Int J Clin Exp Pathol 2018;11(4):2100-2105
www.ijcep.com /ISSN:1936-2625/IJCEP0071887

Original Article

Correlation between overexpression of connective tissue growth factor, tumor progression, and clinical prognosis in endometrial cancer patients

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Received January 1, 2018; Accepted February 5, 2018; Epub April 1, 2018; Published April 15, 2018

Abstract: Objective: To investigate the correlation between overexpression of connective tissue growth factor (CTGF), tumor progression, and clinical prognosis in patients with endometrial cancer. Methods: Tumor samples were obtained from 198 patients with endometrial cancer who underwent hysterectomy, and 50 samples were collected from normal endometrial tissue. Immunohistochemical staining was performed on all samples. Scoring was carried out by two independent pathologists experienced in evaluating immunohistochemical staining. Results: Normal endometrial specimens exhibited little or no CTGF immunoreactivity. CTGF was localized mainly in the cytoplasm of the tumor cells. Of the endometrial cancer specimens examined, 95 (48%) of 198 patients were negative for CTGF, whereas 103 (52%) of 198 patients were positive for CTGF. No significant correlation was noted between the level of CTGF and patient age (P=0.81), blood pressure (P=0.76), blood glucose (P=0.51) or vascular/lymphatic invasion (P=0.15). However, positive CTGF expression showed a strong association with the level of CA125 (P=0.02), histologic grade (P=0.004), depth of myometrial invasion (P=0.028), and FIGO stage (P=0.025). Independent predictive value for overall survival was shown for positive CTGF expression (P<0.001), lymph node status (P<0.001) as well as lymphovascular invasion (P=0.02). Conclusion: CTGF is an independent prognostic factor of endometrial cancer. CTGF expression may play a critical role in progression of endometrial cancer and is significantly associated with poor prognosis.

Keywords: Correlation analysis, endometrial cancer, clinical prognosis, connective tissue growth factor

Introduction

Endometrial cancer is one of the most common malignancies of the female genital tract, yet the molecular genetic events that underlie the development of this cancer remain obscure [1]. The cancer often causes abnormal bleeding as a first symptom and is therefore usually detected in its early stages (FIGO stage I) [2]. However, some patients are diagnosed at stage III or IV. Hysterectomy and bilateral salpingo-oophorectomy is still the primary and most effective treatment for patients with localized cancer. Thus, there is an urgent need for new therapeutic targets and strategies, which may be realized through an increased understanding of the molecular mechanisms governing endometrial tumorigenesis.

Connective tissue growth factor (CTGF), also known as CCN2, belongs to the CCN (CGTF/Cysteine-rich 61/nephroblastoma) family. It is a cysteine-rich 36-38 kDa secreted protein, first described in 1991 [3]. All CCN family members are secreted proteins associated with extracellular matrix (ECM), and they play an important role in normal processes such as implantation, placentation, embryogenesis, differentiation, and development, as well as in pathologic processes including wound healing, fibrotic disorders, and tumorigenesis [4]. CTGF plays different roles in different types of cancer. It has been shown to be an oncogene promoting tumor progression in pancreatic cancer, prostate cancer, liver cancer, breast cancer, and sarcoma [5-9]. Conversely, CTGF functions as a tumor suppressor in lung cancer, ovarian cancer, and oral squamous cell cancer [10-12]. It also serves as a prognostic marker in esophageal squamous cell carcinoma, gastric cancer and lung cancer [13, 14]. However, the expression pattern and functional mechanism of CTGF in endometrial cancer have not been established.

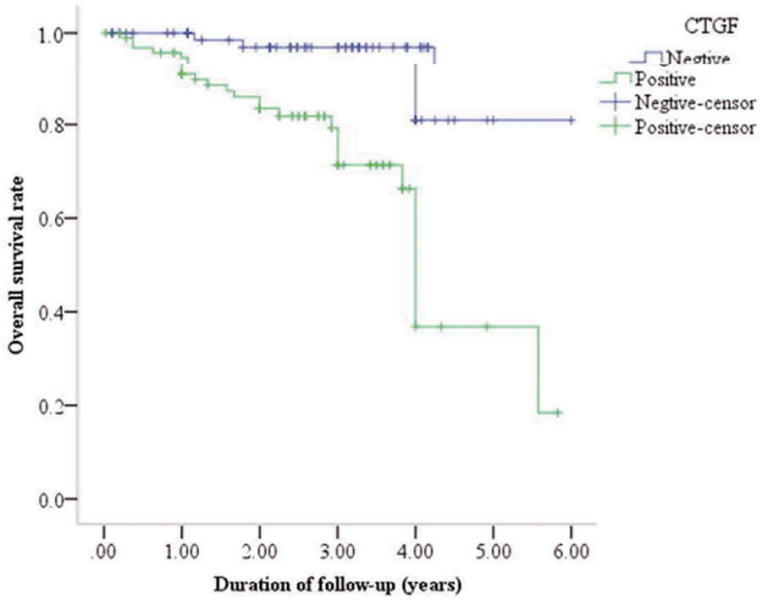

Figure 1. Kaplen-Meier survival curves for 198 patients with endometrial cancer, grouped according to CTGF expression.

Therefore, in the present study we examined the expression of CTGF in normal and malignant endometrial tissue by immunohistochemistry in order to analyze its possible involvement in malignant progression. Additionally, we evaluated whether CTGF expression is associated with clinicopathologic parameters and prognosis in endometrial cancer patients.

Materials and methods

Tissue samples

Tumor samples were obtained from 198 patients with endometrial cancer who underwent surgery at Jining Medical University from January 2015 to October 2017. All patients underwent hysterectomy, and their clinical and pathologic data were available. 50 normal endometrial tissues were collected from other patients whose pathology was leiomyoma from the same hospital. Written informed consents were obtained from all patients. The study procedures were approved by the medical ethics committee of Jining Medical University.

Immunohistochemistry

For immunohistochemical staining, 4 μm histological sections were de-paraffinized with xylene and rehydrated through a graded series of alcohols. The sections were then boiled at 121°C for 4 min in 0.01 M citrate buffer (pH=6.0) to retrieve the antigenicity, and endogenous peroxidase was blocked by incubation in 3% H_2O_2 in methanol for 20 min. The sections were incubated overnight at 4°C with dilution at a dosage of 30 μg/ml of CTGF primary goat-polyclonal antibody (U.S. R&D, AF660). Then, they were exposed to biotin-labeled secondary antibody for 30 min; finally, the slides were counterstained with hematoxylin.

Staining evaluation

Scoring was carried out by two independent pathologists experienced in evaluating immunohistochemical staining according to the WHO classification criteria, who were blinded to the clinical outcome of these patients and had not seen the corresponding hematoxylin-eosin slides. Staining was scored on the following scale: 0, no staining; 1+, minimal staining; 2+, moderate to strong staining in at least 20% of cells; 3+, strong staining in at least 50% of cells. Cases with 0 or 1+ staining were classified as negative, and cases with 2+ or 3+ staining were classified as positive.

Statistical analysis

The number and percentage of categorical variables were calculated, and groups for categorical variables were analyzed by Chi-square test. The *p*-values are shown for the difference of these variables according to CTGF expression. COX regression models were performed to test the risk factors. Significance tests were two-tailed and *p*-values less than 0.05 were considered statistically significant. All analyses were performed using the SPSS 19.0 statistical software SPSS Inc., Chicago, IL, USA).

Results

Expression of CTGF in normal endometrium and endometrial cancer

Normal endometrial specimens exhibited little or no CTGF immunoreactivity, as illustrated in

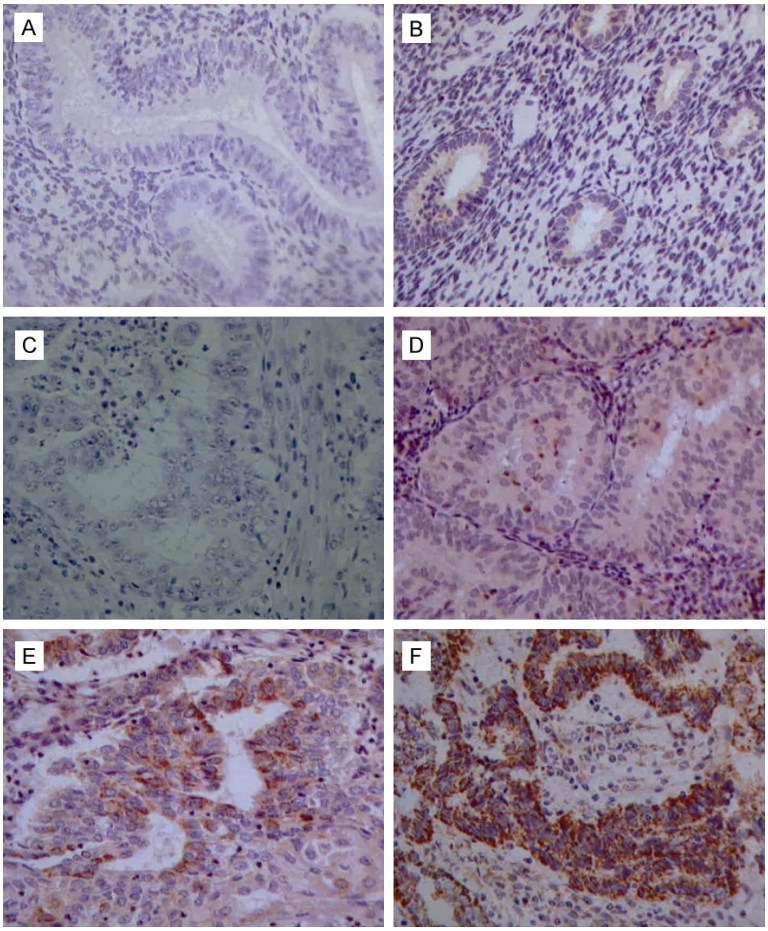

Figure 2. Immunohistochemical staining of CTGF in endometrium: A, B. Low or no expression of CTGF in normal endometrium; C-F. Rising expression of CTGF in endometrial cancer as the grade increases (original magnification × 10).

Table 1. Connective tissue growth factor (CTGF) expression in normal endometrium and endometrial cancer

Tissue Type	Total No.	Positive CTGF No. (%)	X^2	*P*
Endometrial cancer	198	103 (52)	20.95	<0.001
Normal endometrium	50	8 (16)		

Abbreviation: CTGF, connective tissue growth factor.

Figure 2A, **2B**. In tumor cells, CGTF was expressed in the cytoplasm with membranous accentuation. (**Figure 2C**, **2D**). Of the endometrial cancer specimens examined, 95 (48%) of 198 patients were negative for CTGF, whereas 103 (52%) of 198 patients were positive. In the normal control endometrial tissues, percent of CTGF-positive cases was 16% (8 of 50) (**Table 1**). As shown in **Table 1**, the level of CTGF was significantly higher in endometrial cancers than in the normal endometrium (*P*<0.001).

Correlation between CTGF expression and clinicopathologic factors

The relationship between CTGF expression and each clinicopathologic factor was analyzed for endometrial cancer. As shown in **Table 2**, there was no significant correlation between the level of CTGF and patient age (*P*=0.81), blood pressure (*P*=0.76), blood glucose (*P*=0.51), or lymphovascular invasion (*P*=0.15). However, positive CTGF expression showed a strong association with the level of CA125 (*P*=0.02), histologic grade (*P*=0.004), depth of myometrial invasion (*P*=0.028) and FIGO stage (*P*=0.025).

Clinical significance of CTGF expression in endometrial cancer

Positive CTGF expression was significantly associated with poor survival when overall survival (OS) was evaluated. As shown in **Table 3**, multivariate Cox regression analysis shows that positive CTGF expression (*P*<0.001), lymph node status (*P*<0.001) as well as lymphovascular invasion (*P*=0.02) were independent prognostic markers for OS of endometrial cancer patients.

Univariate survival analysis was performed to evaluate the OS of the affected patients, as illustrated in **Table 4**. Multivariate correlation analysis demonstrated that the following factors including histological grade, myometrial invasion, FIGO stage, lymphovascular invasion, and CTGF expression level were correlated to the OS of endometrial cancer patients.

Discussion

CTGF has a defined role in wound-healing and fibrotic disease. Recently, several studies impli-

Table 2. Association between CTGF expression (positive/negative) and clinicopathologic parameters

	CTGF Negative	CTG Positive		*P*
Age (years)				
≤60	80 (51)	77 (49)		
>60	9 (33.33)	18 (66.67)	2.865	0.091
Hypertension				
No	68 (47.55)	75 (52.45)	0.09	0.76
Yes	26 (50)	26 (50)		
Diabetes				
No	88 (48.89)	92 (51.11)	0.44	0.51
Yes	6 (40)	9 (60)		
CA125				
≤35	56 (54.37)	47 (45.63)	5.55	0.02
>35	15 (33.33)	30 (66.67)		
Histological grade				
G3	6 (20.69)	23 (79.31)		0.004
G2	39 (57.35)	29 (42.65)		
G1	50 (49.5)	51 (60.5)		
Depth of myometrial invasion				
<1/2	82 (51.9)	76 (48.1)	4.81	0.028
≥1/2	13 (32.5)	27 (67.5)		
FIGO stage				
I-II	74 (51.39)	70 (48.61)	5.02	0.025
III-IV	11 (30.56)	25 (69.44)		
Vascular/lymphatic invasion #				
No	95 (48.97)	99 (51.03)	2.06	0.15
Yes	0 (0)	4 (100)		

Abbreviation: FIGO, International Federation of Gynecology and Obstetrics; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated; # Chi-square corrected.

Table 3. Multivariate Cox-regression analysis of various clinicopathological parameters and their use as prognostic markers for disease-specific 5-year survival in EC patients

	SE	*P*	Relative risk	95.0% CI
Lymph node status	0.54	<0.001	6.21	2.16-17.88
Lymphovascular invasion	0.80	0.02	6.19	1.28-29.90
CTGF expression	0.64	<0.001	6.38	1.83-22.29

cated CTGF in tumor development and tumor cell survival. However, the exact role of CTGF in tumor progression is not definite, and the function of CTGF in tumor cell biology of endometrial cancer has not been thoroughly investigated. To address these issues, we confirmed high CTGF expression in endometrial cancer compared with matched normal endometrial tissues. Moreover, we further investigated the effects of CTGF on peritoneal dissemination of endometrial cancer cells *in vivo*.

This is the first study to show the prognostic significance of CTGF expression and clinicopathologic features in endometrial cancer using clinical samples. The expression of CTGF was significantly correlated with the level of CA125, histologic grade, depth of myometrial invasion and FIGO stage.

In this study, our results showed that CTGF was highly expressed in endometrial cancer tissues compared with matched normal endometrial tissues. The expression of CTGF in tumor tissue was associated with the level of CA125, histological grade, myometrial invasion and FIGO stage. Furthermore, patients with positive CTGF expression had significantly lower cumulative postoperative 5-year survival rate (36.8%) than those with negative CTGF expression (80.9%), as illustrated in **Figure 1**. Those results suggested that CTGF might be involved in progression and metastasis of endometrial cancer. Moreover, CTGF might be a useful prognostic marker.

Some original research on vascular endothelial cells identified a role for CTGFas a growth factor. CTGF was selectively induced in fibroblasts after activation with TGF. The coordinated expression of TGF1 and CTGF was found in granulation beds of wound repair. Moreover, it was demonstrated in scleroderma lesions that dermal fibroblasts overexpressed CTGF [15]. Besides its profibrotic properties, CTGF has been found to be overexpressed in many tumors. In some instances, the expression of CTGF is correlated with the patient prognosis [16]. High levels of CTGF were associated with invasive glioblastoma [17]. Moreover, increasing expression of CTGF correlated with worse patient survival

Table 4. Univariate Cox-regression analysis of various clinicopathologic parameters and their use as prognostic markers for disease-specific 5-year survival in EC patients

Variables	No. Patients	HR/rr	95% CI	*P*
Age (year)				
≤60	157	1		
>60	27	2.08	0.88-4.91	0.095
Hypertension				
No	143	1		
Yes	52	1.05	0.45-2.47	0.91
Diabetes				
No	180	1		
Yes	15	0.44	0.06-3.2	0.42
CA125				
≤35	103	1		
>35	45	4.22	1.78-10	0.001
Lymph node status				
No	182	1		
Yes	16	4.72	2.01-11.06	<0.001
Histologic grade				
G1	101	1		
G2 vs. G1	68	1.33	0.52-3.43	0.55
G3 vs. G1	29	0.95	0.42-2.14	0.89
Depth of myometrial invasion				
≤1/2	158	1		
>1/2	40	3.2	1.52-6.76	0.002
FIGO stage				
1.2	144	1		
3.4	36	3.12	1.51-6.43	0.002
Lymphovascular invasion				
No	194	1		
Yes	4	5.06	1.18-21.68	0.03
CTGF expression				
Negative	95	1		
Positive	103	6.28	2.39-16.51	<0.001

Abbreviation: CI, confidence interval; HR, hazard ratio; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated; FIGO, International Federation of Gynecology and Obstetrics.

[18]. However, the high CTGF expression levels correlated with improved survival in colorectal cancer and non-small cell lung cancer [19, 20]. Research was lacking about the expression of CTGF in endometrial cancer. In our study, the expression of CTGF gene in endometrial cancer tissue was clearly higher than that in normal endometrium, indicating that CTGF might play an important role in this tumor. Our study demonstrated that positive CTGF expression, lymph node metastasis, and lymphovascular invasion were independent prognostic markers for OS in endometrial cancer patients. That is, the patients who expressed CTGF had a poor prognosis. These results indicated that CTGF may be an independent prognostic factor in endometrial cancer. This is the first study to show the prognostic significance of CTGF expression and its association with clinicopathologic features in endometrial cancer using clinical samples. Therefore, CTGF may serve as a target for both patient prognosis and therapy. Previously, it was reported that several other molecular markers were shown to be of prognostic significance in endometrial cancer. However, their effectiveness is uncertain in clinical application as replacements for, or in addition to, the prognostic parameters currently in use.

Disclosure of conflict of interest

None.

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