Chondoadherin as a biomarker in patients with endometrial cancer

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Early diagnosis and development of newer and more effective treatments for endometrial cancer, which is observed so frequently, continue to be necessary. In the present study, we aimed to show the relationship between the tumorigenesis of endometrial cancer and chondoadherin and its place as a biomarker. A total of 15 patients diagnosed with endometrioid adenocarcinoma in the pathology unit of the present tertiary hospital and 15 patients operated for non-tumor reasons between 2019 and 2020 were included in the study. Pathology tumor blocks were selected for ELISA and PCR study in which chondoadherin gene expression and protein levels were measured. We found increased expression of the chondoadherin-like (CHADL) gene in endometrial cancer cells compared to endometrial cells without tumor diagnosis (2.85 ± 0.44 vs. 1.94 ± 0.33). When the mean value for the protein level in CHADL tissues was examined, we found a higher rate in endometrial cancer tissues (228.83 ± 22.30 vs. 186.66 ± 21.09). The CHADL protein level and gene expression increased as the grade increased. The present study is the first report presenting chondoadherin level in endometrial cancer. Chondoadherin level in endometrial cancer can be a guiding marker in early diagnosis and treatment process and prognosis.

KEY WORDS: Biomarker, Chondoadherin, Endometrial cancer

Introduction

Endometrial cancer is the most common cancer of the female genital tract [1,2]. Although its highest incidence is seen in the seventh decade of life, it ranks fthe presentth after breast, lung and colorectal cancers [2,3]. Rescue treatments such as hormonal agents or cytotoxic chemotherapy that provide short-term remission represent the typical treatment strategy for endometrial cancer [3]. Early diagnosis and development of newer and more effective treatments for endometrial cancer, which is observed so frequently, continue to be necessary.

Chondoadherin (CHAD) is a leucine-rich repeat protein known as the cartilage matrix protein, which is thought to mediate the adhesion of isolated chondrocyte cells [4]. It has the ability to bind triple helix collagen, interact with cells via $\alpha 2\beta 1$ integrin and cell surface heparan sulfate proteoglycans [5-7]. It has been shown that CHAD causes many cellular responses by activating intracellular signaling mechanisms as a result of its interactions with cells, and changes in the cytoskeleton as a result of its reaction with various receptors [7]. Data from CHAD clearly indicate that it has the potential to affect cell metabolism and matrix homeostasis.

Reports on other functions of CHAD, including its role during carcinogenesis and cancer development, are still limited [8-10]. It has been shown that the extracellular matrix proteins (ECM), focal adhesion and ECM receptors in CHAD are associated with metastasis of various cancers [11,12]. In the present study, we aimed to show the relationship between the tumorigenesis of endometrial cancer and CHAD and its place as a biomarker.

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Methods

Patients

A total of 15 patients diagnosed with endometrioid adenocarcinoma in the pathology unit of the present tertiary hospital and 15 patients operated for non-tumor reasons between 2019 and 2020 were included in the study. The study was approved by the Ethics Committee of Zeynep Kamil Women's and Children's Diseases Training and Research Hospital. Three groups, FIGO grades I, II, and III, were formed from the cases diagnosed with endometrioid adenocarcinoma by archive scanning. Five patients were included in each group. All patients underwent hysterectomy and bilateral salpingo-oophorectomy, as well as pelvic and/or paraaortic lymphadenectomy operations according to the frozen result. Tumor preparations of the cases from the pathology archive were re-evaluated and appropriate blocks were selected for ELISA and PCR studies.

Gene expression of Chondroadherin by qRT-PCR

From each paraffin block, 5 tissue sections (each 10-µm thick) were collected into 1.5-ml microfuge tubes. Extraction of total RNA from paraffin-embedded tissues was determined in duplicate by FFPE RNA isolation kit (Invitrogen; Catalogue number K156002) according to manufacturer's recommendation. RNA was reverse transcribed to cDNA and amplification was performed using SYBRGreen PCR Master Mix (Applied Biosytem). The results were analyzed using StepOne Software v2.3 (Applied Biosystems, Foster City, CA), and normalized by GAPDH as an internal control. Data were expressed as fold induction relative to the control.

Protein Levels of Chondroadherin By Elisa

Protein extraction of all samples were performed as previously described [13]. Protein concentrations were measured with Bradford method [14]. The Chondroadherin levels were measured with ELISA in accordance with the manufacturer's protocols (Fine Test; Catalogue number EH1724) using a microplate reader was used (BioTek Epoch, Winooski, VT, USA). Chondroadherin values presented as pg/ µg protein. Results were given as milliliter per milligram of protein.

Histopathologic evaluation

After fixation, samples were embedded in paraffin blocks and cut into 5 µm thick sections using a Leica RM2125RTS microtome device (Leica Biosystems, Nussloch, Germany). Selected paraffin sections were stained with hematoxylin-eosin (H&E) staining for morphological evaluation under a light microscope (Olympus BX-51, Olympus, Tokyo, Japan).

Immunohistochemical Staining

Immunohistochemical staining was performed to evaluate the expression of CHADL in control and cancer tissues. Briefly, after deparaffinization and rehydration the sections were treated with 3% hydrogen peroxide diluted in PBS for 15 min at RT to block endogenous peroxide. After incubation with ultra V block (Thermo Fisher Scientific), the slides were incubated with a polyclonal anti-rabbit antibody (PA5-72888; Invitrogen; 1:100 dilution) overnight at +4°C. The next day, after washing, the sections were incubated with biotinylated secondary antibody and subsequently treated with 3,3-diaminobenzidine (DAB) solution for signal development. The sections were evaluated under the microscope (Olympus BX-51 Japan).

Scoring

We used the semiquantitative scoring system that took into account the intensity of immunoreactivity and area extent as described before [15-17]. Briefly, every tissue was given a staining intensity score of the cytoplasm (no staining=0; weak staining=1; moderate staining=2; strong staining=3) and the extent of stained cells (0%=0; 1-10%=1; 11-50%=2; 51-80%=3; 81-100%=4).

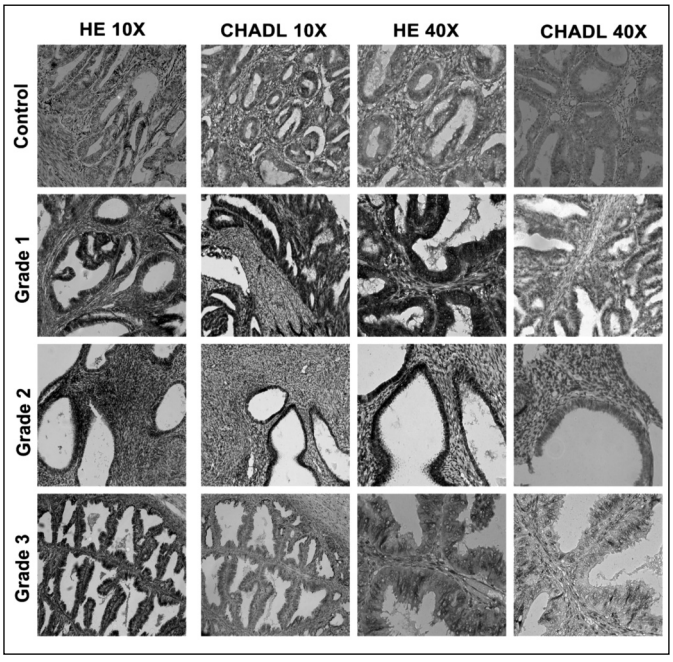

Fig. 1: Immunohistochemical staining of CHADL in tissues diagnosed with endometrial cancer according to grade.

The final immunoreactive score (0-12) was determined by multiplying the intensity and extent of positivity scores of stained cells. Scores of 0-4 were described as no expression or weak; score of 5-8 were described as intermediate expression; and score of 9-12 described as strong expression [18] (Fig. 1).

Statistical Analysis

Data are shown as the means±standard from at least three independent experiments. One-way ANOVA was applied to evaluate the differences among the multiple groups. Student's t-test was used to perform the statistical comparisons between two groups. If the variances were homogeneous, two groups were compared using the least significance difference (LSD) method. Otherwise, Dunnett's T3 method was included to analyze nonhomogeneous variances between two groups. All statistical tests were two-sided, and $p<0.05$ (*) and $p<0.01$ (**) were considered statistically significant. All analyses were performed using SPSS version 18.0.

Results

In terms of demographic data, the mean age was 57 (46-75) in the endometrial cancer group, and 49 (43-67) in the control group. The mean age was 57 (53-75) in those with Grade 1, was 52 (46-65) in those with Grade 2, and was 59 (50-73) in those with Grade 3.
To explore the role of CHADL in endometrium and endometrial cancer tissues, the difference in expression was compared in 15 endometrial cancer and 15 non-pathologically diagnosed hysterectomy tissues using the IHC method (Fig. 1).
CHADL gene expression and protein were mainly localized in the cytoplasm of endometrial cancer cells, and CHADL gene expression was significantly increased in cancer tissues compared to tissues without pathological diagnosis (2.85±0.44 vs. 1.94±0.33). When the mean value for the protein level in the CHADL tissues was examined, it was found to be higher in endometrial cancer (228.83±22.30 vs. 186.66±21.09) (Table I, Fig. 2). In the CHADL protein level and gene expression analysis performed among these groups, it was observed that

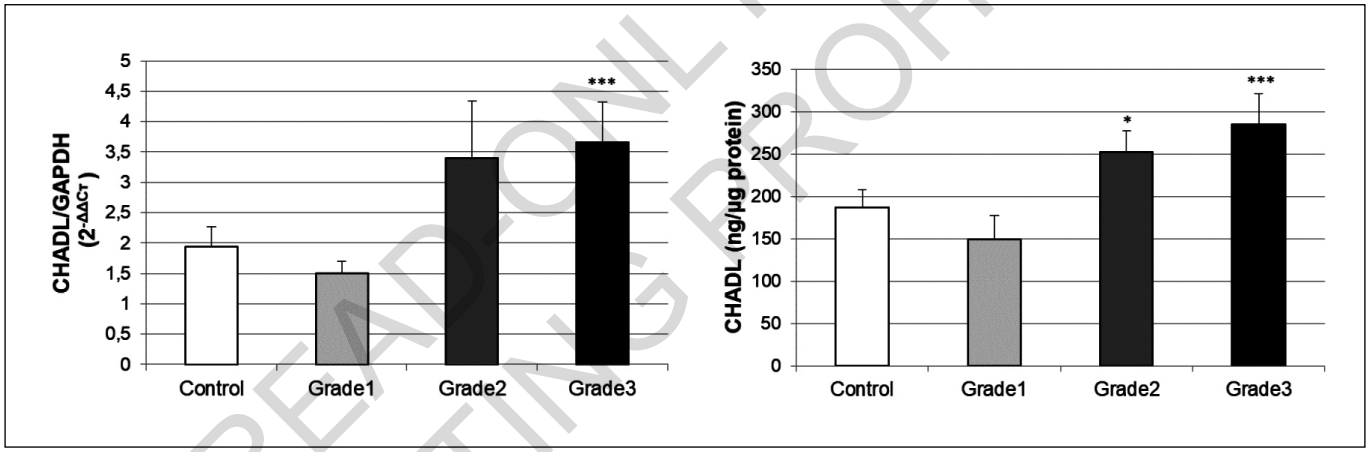

Fig. 2: Graph of gene expression and protein values of mean CHADL according to grades of endometrial cancer patients and between control group.

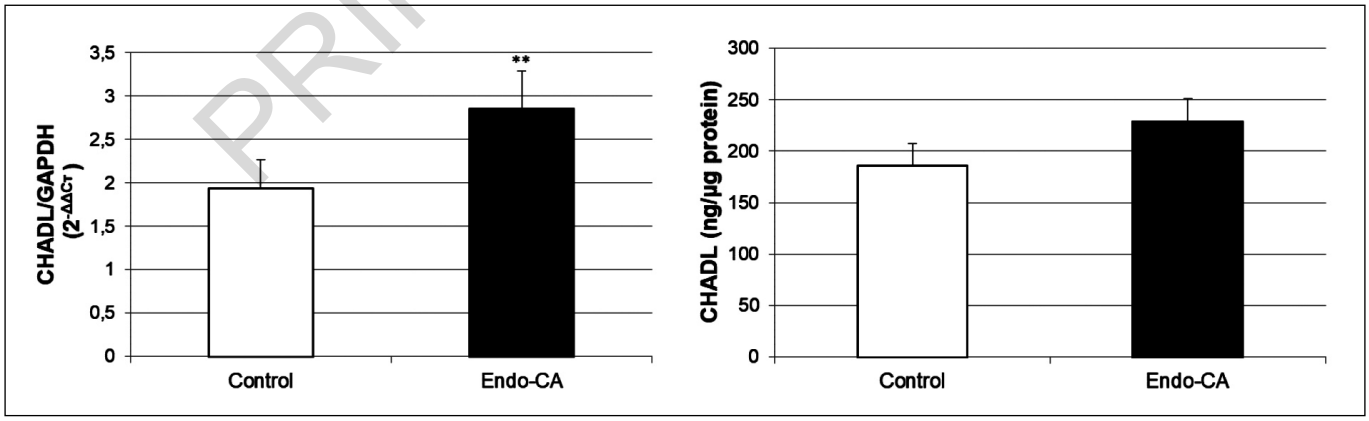

Fig. 3: Graph of gene expression and protein values of mean CHADL between endometrial cancer patients and control group. Endo-CA: Endometrial cancer.

Table I - *Gene expression and protein values of mean CHADL between tissues with endometrial cancer diagnosis and tissues without tumor diagnosis*

	Control (Mean±SE)	Endometrial cancer (Mean±SE)
CHADL/GAPDH	1.94±0.33	2.85±0.44
CHADL (pg/mg protein)	186.66±21.09	228.83±22.30

CHADL: Chondoadherin-like, GAPDH: Glyceraldehyde 3-phosphate dehydrogenase, SE: Standard error.

Table II - *One-way ANOVA test results in comparison of mean gene expression and protein values of CHADL between endometrial cancer patients by grades and control group, and comparison with control group.*

	CHADL/GAPDH			
	Mean	SD	p (in comparison to the control group)	p (comparison among all groups)
Control	1.94	0.33		0.028
Grade1	1.50	0.19	0.928	
Grade2	3.4	0.94	0.195	
Grade3	3.66	0.66	0.098	
CHADL (pg/mg protein)				
	Mean	SE		
Control	186.66	21.1		0.023
Grade1	149.03	28.08	0.769	
Grade2	252.49	24.24	0.348	
Grade3	284.98	36.42	0.079	

CHADL: Chondoadherin-like, GAPDH: Glyceraldehyde 3-phosphate dehydrogenase, SD: Standard deviation, SE: Standard error.

the protein level and gene expression increased as the grade increased. (CHADL protein levels; Control: 186.66±21.1 pg/µg, Grade 1: 149.034±28.08 pg/µg, Grade 2: 252.49±24.24 pg/µg, Grade 3: 284.98±36.42 pg/µg; CHADL gene expression levels; Control: 1.94, Grade 1: 1.50±0.19, Grade 2: 3.4±0.94, Grade 3: 3.66±0.66 (Table II, Fig. 3) 2).

Discussion

The high incidence rate of endometrial cancer among gynecological malignancies has encouraged researchers to explore other ways in the etiology and treatment of endometrial cancer [19,20]. The etiology of endometrial cancer has been tried to be explained by putting forward the hypothesis of two pathogenic pathways. First, tumors with high to moderate differentiation, which develop as a result of a hormone-dependent process, progress to malignancy at the end of hyperplastic processes. The second pathway, on the other hand, develops on the ground of atrophic endometrium with low-grade differentiation [21]. The pathogenesis of endometrial cancer is modeled by examining the latest technology data obtained from molecular-biological specificity studies of tumors. These studies help to identify the molecular-biological changes of endometrial cancer, which are responsible for differences in the aggressiveness of tumor progression [22,23]. Recent studies have shown that endometrial cancer is characterized by significant biological heterogeneity, which determines the different clinical cthe presentse, which complicates the choice of treatment strategy [24-26]. For this reason, new treatment protocols are currently being tried to be established by investigating molecular markers for defining the molecular subtype of endometrial cancer according to grade [22,26-28].

In the present study, we measured all three grade levels to determine the value of CHAD as a prognostic biomarker in the early diagnosis and pathogenesis of endometrial cancer The present study is the first report to detect and show CHAD level in endometrial cancer with these data.

Although CHAD is a matrix protein found close to cells, it is particularly prominent in the cartilage of the growth plate, between proliferative and hypertrophic regions [29]. In the leucine-rich repeat family group in which CHAD is found, CHAD is the only matrix protein with a double disulfide ring near the C-terminus, all other members have a single ring in this region. It does not contain the N-terminal extension common to other leucine-rich repeat proteins. The lack of post-translational glycosylation of CHAD is one of its uniquely important features among proteins [30]. CHAD binds to two separate triple helix collagen regions with high affinity, as well as to the $\alpha 2\beta 1$ integrin on the cell surface of chondrocytes [31]. The change in cells that

adhere to CHAD via α2β1 is that they become rounded, as seen in cells that adhere to the integrin binding site of fibronectin via α5β1 [32]. Rounding the cell is necessary to activate protein kinase C to provide adequate signaling to induce spread with a peptide or phorbol esters, formation of focal adhesions, and stress. Thus, cell proliferation on CHAD becomes inducible [31].

Integrins are the first-line means by which cells sense and respond to their microenvironment [33]. They are considered to be important receptors in regulating the distinctive features of cancer steps such as proliferation, self-renewal, apoptosis, resistance to treatment, angiogenesis and metastasis [34]. Increasing expression profile of integrins on cancer cells and providing ECM composition and organization in tumor stroma are the main factors in cancer development, metastasis and treatment resistance [33,34]. CHAD, which interacts with integrins as one of the ECM proteins and cell receptor, induces cell invasion, adhesion, invasion and angiogenesis, and its role in the carcinogenesis mechanism can be explained. As a result of the data of the present study, the increased CHAD level compared to normal endometrial tissue and grades supports this hypothesis. In the present results, CHADL gene expression was significantly increased in EC tissues compared to normal endometrial tissues without tumor diagnosis (2.85±0.44 vs. 1.94±0.33).

In recent years, the role and effective behavior of ECM in many cancer types has been demonstrated by studies, including the relationship between NFAT, one of the markers investigated for the prognosis of subtypes of ovarian cancer, and CHAD; They found that the proximal promoter of CHAD contains 9 binding sites for NFAT [35]. This study found that NFAT overexpression can further increase CHAD expression level. Thus, they concluded that it is a prognostic factor that may be included in the poor prognosis of patients with clear cell ovarian cancer [35]. In another study, a significant increase in CHAD was found in the metastasis of breast cancer to the bone via α2β1 integrin. MDA-MB-231 is an important integrin for metastasis of breast cancer cells to bone. Thus, with α2β1 integrin, ECM proteins can provide signals to breast cancer cells that have metastasized to the bone for the development of antiapoptotic and treatment resistance in chemotherapy. MDA-MB-231 has been shown to inhibit apoptosis induced by paclitaxel and vincristine in breast cancer [36]. Similar findings have been reported in head and neck cancer cell lines [37].

In conclusion, the present study is the first study report showing CHAD level in endometrial cancer. CHAD level in endometrial cancer will be an important guiding marker in early diagnosis and treatment process and prognosis. More research is needed to address the biological mechanisms in the carcinogenesis pathway of CHAD.

Riassunto

Continuano ad essere necessari sia la diagnosi precoce che lo sviluppo di nuovi e più efficaci trattamenti del carcinoma dell'endometrio, che si osserva così frequentemente.

Con questo studio, abbiamo cercato di dimostrare la relazione tra la tumorogenesi del cancro dell'endometrio e la condoaderina, ed il suo ruolo come biomarcatore. Nello studio sono state incluse 15 pazienti con diagnosi di adenocarcinoma endometriale e 15 pazienti operate per ragioni non tumorali tra il 2019 e il 2020 nell'unità di patologia del nostro ospedale di terzo livello. Le inclusioni di patologia neoplastica sono stati selezionati per lo studio ELISA e PCR, ed in essi sono stati misurati l'espressione genica della chondoaderina e i livelli di proteine.

Abbiamo trovato una maggiore espressione del gene chondoadherin-like (CHADL) nelle cellule di cancro dell'endometrio rispetto alle cellule dell'endometrio senza diagnosi di tumore (2,85±0,44 vs. 1,94±0,33). Quando è stato esaminato il valore medio del livello proteico nei tessuti CHADL, abbiamo riscontrato un tasso più elevato nei tessuti del cancro dell'endometrio (228,83±22,30 vs. 186,66±21,09). Il livello della proteina CHADL e l'espressione genica sono stati rilevati in aumento con l'aumentare del grado.

Questo studio è il primo in letteratura che fa riferimento al livello di chondoaderina nel cancro dell'endometrio. Il livello di condoaderina nel cancro dell'endometrio può essere un indicatore guida nella diagnosi precoce, nel processo di trattamento e nella prognosi.

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