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Loss of Adherens Junction Protein E-Cadherin is a Biomarker of High-Grade Histology and Poor Prognosis in Endometrial Cancer

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Abstract

Background: Loss of cellular polarity is a hallmark of cancer. E-cadherin, which localizes at the adherens junction, is a major cellular polarity determinant to control of tissue architecture. Loss of E-cadherin expression has been reported in many human cancers. Here, we investigated whether loss of E-cadherin expression is involved in endometrial cancer development.

Methods and findings: We investigated the localization and expression of E-cadherin in 152 cases of endometrial cancer using immunohistochemical staining technique. E-cadherin localizes at the cellular membrane in the normal endometrial tissue. Loss of E-cadherin expression was observed significantly in endometrial cancer with high-grade histology. Our analysis revealed that patients with loss of E-cadherin show poorer overall and progression free survival. It was frequently observed in cases with advanced clinical stage, deep myometrial invasion, and p53 mutation, but the difference did not reached to the statistical significance. Loss of E-cadherin showed no obvious relationship with age at onset, lymph-nodal metastasis, vessel involvement, and estrogen and progesterone receptor expression.

Conclusion: Our analysis revealed that disruption of tissue polarity due to loss of adherens junction protein E-cadherin provide more aggressive characters including high-grade histology and poor prognosis to endometrial cancer cells

Keywords: Endometrial cancer, E-cadherin, Adherens junction, Histological grade, Prognosis

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Introduction

Loss of cell polarity due to disruption of tissue architecture is a hallmark of cancer [1]. Mammalian epithelial tumours lose polarity as they progress toward malignancy, but whether polarity loss might causally contribute to cancer has remained unclear [2-4]. Endometrial cancer is classified into two groups-type I and type II based on pathological histology, as well as molecular pathogenesis and clinical profiles [5]. The endometrial type I cancer is estrogen-dependent of low-grade endometrioid histology and arises in the background of endometrial hyperplasia, its precursor lesion [5]. The type 1 cancer usually occurs in the pre and peri-menopausal women and strongly linked to obesity [5]. The endometrial type II cancer is estrogen-independent tumor with high-grade histology, i.e., high-grade endometrioid, serous and clear cell.

The epithelial-mesenchymal-transition is a major histological feature of human cancer. It has been reported in development of endometrial cancer and its clinical behavior [6,7]. The integrity of adherens junction is critical for maintenance of tissue architecture [8]. E-cadherin is a major component of adherens junction and its loss of expression has been reported to be involved in epithelial-mesenchymal-transition [9,10]. Here, we investigated whether loss of E-cadherin is involved in endometrial cancer development.

Methods

Patient characteristics and tumour materials

We analyzed E-Cadherin (CDH-1) expression in 152 cases of endometrial cancer, who underwent surgery at Teikyo University Hospital from January 2003 to December 2012 using immunohistochemical staining. E-Cadherin expression was compared with clinicopathological factors. All patients provided informed consent to participate in this study. Hematoxylin and eosin (H&E)-stained slides of these cases were reviewed. The clinical and pathological characteristics of the cases were obtained from their clinical charts.

Immunohistochemistry (IHC)

Four micrometer-thick paraffin sections from representative tumour blocks were screened for E-Cadherin protein expression using Envision FLEX (DAKO, Glostrup, Denmark). After deparaffinization and warm bath processing, the sections were activated with citric acid buffer and endogenous peroxidase activity was removed by 3% H_2O_2. After blocking, the sections were incubated for 30 min with primary monoclonal mouse anti-E-cadherin antibody (36B5, NCL-E-Cad, dilution: 1:50; Novocastra, Newcastle, UK), primary monoclonal mouse anti-p53 antibody (DO-7, M7001, dilution: 1:50; DAKO), primary monoclonal mouse anti-estrogen receptor antibody (1D5, M7047, dilution: 1:50; DAKO), or primary monoclonal mouse anti-progesterone receptor antibody (PgR636, M3569, dilution: 1:800; DAKO). The sections were then incubated with a secondary antibody (anti-rabbit, anti-mouse: DAKO) for 30 minutes. Antibody binding was visualized using a 3,3'-diaminobenzidine solution (DAKO) for 10 minutes. Finally, the tissues were counterstained by standard H&E staining and mounted using a conventional mounting medium. All steps of IHC were performed at room temperature. An additional section was used as a case specific negative control without incubation with the primary antibody. As a positive control, normal endometrial tissue was investigated. The evaluation of E-Cadherin IHC was performed by light microscopy. The patients with endometrial cancer were divided into 3 groups according to the previous report [11]. It was evaluated as homogeneous group when immunostaining of E-Cadherin was observed more than 70% of epithelial cells for each tissue section in (with strong membrane immunostaining), and normal endometrium showed the pattern. It was evaluated as heterogeneous expression when immunostaining of less than 70% of epithelial cells was observed. It was evaluated as negative when immunostaining of no epithelial cells was observed. Homogeneous group was defined as normal expression. Heterogeneous and negative groups were defined as down-regulation.

Statistical analysis

JMP 12 (SAS Institute, Tokyo, Japan) was used for statistical analysis. To analyse correlations between categorized variables, multi-field tables were calculated and interpreted using the Pearson χ^2-test of independence. Risk ratios were estimated for clinical and pathological factors. Survival curves were estimated with Kaplan-Meier methods, and the respective curves were tested for significant differences by a log-rank test. Hazard ratios were estimated by a Cox proportional hazards model. The level of statistical significance was set at $p<0.05$.

Results

E-cadherin expression in normal and malignant endometrial tissues

E-cadherin expression was observed at the membrane in the normal endometrial tissues (**Figure 1A**). Nest, we analyzed the expression of E-cadherin in 152 endometrial cancer tissues. The membrane-bound expression of E-cadherin in the endometrial cancer was evaluated as homogeneous group (**Figure 1B**). It was evaluated as heterogeneous expression when immunostaining of less than 70% of epithelial cells was observed (**Figure 1C**). It was evaluated as negative when immunostaining of no epithelial cells was observed (**Figure 1D**). The patients with homogeneous E-cadherin expression were categorized as a normal E-cadherin expression group. The patients with heterogeneous or negative expression of E-cadherin were categorized as a E-cadherin down-regulation group.

Relationship between E-cadherin expression and clinicopathological factors

E-cadherin down-regulation was observed significantly in endometrial cancer with high-grade histology (**Table 1**). It was frequently observed in cases with advanced clinical stage, deep myometrial invasion, and p53 mutation, but the difference did not reached to the statistical significance (**Table 1**). Loss of E-cadherin showed no obvious relationship with age at onset, lymph-nodal metastasis, vessel involvement, and estrogen and progesterone receptor expression (**Table 1**). Next, we evaluated whether E-cadherin down-regulation is involved in prognosis of

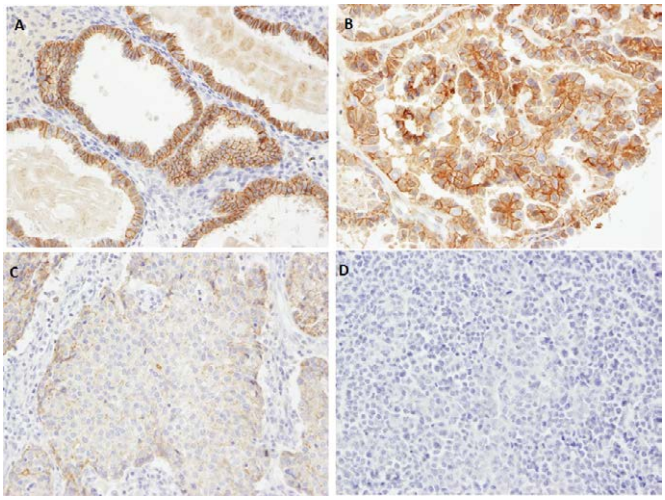

Figure 1 E-cadherin expression in normal and malignant endometrial tissues. (A) E-cadherin expression in normal endometrial tissue. It localizes at the cellular membrane. (B) The membrane-bound normal E-cadherin expression in endometrial cancer tissue (homogeneous group). (C) Heterogeneous E-cadherin expression. The immunostaining of E-cadherin in less than 70% of epithelial cells was observed. (D) Negative E-cadherin expression in endometrial cancer tissue.

Table 1 E-cadherin in endometrial cancer.

Clinicopathological factors	Total	E-Cadherin normal	E-Cadherin down regulation	P-value
Overall survival				0.0019
Progression free survival				0.0093
Age				
<50	46	32(69.6%)	14(30.4%)	0.308
≧50	106	82(77.4%)	24(22.6%)	
Stage				
pT1-2	123	96(78.0%)	27(22.0%)	0.0738
pT3-4	29	18(62.1%)	11(37.9%)	
Lymphonodal metastasis				
pN0	88	66(75.0%)	22(25%)	0.3933
pN1	27	18(66.7%)	9(33.3%)	
Distant metastasis				
M0	140	107 (76.4%)	33 (23.6%)	0.1647
M1	12	7 (58.3%)	5 (41.7%)	
Vessel involvement				
-	93	73 (78.5%)	20 (21.5%)	0.2116
+	59	41 (69.5%)	18 (30.5%)	
Myometrial invasion				
<1/2	91	73 (80.2%)	18 (19.8%)	0.0695
≧1/2	61	41 (67.2%)	20 (32.8%)	
Histlogical grade				
Low grade	90	74 (82.2%)	16 (17.8%)	0.0132
High grade	62	40 (64.5%)	22 (53.5%)	
Estrogen receptor				
-	26	17 (65.4%)	9 (34.6%)	0.2136
+	126	97 (77.0%)	29 (33.0%)	

patients with endometrial cancer. Patients with E-cadherin down-regulation showed poorer overall and progression-free survival (**Figures 2** and **3**).

Discussion

The relationship between loss of epithelial polarity and development of malignant tumor has long been known, but it has not been revealed whether loss of tissue architecture have a causative role in tumor development and growth [2]. Adherens junction has an essential role in establishment of cell polarity and tissue architecture [8,9]. Many junctional protein complexes are involved in construction of the adherens junction [9]. E-cadherin has a critical role in establishment of integral adherens junction [8,9]. Loss of E-cadherin expression leads to epithelial-mesenchymal-transition, which is a representative malignant phenotype of human cancer [6,7].

We evaluated E-cadherin expression in 152 endometrial cancer cases. Our analysis revealed that loss of E-cadherin is linked to the high-grade histology **(Table 1)**. Endometrial cancers are subdivided into 2 groups according to clinicopathological factors. Although the type II endometrial cancers contribute only 10% of endometrial cancer incidence, they cause about 50% of disease recurrence [12]. The 5-year survival rate of endometrial cancer is 96% if the cancer diagnosed at a local stage, but decreases to 17% if diagnosed at an advanced stage [13]. Deep myometrial invasion, nodal involvement and distant metastasis worsen prognosis of endometrial cancer [14]. Awareness of the biomarkers that predict prognosis in endometrial cancer is warranted. Recent study showed the genomic features of endometrial cancer permit a reclassification of endometrial cancer patients for their prognosis [15]. The overexpression of p53 based on genetic mutation is a molecular signature of type II endometrial cancer [16,17]. The previous studies and ours suggest the possibilities that loss of E-cadherin is also molecular signature of type II endometrial cancer [11,18-25]. The prognosis of patients with type I endometrial cancer is relatively excellent, whereas prognosis of patients with type II cancer is poor [5]. Survival of patients with loss of E-cadherin was poorer than that with normal E-cadherin expression **(Figures 2** and **3)**. These data also support the possibility that loss of E-cadherin is a key molecular event during development of type II endometrial cancer. Loss of E-cadherin has been reported to be associated with aggressive behavior and metastasis in many human cancers [1,26,27]. These data support the critical significance of loss of tissue architecture in cancer development and tumor progression.

Conclusion

Our study revealed that loss of E-cadherin is linked to high-grade histology and poor prognosis. It shed light on molecular mechanism of endometrial carcinogenesis. The development of cancer is very complicated and based on multiple steps. Further study will be necessary to fully understand the detail molecular events during endometria carcinogenesis.

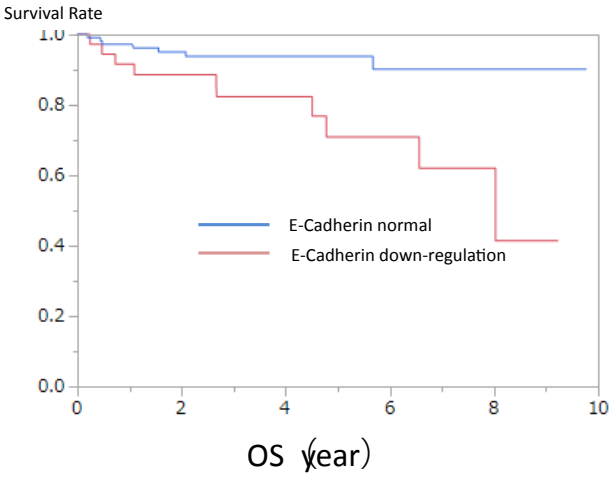

Figure 2 Overall-survival of patients with endometrial cancer between normal E-cadherin expression group and E-cadherin down-regulation group.

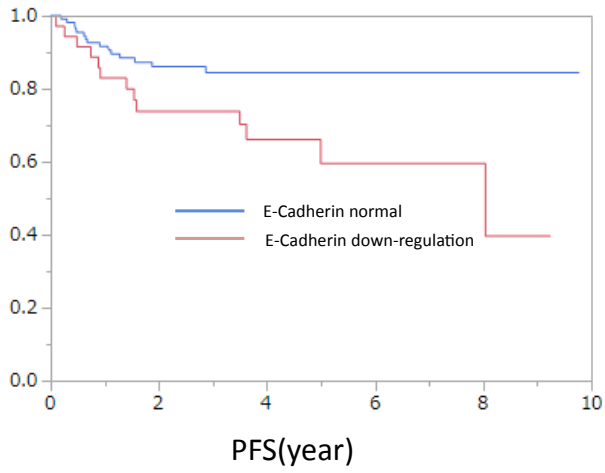

Figure 3 Progression-free survival of patients with endometrial cancer between normal E-cadherin expression group and E-cadherin down-regulation group.

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