past, various risk factors for LN metastasis were described, including a higher grade tumor, deep myometrial invasion, and tumor size (Barrena Medel et al. 2011; Guntupalli et al. 2012). Lymphovascular space invasion (LVSI) correlates with LN involvement, and is reported to be a predictor of prognosis (Guntupalli et al.

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2012). Recent medical advances in detecting micrometastases have resulted in developing minimally invasive surgery techniques that induces the higher quality of postoperative life, but such procedures rely on the diagnosis of the perioperative biopsies (Daraï et al. 2015). The accuracy of LN metastasis detection using computed tomography (CT), magnetic resonance imaging (MRI), ultrasonography (US) or 18F-2-fluoro-2-deoxy-D-glucose-positron emission tomography-CT (PET-CT) is not clinically satisfactory (Pelikan et al. 2013). Novel or more reliable methods that can identify those at greater risk for LN metastasis are therefore desirable.

We previously showed the association between C-reactive protein (CRP) 1846C>T polymorphism and LN involvement in esophageal, non-small lung, and breast cancers. (Motoyama et al. 2009, 2013; Minamiya et al. 2010; Terata et al. 2014). The CRP gene is located on chromosome 1q21-q23, and polymorphism in the noncoding region (1846C>T) of this gene is associated with variation in serum CRP levels (Nimptsch et al. 2015). Patients with the CRP 1846 C/C genotype produce larger amounts of CRP protein than those with the C/T or T/T genotype, and have greater exposure to CRP throughout their lifetimes. Using a mouse LN metastasis model, we showed that CRP itself inhibits tumoral lymphangiogenesis and consequently suppresses LN metastasis (Sasaki et al. 2013). Moreover, we demonstrated that CRP has therapeutic potential against cancer through reducing the accumulation of M2 macrophages and inhibiting angiogenesis within tumors in mice (Kuribayashi et al. 2014).

In the present study, we investigated the relation between CRP 1846C>T genetic polymorphism and LN metastasis or LVSI in EC, and assessed its clinical significance.

Patients and Methods

This study was approved by the Ethics Committees of the Akita University Graduate School of Medicine. All of the participants provided informed consent and signed a human subject institutional review board consent form at Akita University Hospital.

The study participants were 130 consecutive Japanese patients diagnosed with pathological EC treated with hysterectomy with systematic LN dissection or biopsy, at Akita University Hospital and affiliated hospitals between April 1998 and October 2010. Patients who were unable to undergo systematic LN dissection because of older age, complications, or more advanced metastasis underwent nonsystematic LN dissection or LN biopsy instead. They were followed up thereafter at Akita University Hospital, which obtained informed consent from all patients. Patients in whom pathological lymphatic invasion was not analyzed were excluded. Patients who received neoadjuvant chemotherapy were also excluded. The disease was classified according to the FIGO 2008 staging system and the Union for International Cancer TNM Classification of Malignant Tumors (seventh edition).

Peripheral blood samples were collected from the patients after surgery and stored at −20°C. DNA was later extracted using the QIAamp Blood Kit (Qiagen, Hilden, Germany) and stored at −80°C until analysis. We investigated the CRP 1846C>T (rs1205) genetic polymorphism at the Akita University Graduate School of Medicine. Genotyping was performed using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method. Methods of identification for genotyping and details of the primers and PCR conditions have been published elsewhere (Russell et al. 2004; Motoyama et al. 2009, 2013).

The patients were divided into 2 groups, those with pathological LN metastasis and/or LVSI, and those without. LN metastasis and LVSI were determined based on hematoxylin-eosin staining and, in most cases, Elastica-Masson staining. The influence of CRP 1846C>T polymorphism on LN involvement and LVSI in EC was investigated retrospectively. LVSI cases were documented based on the general rules of Japanese Gastric Cancer Association (2011), and classified into degrees: nil, minimal, moderate, and prominent. Nil (−) reflects no channel involvement, minimal (+) refers to a maximum of 2 channels being involved on the border of the invading front of the tumor, moderate (++) refers to the involvement of 3 or more vessels in a wider area surrounding the invading tumor, and prominent (+++) signifies many vessels diffusely involved deeper into the myometrium (Karube et al. 2010).

Statistical analysis

Values are expressed as medians (range). Continuous data were compared using the Mann-Whitney-*U* test. Differences between groups or genotype frequencies were analyzed using the Pearson χ^2 test or Fisher exact probability test. The relationship between CRP genotypes and confounders affecting the apparent likelihood of LN metastasis or lymphatic invasion was tested using univariate and multivariate logistic regression analyses. All analyses were performed using JMP 10 (SAS Institute Inc., Cary, NC) and yielded two-sided *P* values. Values of $P < 0.05$ were considered significant.

Results

The patient population included 130 women with a median age of 56 (30-76) years. The majority of these women (107 [82%] patients) had endometrioid adenocarcinomas, of whom 62 had Grade 1 tumors, 32 had Grade 2 tumors, and 12 had Grade 3 tumors; moreover, 1 patient was diagnosed by endometrial curettage with no residual tumor detected in the surgical specimen. As for the remaining patients, 8 (6%) had clear cell adenocarcinomas, 5 (4%) had carcinosarcomas, 4 (3%) had mixed adenocarcinomas, 3 (2%) had serous adenocarcinomas, 2 (2%) had undifferentiated adenocarcinomas, and 1 had mucinous adenocarcinoma. Nine (7%) patients (1 with endometrioid adenocarcinoma Grade 1 tumor, 4 with Grade 2 tumors, 1 with Grade 3 tumors, 1 with clear adenocarcinoma, and 2 with mixed adenocarcinomas) had LN metastases and 18 (13%) patients (7 with endometrioid adenocarcinoma Grade 1 tumors, 5 with Grade 2 tumors, 3 with Grade 3 tumors, 1 with clear adenocarcinoma, 1 with mixed adenocarcinoma, and 1 with mucinous adenocarcinoma) had moderate/prominent (severe) lymphatic invasion. Eighty patients (61%) had FIGO pStage IA, 26 (20%) had pStage IB, 10 (8%) had pStage II, 12 (9%) had pStage III, and 2 (2%) had pStage IV disease. To our knowledge, 1 patient had died at the

time of this writing.

The frequencies of the CRP 1846C>T genotypes were all consistent with what would be expected if the population was in Hardy-Weinberg equilibrium; they were similar to the genotype frequencies reported in the National Cancer Institute SNP500 database. Furthermore, the genotype frequencies for this CRP polymorphism among our patients did not differ from those of the controls at Akita University Hospital (Motoyama et al. 2009). Of the 130 EC patients studied, the CRP 1846C/T genotype was C/C in 11 patients, C/T in 58 patients and T/T in 61 patients (i.e., C/T + T/T = 119 patients).

The clinical characteristics of the C/C vs. C/T + T/T groups are compared in Table1. None of the characteristics (age, LN involvement, number of involved LNs and dissected lymph nodes, lymphatic invasion, vascular invasion, preoperative carbohydrate antigen (CA) 125, CA19-9, serum CRP, and the number of patients with recurrence) showed statistically significant differences; however, the C/C group had no LN metastasis or severe lymphatic invasion. The sensitivity and negative predictive value of the CRP genetic polymorphism test were both 100% for detecting LN metastasis. The relationship between lymphatic invasion and LN involvement is shown in Table 2; there was a significant correlation between LN metastasis and lymphatic invasion.

Table 1. Characteristics of patients carrying the CRP 1846 C/C or C/T + T/T genotypes.

		Genotype		*P*
		C/C (N = 11)	C/T + T/T (N = 119)	
Age (years)		56 (44-71)	56 (30-76)	0.8212
Lymph node involvement (pN)	Negative	11	110	
	Positive	0	9	1.0000
Number of involved lymph nodes		0 (0-0)	0 (0-20)	0.3747
Number of dissected lymph nodes		66 (29-131)	51 (9-115)	0.1735
Lymphatic invasion	Negative	6	57	
	Positive	5	62	0.7568
Lymphatic invasion (classification)	Nil/Minimal	11	101	
	Moderate/Prominent	0	18	0.3602
Vascular invasion	Negative	5	89	
	Positive	6	30	0.0709
Vascular invasion (classification)	Nil/Minimal	11	113	
	Moderate/Prominent	0	6	1.0000
Preoperative serum CA125 (U/mL)		9.7 (5.8-17.8)	14.7 (3.3-814.3)	0.1575
Preoperative serum CA19-9 (U/mL)		11.5 (6.5-28.3)	18.3 (0.8-887.5)	0.2126
Preoperative serum CRP (mg/L)		0.95 (0-13.2)	0.45 (0-42.9)	0.1992
Histology	Endometrioid	9	98	
	Others	2	21	1.0000
Depth of tumor invasion, pT	pT1a	5	77	
	pT1b	5	25	
	pT2	1	12	
	pT3	0	5	0.2992
Recurrence	Recurred	1	6	
	Did not recur	10	113	0.4697

CA, carbohydrate antigen; CRP, C-reactive protein.

Table 2. Relationship between lymph node involvement and lymphatic invasion.

		Lymph node involvement (pN)		*P*
		Negative	Positive	
Lymphatic invasion	Negative	109	3	
	Positive	12	6	0.0002*
Lymphatic invasion (classification)	Nil/Minimal	62	1	
	Moderate/Prominent	59	8	0.0335*

*Statistically significant.

Table 3. Univariate and multivariate analysis of lymph node involvement and/or severe lymphatic invasion (logistic regression analysis).

	Univariate analysis			Maltiivariate analysis		
	95% CI	*P*	Odds ratio	95% CI	*P*	Odds ratio
Age (< 56 vs. ≥ 56)	0.5565 - 3.8687	0.4652	–			
CRP 1846C/T genotype (C/C vs. C/T + T/T)	1.0673 - ♦	0.0437*	♦	1.1173 - ♦	0.0410*	♦
Pathological T (T1a vs. T1b-T3)	2.1406 - 17.366	0.0004*	5.7575	0.8429 - 9.5975	0.0927	–
Histology (Endometrioid (G1, G2, G3) vs. non-endometrioid)	0.4669 - 4.6424	0.4370	–			
Venous invasion (Negative vs. Positive)	2.2915 - 17.074	0.0003*	6.0760	1.3954 - 14.523	0.0116*	4.4140
Preoperative CA 125 (< 35.0 vs. ≥ 35.0 ng/mL)	2.4840 - 19.264	0.0002*	6.8200	1.0106 - 10.407	0.0480*	3.2364
Preoperative CA 19-9 (< 37.0 vs. ≥ 37.0 ng/mL)	1.7756 - 14.233	0.0028*	5.0357	0.7075 - 8.1366	0.1555	–

*Statistically significant.
♦Estimated values were infinitely large, because the CRP 1846C/C group had no lymph node involvement or severe lymphatic invasion.
CA, carbohydrate antigen; CI, confidence interval.

Table 4. Odds ratios for lymph node involvement and/or severe lymphatic invasion: C-reactive protein 1846 C/C vs. C/T + T/T genotypes (logistic regression analyses).

	95% CI of odds ratio	*P*
Crude	> 1.0673	0.0437*
Adjusted for age (< 56 vs. ≥ 56)	> 1.0701	0.0435*
Adjusted for age and tumor depth (T1a vs. T1b-T3)	> 1.5982	0.0176*
Adjusted for age, tumor depth and venous invasion (Negative vs. Positive)	> 2.7557	0.0042*
Adjusted for age, tumor depth, venous invasion and CA125 (< 35.0 vs. ≥ 35.0 ng/mL)	> 1.4534	0.0242*
Adjusted for age, tumor depth, venous invasion, CA125 and CA19-9 (< 37.0 vs. ≥ 37.0 ng/mL)	> 1.1402	0.0395*

*Statistically significant.
CA, carbohydrate antigen; CI, confidence interval.

We next used univariate and multivariate logistic regression models to further investigate the extent to which CRP 1846C>T polymorphism independently predicts the occurrence of LN metastasis and/or severe lymphatic invasion (Table 3). Univariate and multivariate analysis revealed that the CRP 1846C>T polymorphism was associated with LN involvement and/or severe lymphatic invasion. In the crude and multivariate-adjusted model, CRP 1846C>T polymorphism is an independent predictive factor of LN metastasis and/or severe lymphatic invasion (Table 4).

Discussion

Inflammation, such as that associated with prolonged menstruation, obesity, and unopposed menopausal estrogen use, plays an important role in endometrial carcinogenesis (Delahanty et al. 2013). Inflammation influences not only development but also progression of a variety of common solid tumors (Nakatsu et al. 2012). However, its role in EC metastasis is not well understood.

CRP is known as one of the acute-phase proteins, and serum CRP levels are indicative of the presence of inflammation. In ovarian cancer patients, elevated serum CRP levels were reported to be a poor prognostic factor (Kodama et al. 1999). Moreover, elevated preoperative serum CRP levels were reported to correlate with less favorable prognoses in surgically treated EC patients (Schmid et al. 2007). Such data appear to suggest that high CRP levels are indicative of EC development and metastases. However, Nimptsch et al. (2015) reported the CRP 1846C>T polymorphism decreased CRP levels, and Sasaki et al. (2013) reported that CRP may actually reduce the risk of metastasis in certain mouse models. In this study, we did not demonstrate an association between the polymorphism and CRP levels or EC prognoses. One reason may be that advanced EC already has a better prognosis than some other types of cancers. Moreover, according to the above-mentioned studies, reduced serum CRP levels due to the 1846C>T polymorphism may actually increase the risk of metastasis, meaning that patients with CRP 1846C/C would actually have the smaller risk of LN metastasis. Nevertheless, it is far from clear that advanced EC patients would necessarily have high serum CRP levels and poorer prognosis.

Many reports on the association between gene mutations and prognosis have been published in the past two decades (Diaz-Padilla et al. 2012), but there is little knowledge about the relationship between genetic polymorphism and metastasis in gynecological cancers. Several types of CRP single nucleotide polymorphisms (SNPs) combined with central obesity have an influence on EC risk (Wen et

al. 2008). SNPs are not considered a main factor in carcinogenesis generally, but are assumed to influence disease outcome (Yang et al. 2011). In several cancers including colorectal, lung and prostate malignancies, CRP genetic polymorphism influences the incidence, poor prognosis, or higher grade of the disease (Minamiya et al. 2010; Yang et al. 2011; Markt et al. 2014). In cervical cancer, CRP genetic polymorphism was reported to influence serum CRP levels and prognosis (Polterauer et al. 2011). Considering these data from previous SNP studies, we found it reasonable to hypothesize the existence of an association between the polymorphism and EC progression. Our study is the first to investigate the correlation between CRP genetic polymorphism and lymphogenous progression in EC.

LVSI is an independent predictor of nodal disease and poor outcomes in endometrioid EC (Guntupalli et al. 2012). The existence of severe LVSI in EC was reported to be a useful indicator of LN metastasis (Hachisuga et al. 1999). These reports recommend classifying LVSI into degrees; thus, we adopted a four-degree classification according to the general rules of the Japanese Research Society for Gastric Cancer and previous literatures (Sakuragi et al. 2000; Karube et al. 2010).

While we showed that the CRP 1846C>T genetic polymorphism was significantly related to the risk of LN involvement and/or severe lymphatic invasion in EC, our results did not demonstrate that the presence of the polymorphism would definitely lead to LN metastasis; our sample size of patients with LN metastases was too small to make such a determination. Because LN metastasis results in a poorer outcome in EC patients than early stage patients, and patients with advanced ECs are ineligible for surgery, we were unable to perform CRP genetic polymorphism analysis in all patients with pathologically diagnosed LN metastasis. On the other hand, testing for the CRP 1846C>T polymorphism is very sensitive and has a high negative predictive value; we believe the latter to be the most relevant and useful factor in clinical settings. Predicting negative LN metastasis can lead to minimally invasive surgery without the need for LN dissection because the patient is very unlikely to have LN metastasis potential. Our finding of a strong relationship between the CRP 1846C>T polymorphism and the risk of LN metastasis encourages a new approach of limiting lymphadenectomies to a particular subgroup of patients. Considering the polymorphism's close correlation with LVSI and LN metastasis, we propose that it be considered a novel risk factor for LN metastasis in EC.

Since the establishment of FIGO surgical staging for EC, the necessity and therapeutic value of pelvic and paraaortic lymphadenectomy have been evaluated (Barrena Medel et al. 2011). Based on retrospective verification, systemic lymphadenectomy cannot be recommended for low-risk (FIGO (2008) stage IA and low grade pathology) EC (ASTEC study group et al. 2009). Because some risk factors become clearer after surgery, most low-risk EC patients are practically treated with lymphadenectomy; however, lymphadenectomy in gynecological cancer often causes post-operative complications, such as lymphocysts, lymphedema, or deep vein thrombosis. These complications cause severe distress to the patient; therefore, more reliable methods to estimate the risk of LN metastases are needed. Various imaging modalities and tumor markers are currently being used for preoperative clinical staging, but they cannot always detect actual LN metastases (Pelikan et al. 2013). Moreover, micrometastasis cannot be reliably detected during preoperative evaluation. Yamagami and Aoki (2015) reported that approximately 4,500 women were diagnosed with surgical stage IA EC in Japan in 2006. According to this study, approximately 10% of Japanese individuals may have the CRP 1846C/C polymorphism. Hence, it follows that approximately 450 EC patients per year could avoid lymphadenectomy after testing for this polymorphism. By adding CRP genetic polymorphism to the list of existing LN metastasis predictors, lymphadenectomies can be limited to those EC patients who would benefit from their remedial effects.

Many Japanese EC patients have family histories or genetic predispositions to cancers (Sugawara et al. 2015). We suggest considering gene analysis, including for polymorphism, when devising disease management strategies. Genetic surveillance, including but not limited to CRP genetic polymorphism detection, may be able to better identify those EC patients who ought to undergo lymphadenectomy.

Conclusion

Our study suggests that the CRP 1846C>T genetic polymorphism is a novel risk predictor of LN involvement and/or lymphatic invasion in EC. Testing for this polymorphism may offer a more accurate assessment of the risk of LN metastasis. Given the high sensitivity and negative predictive value of this test, its administration can lead to better individualized strategies for disease management.

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Conflict of Interest

The authors declare no conflict of interest.

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