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Immunohistochemical Analysis of CD44s and CD44v6 in Endometriosis and Adenomyosis

: Comparison with normal, hyperplastic, and malignant endometrium

The expression patterns of CD44s and CD44v6 were immunohistochemically compared with those of normal, hyperplastic and malignant endometrium. In normal endometria (n=37), endometrioses (n=46) and adenomyoses (n=20), the surface and glandular epithelial cells were negative for CD44s and CD44v6 in a proliferative pattern and positive in a secretory pattern, whereas the stroma was only positive for CD44s in both proliferative and secretory patterns. The endometrial hyperplasia (4 simple and 9 complex) had the identical patterns with normal proliferative phase of endometrium. Only one case showing complex hyperplasia with atypia was focally positive for CD44s and CD44v6 in glandular epithelia. CD44s and CD44v6 were positive in all endometrial adenocarcinomas (13), except one CD44s-negative case. In summary, the expressions of CD44s and CD44v6 in endometriosis and adenomyosis recapitulated those of normal cyclic endometrium. The expression patterns in endometrial hyperplasia were similar to those in normal proliferative endometrium, whereas the endometrial adenocarcinoma showed abnormal expressions for CD44s and CD44v6. Thus it was considered that the ectopic endometrium in endometriosis and adenomyosis was not aberrant as in endometrial carcinoma on the aspects of immunohistochemical expressions of CD44s and CD44v6.

Key Words : *Antigens, CD44; Endometriosis; Endometrial Hyperplasia; Endometrial Neoplasms*

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INTRODUCTION

The etiology and pathogenesis of endometriosis and adenomyosis, defined as the ectopic location of endometrium-like glandular epithelium and stroma outside the uterine cavity, have been poorly understood (1, 2). However, a couple of theories have been proposed to explain these diseases. One is the implantation theory, in which the majority of endometriotic lesions are believed to be derived from eutopic endometrial cells that are transported to the peritoneal cavity by retrograde menstruation, adhere to the peritoneal wall and proliferate. Another is the metaplastic theory, in which endometriosis is established by hormone-dependent transformation of peritoneal cells into Mûllerian-type epithelium. Endometriotic lesions can be located superficially on the peritoneum, in ovary, in myometrium of uterus (adenomyosis), or at other sites. Clinical features and in vitro experiments have suggested that endometriotic cells are invasive and able to metastasize, and that common molecules are operative in the invasion and metastasis of both endometriotic and cancer cells (3, 4). However, their underlying molecular mechanisms have not been fully determined (5-7). Analogous to the tumor metastasis, it is likely that cell adhesion molecules are central for the invasion and metastasis of endometriotic cells. Adhesion molecules, such as cadherins and integrins, have been investigated in endometriosis, however, few studies have been done about CD44 expression in endometriotic lesions (2, 8-13).

CD44 is a widely expressed cell-surface glycoprotein, and is postulated to be involved in cell-to-cell and cell-to-matrix interactions. *CD44* gene has at least 20 exons, occupying a length of 50-80 kbp located on the short arm of chromosome 11 (11p13). The standard form of CD44 (CD44s) comprises exons 1-5 joined to exons 16-20 to give a molecular weight of 80-90 kDa, while the variant forms (CD44v) are generated by alternative

splicing of exons 6-15 (variant exons 1-10) with higher molecular weights (14, 15). The increased expressions of these CD44 isoforms were shown to be associated with the increased potential of invasion and metastasis in human malignant cells (16-19).

In this study, the expression profiles of CD44s and CD44v6 in ectopic endometrium of endometriosis and adenomyosis were compared with those in eutopic endometrium, endometrial hyperplasia, and adenocarcinoma to verify whether the ectopic endometrium has the same phenotypes as a malignant lesion.

MATERIALS AND METHODS

Materials

One hundred and twenty-nine cases were selected from the pathology file in the Department of Pathology, Anam Hospital of Korea University Medical College. The tissues were routinely processed with 10% buffered formalin fixation and paraffin embedding. The H-E stained slides were reviewed and appropriate blocks were selected for the immunohistochemical staining. The cases consisted of 46 ovarian endometriosis, 20 adenomyosis, 13 endometrial hyperplasia (4 simple and 9 complex) and 13 endometrial adenocarcinomas (10 endometrioid and 3 serous). Thirty-seven normal endometria, 10 obtained for the diagnostic procedure such as dating and 27 obtained from the patients of endometriosis and adenomyosis, were included in this study.

Immunohistochemical staining

For an immunohistochemical study with DAKO LSAB kit (DAKO A/S, Denmark), 4-μm thick tissue sections were deparaffinized and rehydrated. Endogenous peroxidase activity was eliminated by incubation with 3% H_2O_2 in methanol for 15 min. The antigen was retrieved at 103 kPa for 2 min by placing the slides in 0.01 M sodium citrate buffer (pH 6.0). The slides were then incubated with the primary monoclonal antibodies for CD44s (1:100, Zymed Laboratories, CA, U.S.A.) and CD44v6 (1:50, NeoMarkers, CA, U.S.A.) for one hour at room temperature. After incubation at room temperature for 30 min with biotinylated link, the sections were incubated with streptavidin-peroxidase complex at room temperature for 30 min. Immunostaining was visualized by using 3,3'diaminobenzidine. The sections were counterstained with hematoxylin. As a negative control, 0.1 M Tris buffer (pH 7.6) replaced the primary antibody, and the tonsil was used as a positive control.

Interpretation

Only the cell membranous staining was considered to be positive. The expressions of CD44s and CD44v6 were separately analyzed in surface and glandular epithelium and stroma.

RESULTS

CD44 immunostaining in normal endometrium

In 18 cases of proliferative phase endometria, the surface and glandular epithelia were negative for both CD44s and CD44v6, whereas the stroma was positive for CD44s and negative for CD44v6. In 19 cases of secretory phase endometria, the surface and glandular epithelia were positive for CD44s and CD44v6, but the stroma was only positive for CD44s (Table 1, Fig. 1).

CD44 immunostaining in endometriosis

The expression patterns were evaluated on the basis of the histologic features of endometriosis. In all 7 simple proliferative patterns of ectopic endometrium, the epithelial cells were negative for both CD44s and CD44v6, whereas the stroma was positive for CD44s and negative for CD44v6.

In 33 endometriosis showing secretory changes, all surface and glandular epithelial cells were positive for both CD44s and CD44v6. The stroma showed positivity for CD44s and negativity for CD44v6. In 6 cases containing almost exclusively hemosiderin-laden macrophages, the macrophages were strongly positive for CD44s, and the residual epithelial components were positive for both CD44s and CD44v6 (Table 1, Fig. 2).

In 9 cases, the paired eutopic and ectopic endometria were analyzed. Six cases among them were in a proliferative phase, while the ectopic endometrium showed a proliferative change in 3, secretory change in 2, and stroma only in one. Three cases were in a secretory phase, whereas the ectopic endometrium showed a secretory change in 2 and a proliferative change in one. The expression patterns of CD44s and CD44v6 in ectopic endometrium followed its phase of the cycle and was not influenced by the status of the matching eutopic endometrium.

CD44 immunostaining in adenomyosis

In all 20 cases of adenomyosis, the ectopic endometrium was in the same cyclic phases with eutopic endometrium, except one case in which eutopic endometrium

Table 1. Expression of CD44 in normal endometrium and endometrial lesions

Groups	No. cases	CD44s(+)		CD44v6(+)	
		Epithelium	Stroma	Epithelium	Stroma
Normal endometrium	37				
Proliferative	18	0	18	0	0
Secretory	19	19	19	19	0
Endometriosis	46				
Proliferative	7	0	7	0	0
Secretory	33	33	33	33	0
Hemosiderin-laden macrophage	6	6†	6	6†	0
Adenomyosis	20				
Proliferative	13	0	13	0	0
Secretory	7	7	7	7	0
Endometrial hyperplasia	13				
Simple	4	0	4	0	0
Complex	9	1*	9	1*	
Endometrial carcinoma	13				
Endometrioid	10	9	10	10	0
Serous	3	3	3	3	0

*Complex hyperplasia with atypia; †The residual epithelial components

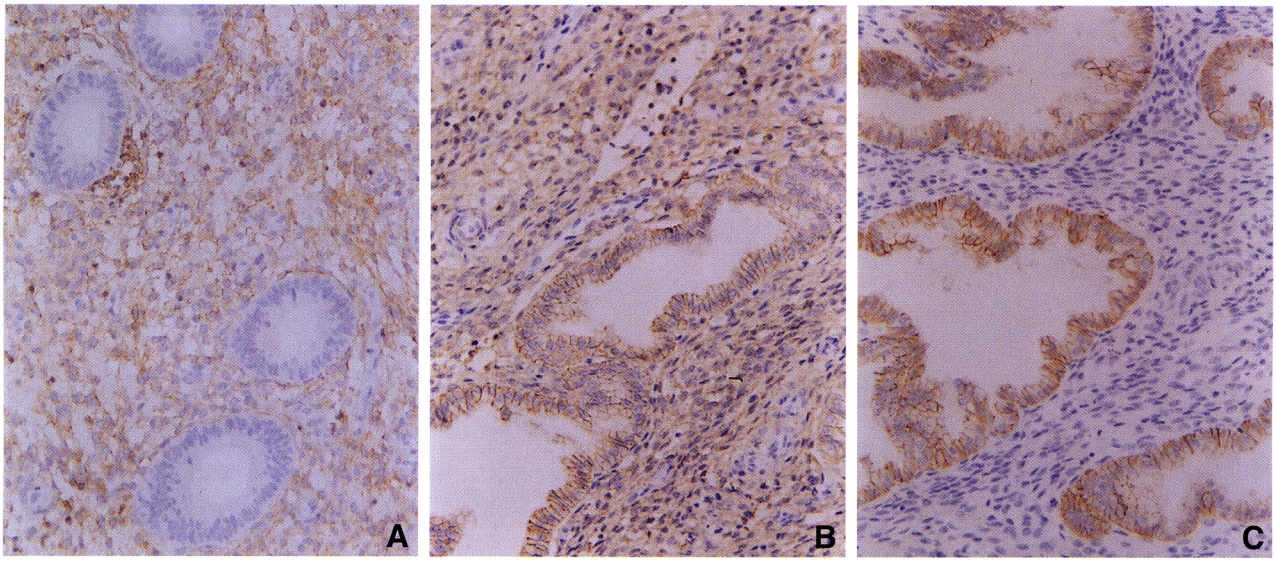

Fig. 1. Immunohistochemical stainings for CD44s and CD44v6 in normal endometrium. The positive membranous staining of CD44s is seen in the stroma of proliferative phase for CD44s, but negative staining in the epithelia (A). Both the epithelial and stromal cells in secretory phase are positive for CD44s (B), but only the epithelial cells are positive for CD44v6 and the stromal cells are negative (C). (LSAB, ×200).

was in a secretory phase and ectopic endometrium in proliferative phase. The adenomyotic tissues were classified as proliferative change in 13 cases and secretory change in 7 cases. The glandular epithelium with proliferative change was negative for both CD44s and CD44v6, whereas the stroma was positive for CD44s. The glandular epithelium of ectopic endometrium in secretory change was positive for both CD44s and CD44v6, whereas the stroma was only positive for CD44s and negative for CD44v6 (Table 1, Fig. 2).

So, the expression patterns of CD44s and CD44v6 in adenomyotic tissues also followed those of phase of normal cyclic endometrium as in the endometriotic tissue.

CD44 immunostaining in endometrial hyperplasia and adenocarcinoma

Thirteen endometrial hyperplasias comprising 4 simple and 9 complex hyperplasias showed identical CD44s immunostaining patterns as in the normal endometrium of

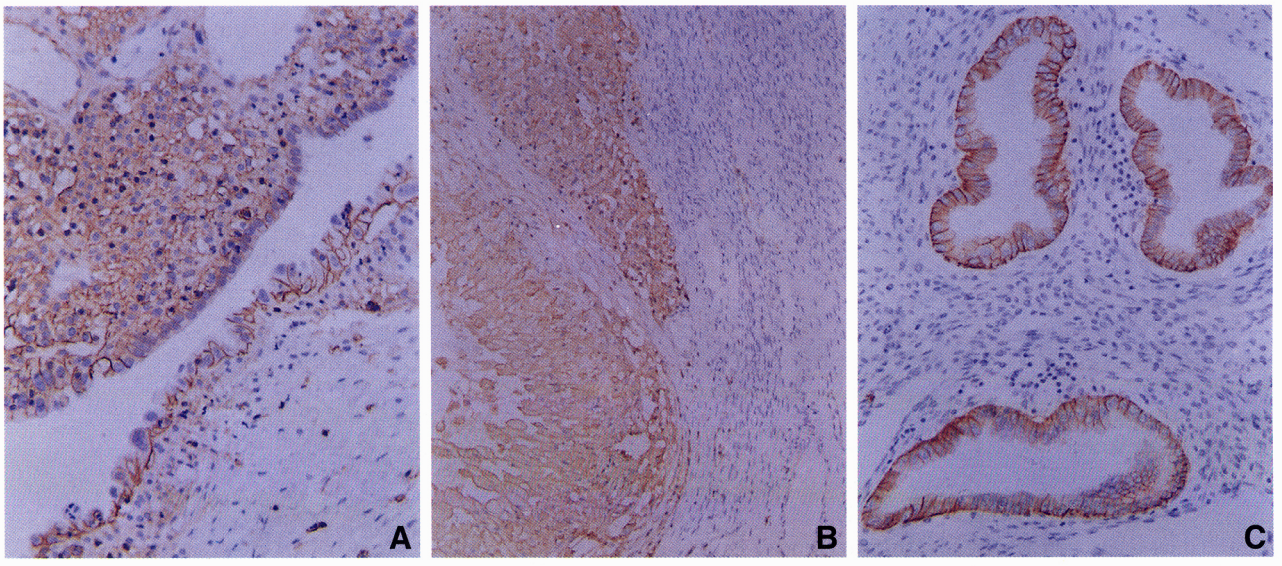

Fig. 2. Immunohistochemical stainings for CD44s and CD44v6 in ovarian endometriosis and adenomyosis. Ectopic endometriotic glands and stromal cells showing secretory cystic change are positive for CD44s. Some leukocytes are also positive **(A)**. The hemosiderin-laden macrophages are also strongly positive for CD44s **(B)**. In adenomyosis with secretory change, the epithelial cells are positive for CD44v6, but the stromal cells are negative **(C)** (LSAB, ×200).

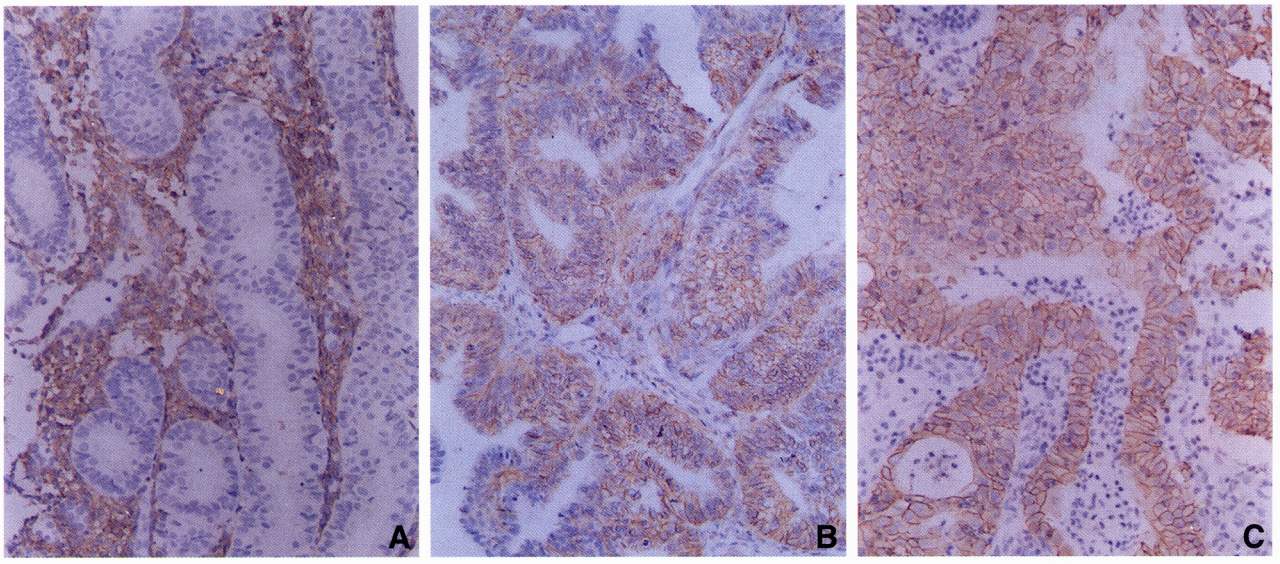

Fig. 3. Immunohistochemical stainings for CD44s and CD44v6 in endometrial hyperplasia and adenocarcinoma. The endometrial hyperplasia shows negative staining for CD44s in epithelial cells, but positive in stroma **(A)**. In adenocarcinoma, the neoplastic glands are positive for CD44s **(B)** and CD44v6 **(C)** (LSAB, ×200).

proliferative phase; the epithelium was negative for both CD44s and CD44v6, whereas the stroma was positive for CD44s and negative for CD44v6. Only one case of complex hyperplasia showing cytological atypia revealed a focally positive stainings for CD44s and CD44v6 in glandular epithelium (Table1, Fig. 3).

All 10 endometrioid and 2 out of 3 serous endometrial adenocarcinomas showed positive staining for CD44s. All cases were positive for CD44v6, in which 2 cases were weakly positive and one was focally positive. As for the stroma, all cases were positive for CD44s and negative for CD44v6 (Table 1, Fig. 3).

DISCUSSION

CD44 is a cell adhesion molecule that is often expressed in the form of various splice variants. It appears to play a role in many biological processes, including lymphocyte homing and activation, cell motility, tumor

growth regulation and metastasis (20-24). Kaufmann et al. demonstrated that CD44v6 is a good prognostic marker in breast carcinoma (25). Ishida reported that normal epithelial cells of the colon were immunonegative for CD44v6 and positive for CD44s, suggesting the expression of CD44v6 as a characteristic of neoplastic cells (26). Ibrahim et al. studied various isoforms of CD44 in endocervical lesions and considered CD44v5 as a useful diagnostic marker of endocervical neoplasia (27). In 1998, Saegusa et al. reported that the CD44 expression in normal cyclic endometrium was closely related to the secretory differentiation of the glandular epithelium and they suggested that the detection of aberrant expression of CD44 may be useful for the early diagnosis of endometrial carcinoma (28). The study of Fujita et al. (7) only showed an intense staining of CD44 in the normal glandular epithelial cells, and a reduced expression in endometrial carcinomas. In this study, we demonstrated up-regulation of CD44s and CD44v6 in the epithelium of secretory phase. Endometrial hyperplasia showed the same expression patterns with those of proliferative phase, whereas only one case with glandular atypia and all endometrial adenocarcinomas expressed both CD44s and CD44v6. We suggest that immunohistochemical expressions of CD44s and CD44v6 could be used for the early diagnosis of endometrial adenocarcinoma. These results turned out to be the same as those of Saegusa et al. (28).

The endometriosis is a frequent occurring disease affecting 15-20% of women in their reproductive life (1). The cyclic changes of ectopic endometrium result in local inflammatory reactions, giving rise to the symptoms related to the affected organs. Most of the endometriosis is histologically benign, however, it can be deeply invasive and spread to the distant sites via the bloodstream or lymphatics.

Recently, these phenotypic similarities of ectopic endometrium to those of malignant tumor cells raised a possibility of common underlying molecular mechanisms and there has been several studies on the expression of adhesion molecules in endometriosis. Starzinski-Powitz et al. studied the expression of adhesion molecules implicated in malignant tumors, and demonstrated the absence of E-cadherin and aberrant expression of integrins in endometriotic cells (2). Darai et al. (13) reported a higher immunohistochemical expression of CD44s in ovarian endometriosis as well as higher concentrations of soluble CD44s in their cystic fluids than in cystadenomas. The ectopic endometrium of endometriosis and adenomyosis in our study recapitulated the expression patterns of the normal counterpart; positive for CD44s and CD44v6 in all glandular epithelium with secretory changes, and negative in epithelium with proliferative changes. It indicated that the expressions of adhesion molecules, CD44s and CD44v6 in endometriosis and adenomyosis were regulated as in eutopic endometrium, in contrast to endometrial adenocarcinomas which constantly showed high levels of expression.

To summarize, the expressions of CD44s and CD44v6 in endometriosis and adenomyosis recapitulated those of normal cyclic endometrium. The endometrial hyperplasia showed similar expression patterns to those of proliferative endometrium, whereas the endometrial adenocarcinomas constantly showed abnormal expressions for CD44s and CD44v6. The expressions of CD44s and CD44v6 in ectopic endometrium of endometriosis and adenomyosis were different from those of endometrial adenocarcinomas.

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