Soluble CD44 concentration in the serum and peritoneal fluid samples of patients with different stages of endometriosis

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Abstract

Purpose Endometriosis is a gynecological disease defined by the histological presence of endometrial glands and stroma outside the uterine cavity, most commonly implanted over visceral and peritoneal surface within the female pelvis. CD44 is a membrane protein expressed by human endometrial cells, and it has been shown to promote the adhesion of endometrial cells. The aim of this study was to determine the levels of soluble CD44 (sCD44) in the serum and peritoneal fluid (PF) samples of patients with different stages of endometriosis.

Methods 39 PF and serum samples from normal healthy and 130 samples from different stages of patients with endometriosis (33 cases of stage I, 38 stage II, 30 stage III and 29 stage IV) were included in this study. Total protein concentration (TPC) and the level of s-cMet in the serum were determined by Bio-Rad protein assay based on the Bradford dye procedure and enzyme-linked immunosorbent assay, respectively.

Results No significant change in the TPC was seen in the serum of patients with endometriosis when compared to normal controls. Results obtained demonstrated that all serum and peritoneal fluid samples, presented sCD44 expression, whereas, starting from stages I to IV endometriosis, a significant increase of sCD44 expression was observed as compared to control group.

Conclusions The results of this study show that a high expression of sCD44 is correlated with advanced stages of endometriosis. It is also concluded that the detection of serum and/or peritoneal fluid sCD44 may be useful in classifying endometriosis.

Keywords Soluble CD44 · Serum · Endometriosis

Introduction

Endometriosis is an enigmatic disease, which affects up to 5% of fertile females and 25–40% of fertile women of reproductive age [1]. Implantation of endometrial tissues in retrograde menstrual flux is a widely accepted etiology of this disease. The pathogenesis of endometriosis is not fully understood, although researches have revealed the involvement of estrogen dependency, inflammatory mediators, and the immune response in the development of endometriosis and its symptoms [2]. Endometriosis shows a drastically elevated frequency in industrial area and possible genetic predisposition. It possesses many features of a benign neoplastic process with the potential for malignant transformation [3].

Several factors are thought to be involved in the development of endometriosis. Retrograde menstruation remains the dominant theory for the development of pelvic endometriosis [4], however, the actual cellular and molecular mechanisms responsible are unclear. A number of
genetic studies have been found to be associated with endometriosis in selective populations [5, 6]; however, the exact genes that play a role in endometriosis are unknown.

CD44 is a membrane protein expressed by human endometrial cells, and it has been shown to promote the adhesion of endometrial cells [7]. CD44 has been demonstrated to bind to the cytoskeleton and membrane proteins. It was first identified transmembrane hyaluronan-binding protein, and interest in its receptor stemmed from the demonstration that CD44-hyaluronan interactions mediate cell adhesion and migration in a variety of physiological and pathophysiological processes including tumor metastasis, wound healing and leukocyte extravasation at sites of inflammation [8]. CD44 is widely distributed, which makes it the major hyaluronan receptor on mast cell types. It has been shown to be involved in the cell migration or cell chemotaxis and metastasis [9].

Cell adhesion molecules exist in two forms including a membrane form, detectable in the cell surface, and a soluble form, detectable in the serum and other biological fluids. The soluble form of CD44 (sCD44), which consists of the ectodomain of CD44, has recently emerged as a key regulator of CD44 action. Although there is growing evidence that CD44 is involved in endometriosis [10], the importance of sCD44 is still unknown.

A variety of internal membrane receptors, including CD44, can be released from the lipid bilayer by proteolysis to form soluble, truncated proteins. The protease that generate soluble forms of membrane proteins are predominantly metalloproteinases or serine proteinases. The soluble receptors are smaller, consisting of the extracellular origin of the membrane-bound receptor and, in general, are able to bind to ligand with reduced affinity [11]. The aim of this study was to analyze the concentrations of CD44 in the serum of patients with different stages of endometriosis.

Materials and methods

Patient samples

After ethic committee’s approval and informed consent, the samples of serum and peritoneal fluid from normal subjects and patients with endometriosis were collected. 39 serum and peritoneal fluid samples from healthy normal and 130 samples from different stages of patients with endometriosis (33 cases of stage I, 38 stage II, 30 stage III and 29 stage IV) were included in this study. The controls consisted of women undergoing laparoscopic tubal ligation or diagnostic laparoscopy with no pelvic findings of endometriosis, inflammatory disease, or uterine fibroids. Patients with previous medical treatment for endometriosis prior to serum and peritoneal fluid collection were excluded from this study.

Samples were age matched between the two groups, analyzed and ranged in age between 24 and 39 years. None of the patients suffered from known diabetes mellitus or infection. All participants were asked to fill out a questionnaire on their medical history, family history of disease, infertility, surgical history, and prescribed. This project has been approved by the local licensing committee and has been performed according the Helsinki Declaration of 1975, as revised in 1983. The samples were stored at $-70 \degree\text{C}$ until used.

Protein analysis

Total protein concentration

The total concentration of proteins in serum was determined by the Bio-Rad protein assay based on the Bradford dye procedure.

Analysis of sCD44 concentration by ELISA

Soluble CD44 (sCD44) in serum and peritoneal fluid was measured using the sensitive two-sided enzyme-linked immunosorbent assay (ELISA) and antiserum against human sCD44 Kit (ab45912, abcam, Cambridge, UK) were first coated with 80 ng primary anti-sCD44 antibody per well in 0.1 M Tris buffer. After overnight incubation, the plates were blocked with EIA buffer (50 mM Tris, pH 7.5, 0.3 M NaCl, 0.1 % Triton X-100, 1 % BSA and 1 % Gelatine). The samples and standards were placed in triplicate wells and incubated overnight at room temperature. After washing, a biotinylated secondary antibody (8 ng/ml) was added to each well and the incubation was carried out overnight at room temperature. b-Galactosidase coupled to avidin was then added and after 2 h was followed by washing. Finally, 200 lM 4-methylumbelliferyl-b-galactoside (Sigma, Poole, UK) in 50 mM sodium phosphate and 10 mM MgCl$_2$ buffer were added and the amount of fluorescence was measured after 40-min incubation at 37 $\degree\text{C}$ using a fluorimeter (Dynatech).

Statistical analysis

All data presented are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA and only values with $P \leq 0.05$ were considered as significant.

Results

Characteristics of subjects

A total of 169 females (39 controls and 130 patients with different stages of endometriosis) were included in the
study. Demographic and clinical characteristics of patients and controls are shown in Table 1. The endometriosis patients were also classified according to the American Society of Reproductive Medicine (ASRM) classification (stage I–IV). The average age of the women with endometriosis was 28.6 ± 5.83, and the average age of the normal participants was 29.2 ± 5.62. All endometriosis participants had a history of pelvic and low abdominal pain. None of the normal participants endorsed a history of pelvic pain. The endometriosis patients were classified according to ASRM classification (stage I–IV). Of the 130 patients, 33 (25.3 %) had stage I, 38 (29.2 %) had stage II, 30 (23.07 %) had stage III, whereas 29 (22.3 %) patients had stage IV (Table 1).

Total protein concentration

The total protein concentration in the serum from patients with endometriosis and normal subjects was determined by the Bio-Rad protein assay based on the Bradford dye mixture. Results obtained demonstrated that no significant changes in all serum samples, starting from stages I to IV endometriosis, were observed [levels of 0.343 ± 0.04, 0.346 ± 0.03, 0.347 ± 0.44, 0.345 ± 0.51 g/l as compared to controls (0.343 ± 0.04 g/l) (P = 0.86, 0.84, 0.86 and 0.90, respectively)] (Fig. 1).

Analysis of sCD44 concentration by ELISA

Using ELISA, it was shown that the concentration of serum and peritoneal fluid sCD44 in the patients with endometriosis was higher than in normal subjects. Results obtained demonstrated that all serum and peritoneal fluid samples presented sCD44 expression, whereas, starting from stages I to IV endometriosis, a significant increase of protein expression was observed [serum levels of 374.18 ± 286.63, 50.79 ng/ml as compared to controls (462.63 ± 90.65) (P = 0.0004, 0.0001, 0.0007 and 0.00002, respectively) (Figs. 2, 3)].

<table>
<thead>
<tr>
<th>Stage of diseasea</th>
<th>No. of patients</th>
<th>Average age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I minimal disease</td>
<td>33</td>
<td>28.6</td>
</tr>
<tr>
<td>Stage II mild disease</td>
<td>38</td>
<td>28.6</td>
</tr>
<tr>
<td>Stage III moderate disease</td>
<td>30</td>
<td>28.6</td>
</tr>
<tr>
<td>Stage IV severe disease</td>
<td>29</td>
<td>28.6</td>
</tr>
<tr>
<td>No. of controls</td>
<td>39</td>
<td>29.2</td>
</tr>
</tbody>
</table>

Discussion

Endometriosis is a frequent gynecological disorder that commonly presents with infertility and pelvic pain in reproductive age. Risk factors of this disease are age, social class, smoking, diet and other life style issues [12]. Endometriosis associated with an inflammatory reaction, developed by secretion of growth factors, cytokines and chemokines in the peritoneal cavity, is harmful especially to successful reproductive activity [13]. It has been shown that a high expression of soluble cMet (s-cMet) is correlated with advanced stages of endometriosis [14]. The present study demonstrated that the concentration of sCD44 in serum and peritoneal fluid of women with endometriosis increased compared to normal control group. Moreover, a high expression of sCD44 in serum and PF is correlated with advanced stages. The potential involvement of the sCD44 pathway in the pathogenesis of the endometriosis has been demonstrated [15].

Shedding of cell surface receptors plays an important role in modulating cell–cell and cell–matrix adhesion, as well as in signaling initiated by interactions of adhesion receptors and their ligands. Elevation of CD44 shedding has been seen in gastric cancers [16]. In addition, increased concentrations of sCD44 are associated with increased tumor size and lymph node metastasis in the patients with breast cancer [17].

Epidermal growth factor (EGF) and matrix metalloproteinase-1 (MMP-1) can induce CD44 shedding [18, 19]. CD44 shedding is thought to result in enhanced cell motility. This notion is supported by the observation that migration of highly aggressive melanoma cells is associated with increased shedding and turnover of CD44 [20]. MMP9 has been shown to be involved in the CD44 shedding from normal and cancer cells, which would promote the malignant potential of tumor cells [21]. High expression of MMP existed in eutopic and ectopic endometrial tissues of endometriosis, which could enhance the ectopic invasion and transplant of endometrial cells [22].

In the light of evidence on the importance of soluble receptors in various physiological settings and in the pathophysiology of various diseases, it is tempting to speculate that sCD44 in serum may have a role in the pathogenesis of endometriosis. Given that sCD44 can act as a competitive inhibitor for interaction of membrane-bound CD44 and hyaluronic acid, up-regulation of sCD44 in the serum of patients with endometriosis may inhibit attachment of endometrial cells.
In conclusion, the results of this study show that CD44 may be involved in the pathogenesis of endometriosis. They also suggest that a high expression of sCD44 is correlated with advanced stages of endometriosis. Therefore, the detection of serum and/or PF sCD44 may be useful in classifying endometriosis. Further study is needed.
to indicate the mechanism of sCD44 up-regulation in patients with endometriosis and to understand the action of sCD44 in the pathogenesis of this disease.

Acknowledgments This study was supported by the University of Guilan. The authors thank Dr. Nouri for the serum samples.

Conflict of interest We also declare that we have full control of all primary data and agree to allow the journal to review our data if requested. The authors declare that they have no conflict of interest.

References

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