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Similar Characteristics of Endometrial and Endometriotic Epithelial Cells

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Abstract

Epithelial–mesenchymal transition (EMT) is characterized by the loss of epithelial and acquisition of mesenchymal cell characteristics. Our aim was to assess the epithelial phenotype in the pathogenesis of endometriosis with epithelial and mesenchymal markers. We used 2 structural (keratin-18, -19 [K18, K19]), 1 membrane-associated (mucin-1 [MUC1]), and 2 mesenchymal proteins (vimentin; zinc finger E-box-binding homeobox 1, [ZEB1]) to compare epithelial and mesenchymal characteristics in eutopic endometrium with the 3 endometriotic entities, peritoneal, ovarian, and deep infiltrating endometriosis (DIE). Quantitation showed no differences for K18, K19, and MUC1 between endometrium with and without endometriosis. Also, K18 was not different between endometrium and endometriotic lesions. In contrast, K19 and MUC1 were modestly but significantly decreased in the endometriotic lesions compared to endometrium. However, the maintained expression of epithelial markers in all investigated tissues, regardless of the pathological condition, clearly indicates no loss of the epithelial phenotype. This is further supported by the reduced presence of epithelial vimentin in endometriotic lesions which is in contrast to an increase in stromal vimentin in ectopic endometrium, especially in ovarian endometriosis. The ZEB1 increase in endometriotic lesions, especially in DIE, on the other hand suggests a role of partial EMT in the development of endometriotic lesions, possibly connected with the gain of invasive capabilities or stemness. Taken together, although we found some hints for at least a partial EMT, we did not observe a severe loss of the epithelial cell phenotype. Thus, we propose that EMT is not a main factor in the pathogenesis of endometriosis.

Keywords

endometrium, endometriosis, epithelial marker, mesenchymal marker, epithelial-mesenchymal transition

Introduction

Endometriosis is defined histologically by the presence of endometrial glands and stroma outside the uterine cavity.[1] Endometrial-like tissue can be found in the myometrium (internal endometriosis), peritoneum, ovaries, and other more distant loci.[2] Retrograde menstruation followed by implantation of the endometrial tissue on different surfaces in the pelvic or abdominal cavity[3] is generally accepted as the main cause of endometriosis.[1] Despite the high rate of retrograde menstruation, only approximately 10% of reproductive-age women acquire endometriosis.[2]

It was hypothesized that peritoneal endometriosis, endometriomas, and deep infiltrating endometriosis (DIE) could represent 3 distinct entities, which do not share a common pathogenesis.[4] However, irrespective of location, endometriotic glands almost always have an overtly endometrioid appearance and resemble histologically uterine endometrial glands.[1]

Epithelial–mesenchymal transition (EMT) is a phenotype switch of cells characterized by the loss of epithelial characteristics and acquisition of a mesenchymal nature.[5] Some aspects of EMT are disruption in intercellular contacts, changes in cell polarity, and increased cellular motility resulting in accelerated migratory, invasive, and metastatic properties.[5,6] Epithelial–mesenchymal transition plays a role in many biological functions such as development, tissue regeneration, wound healing, inflammation, fibrosis, and tumorigenesis.[5-7] However, there are also observations that challenge the viability of the EMT concept in tumorigenesis[6,8] or kidney fibrosis.[9]

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Table 1. Overview of The Tissue Samples Used for K18, K19, and MUC1.[a]

Tissue	Endometrium	Ovarian Endometriosis	Peritoneal Endometriosis	DIE
All samples median age	n = 67 41	n = 24 (26) 33	n = 20 (32) 32	n = 15 (20) 33
Proliferative (median age)	n = 31 (41)			
Secretory (median age)	n = 36 (41)			
Leiomyoma	n = 26			
Adenomyosis	n = 14			
Bladder			9	3
Uterosacral lig.			4	7
Ovarian fossa			4	1
Pouch of douglas			2	3
Round lig. of uterus			3	
Peritoneum			6	
Infundibulo pelvic lig.			1	
Pelvic wall			2	
Rectum				1
Rectovaginal septum			1	2
Paraurethral				1
Sigmoid colon				2

Abbreviations: DIE, deep infiltrating endometriosis; lig, ligament; ZRB1, zinc finger E-box-binding homeobox 1.
[a]n = 24 (26) means 26 lesions from 24 patients.

Table 2. Overview of The Tissue Samples Used for Vimentin and ZEB1.

Tissue	Endometrium	Ovarian Endometriosis	Peritoneal Endometriosis	DIE
All samples median age	n = 50 41	n = 29 (30) 33	n = 25 (27) 31	n = 21 (21) 31
Proliferative (median age)	n = 25 (40)			
Secretory (median age)	n = 25 (42)			
Leiomyoma	n = 26			
Adenomyosis	n = 9			
Bladder			8	2
Uterosacral lig.			1	5
Ovarian fossa			3	
Pouch of Douglas			5	1
Round lig. of uterus			1	
Peritoneum			3	
Infundibulo pelvic lig.			1	1
Pelvic wall			1	
Rectum				4
Rectosigmoid				2
Rectovaginal septum			1	3
Paraurethral			1	1
Sigmoid colon				1
Intestine				1
Mesovarium			1	
Lig. latum uteri			1	

Abbreviations: DIE, deep infiltrating endometriosis; lig, ligament.
[a]n = 29 (30) means 30 lesions from 29 patients.

Some hallmarks in EMT are the loss of epithelial markers, such as keratins, mucins, or E-cadherins[6,8,10] and de novo or increased expression of EMT markers, such as vimentin, S100A4, Slug, Snail, transforming growth factor-βs (TGF-βs), zinc finger E-box-binding homeobox 1 (ZEB1), or wingless-type (WNTs) in epithelial cells.[6,10,11,12]

Recently, EMT has also been proposed to be involved in the pathogenesis of endometriosis. Downregulated messenger RNA (mRNA) expression of epithelial markers, such as keratins or E-cadherin,[13,14] as well as upregulated mRNA expression of EMT markers, such as N-cadherin, Twist, Snail, vimentin, S100A4, or TGF-βs, were reported.[11,13-15] Especially, ZEB1 was found to be increased in invasive endometriosis.[16] However, in only very few studies, the localization of keratins in the endometrial[17-19] and endometriotic glands[18] was analyzed. Similarly, the number of keratin or MUC1 positive or negative endometrial and endometriotic glands has not yet been quantified in any study.

In this study, we investigated the epithelial and mesenchymal phenotype in human endometrial and endometriotic lesions using immunohistochemistry (IHC). We quantified K18, K19, MUC1, ZEB1, and vimentin in all eutopic endometrial and endometriotic glands from peritoneal, ovarian, and DIE in order to characterize the cellular phenotype.

Materials and Methods

Patients

This study has been approved by the Ethics Committee of the Medical Faculty of the Justus-Liebig-University, Giessen, Germany (95/09). The participants gave written informed consent. All specimens (Tables 1 and 2) were obtained by hysterectomy (uteri, n = 117) or laparoscopy (endometriotic tissues, n = 134 patients with 156 lesions) from patients mainly having pain (~60%). Because of the scarcity of the samples, it was necessary to use a second set for vimentin and ZEB1 (Table 2).

The intraoperative findings were classified according to the American Society for Reproductive Medicine score (rASRM) and ENZIAN score in cases of DIE.[20] Dating of the endometrial tissue was based on the dates of the last menstrual period and histological evaluation by the pathologist.

Table 3. Antibodies Used for Characterization of Eutopic and Ectopic Cells.

Protein	Source	Cat-No	Species	Clonality	Dilution
K18	Epitomics	AC-0001	Rabbit	Monoclonal	1:200
K19	Novus Biologicals	NB100-687	Rabbit	Polyclonal	1:300
MUC1	DAKO	M0613	Mouse	Monoclonal	1:200
Vimentin	DAKO	M7020	Mouse	Monoclonal	1:200
ZEB1	Sigma	HPA027524	Rabbit	Polyclonal	1:200
Anti-mouse HRP	DAKO	K4000	Goat	IgG	Ready
Anti-rabbit HRP	DAKO	K4002	Goat	IgG	Ready

Abbreviations: Cat-No, catalog number; HRP, horse radish peroxidase; IgG, immunoglobulin G; MUC1, mucin-1; ZEB1, zinc finger E-box-binding homeobox 1.

Specimens were fixed in Bouin solution (and partly in formaldehyde for the histological evaluation by the pathologist) and embedded in paraffin. After staining 5-µm sections with hematoxylin and eosin, the histological evaluation was performed.

Immunohistochemical Analysis and Quantification

Serial sections of 5 µm were cut to ensure that in most cases, the same lesions could be examined. The IHC of bouin-fixed or formalin-fixed specimens was performed as published previously.[21] The EnVision Plus System from DAKO (Hamburg, Germany) was used according to the manufacturer's instructions. Briefly, antigen retrieval was performed with a citrate buffer (pH 6, DAKO), and then the jars containing the slides were put into a steamer (Braun, Multi Gourmet) at 100°C for 20 minutes and remained in the steamer for cooling for 20 minutes. Primary antibodies against K18, K19, MUC1, vimentin, and ZEB1 (Table 3) were used, and incubation was done in a humidified chamber overnight at 4°C. After washing with phosphate-buffered saline, incubation with the appropriate secondary antibody (Table 3) was done for 30 minutes at room temperature. Then staining was visualized with diaminobenzidine (Liquid DAB K3467; DAKO). Counterstaining was performed with Mayer hematoxylin (Waldeck, Germany), and after dehydration in ethanol, the slides were mounted with Eukitt. Negative controls for IHC were prepared by omission of the primary antibody. Digital images were obtained with the inverse microscope FSX100 (Olympus, Hamburg, Germany) using the Olympus FSX-BSW software and Leica DM 2000/Leica MC170/Leica application suite LAS 4.9.0. Images were processed with Adobe Photoshop, CS6. Quantification of IHC was done by use of the HSCORE (0, no staining; 1+, weak, but detectable; 2+, moderate or distinct; 3+, intense). An HSCORE value was calculated for each tissue by summing the percentages of cells grouped in 1 intensity category and multiplying this number with the intensity of the staining. In each slide, every gland or cyst was used for evaluation of the HSCORE. Additionally, we also counted all stained and unstained endometrial and endometriotic glands in all endometrial and endometriotic lesions for the epithelial marker proteins.

Statistics

All values are presented as means $\pm$ standard error of the mean (SEM) or median. HSCORE values between the different groups were analyzed using 1-way analysis of variance (ANOVA). Then, comparison between 2 groups was done with the nonparametric test of Mann-Whitney U test. Kruskal-Wallis test was used for comparisons between more than 2 groups. P values $\leq .05$ were considered to be significant. GraphPad Prism 6.01 (www.graphpad.com) was used for the statistics.

Results

To examine the epithelial phenotype, we selected K18, K19, and MUC1, because for all 3 proteins, a very high expression in eutopic endometrial glands[17-19] and some ectopic endometriotic lesions has been demonstrated.[18,22] We extended these observations to more patients and analyzed all 3 endometriotic entities.

Immunohistochemistry analysis of the 3 proteins in patients with and without endometriosis demonstrated stable expression in nearly all endometrial glands as well as in nearly all endometrial epithelial cells (Figure 1). Similarly, quantification with the HSCORE also did not reveal any differences between patients with and without endometriosis (Table 4) and proliferative and secretory phases (data not shown). Based on these findings, we also analyzed the localization in DIE and endometriotic lesions of the peritoneum and ovary.

Nearly all endometriotic epithelial cells in nearly all endometriotic lesions demonstrated positivity for K18, K19, and MUC1 in peritoneal (Figure 2A-C), ovarian (Figure 2D-F) as well as deep infiltrating lesions (Figure 2G-I). We merged both data sets for comparison with the 3 endometriotic ectopic entities, because eutopic endometrium with and without endometriois showed no differences in the HSCORE (Table 4). No differences in the HSCORE between eutopic endometrium and the 3 endometriotic ectopic entities were found for K18 (Table 5). In contrast, the HSCORE for MUC1 and K19 was modestly reduced with significant differences between eutopic endometrium and the 3 endometriotic entities (Table 5). However, although some HSCOREs were reduced in the 3 endometriotic entities, the number of immunostained glands and cysts was similar between eutopic endometrium and the 3 endometriotic entities (Table 6).

To examine the mesenchymal phenotype of the epithelial cells, we selected vimentin, which is a marker for mesenchymal cells,[6,10] and ZEB1, which is a transcription factor strongly

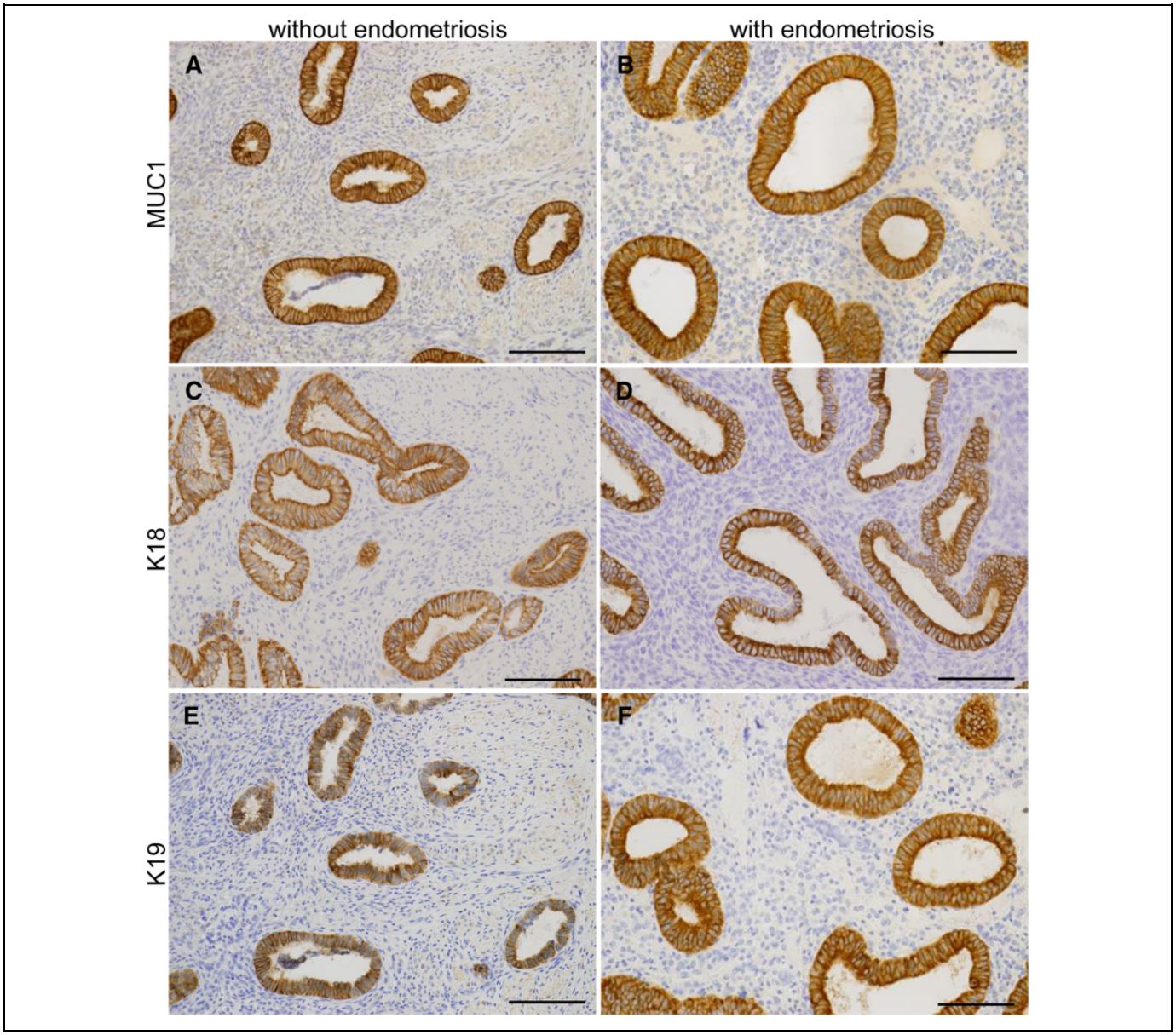

Figure 1. Representative microphotographs of MUC1 (A and B), K18 (C and D), and K19 (E and F) in the endometrium of patients without endometriosis (A, C, and E) or with endometriosis (B, D, and F). One patient with a normal endometrium showed ovarian and rectovaginal endometriosis (B). One patient also had adenomyosis (D). One patient showed besides adenomyosis also endometriosis in the fallopian tubes (F). Scale bars 100 μm.

involved in EMT.[12] Protein expression of vimentin was nearly identical in epithelial and stromal cells of the endometrium when compared to endometrium with endometriosis (Figure 3A-D, Table 4) and also did not show significant differences between proliferative and secretory phases (data not shown). The presence of vimentin was significantly lower in the epithelial cells in ovarian lesions, whereas only a moderate reduction was found in peritoneal endometriosis (Figure 3E-F, Table 5). In deep infiltrating endometriotic lesions (Figure 3G), a slight but not significant increase in the epithelial vimentin HSCORE was found compared to eutopic endometrium (Table 5). In contrast, the stromal vimentin HSCORE (without endothelial cells) showed an increase in ectopic endometrium, especially in ovarian endometriosis (Table 5).

Localization of ZEB1 was found in all samples in the endometrial stroma and also in the glands but not in all (Figure 4A-D) and was similar in proliferative compared to secretory phases (data not shown). In the endometriotic ectopic lesions, a strong nuclear and sometimes a nuclear together with cytoplasmic ZEB1 localization could be found in the glands and cysts (Figure 4E and F). In the surrounding stromal cells, a nuclear localization was apparent (Figure 4E and F). The HSCORE was slightly but not significantly higher in endometrium when compared to endometrium with endometriosis

Table 4. HSCORE Comparison of Endometrium With and Without Endometriosis.

Proteins	Endometrium Without Endometriosis	Endometrium With Endometriosis
MUC1		
Mean HSCORE	288	289
SEM	6.2	3.8
N (median age)	20 (40.5)	47 (41)
P Value		NS
K18		
Mean HSCORE	238	220
SEM	15	10
N (median age)	20 (40.5)	47 (41)
P Value		NS
K19		
Mean HSCORE	293	282
SEM	4.6	5.3
N (median age)	20 (40.5)	47 (41)
P Value		NS
Vimentin		
Mean HSCORE	65	76
SEM	4.6	12.7
N (median age)	20 (40.5)	30 (41.5)
P Value		NS
Vimentin (Stroma)		
Mean HSCORE	11	6.9
SEM	3.8	2.4
N (median age)	20 (40.5)	30 (41.5)
P Value		NS
ZEB1		
Mean HSCORE	104	71
SEM	11.9	15.5
N (median age)	20 (40.5)	30 (41.5)
P Value		NS

Abbrreviations: MUC1, mucin-1; NS, not significant; SEM, standard error of the mean; ZEB1, zinc finger E-box-binding homeobox 1.

(Table 4). Additionally, the ZEB1 HSCORE was moderately but not significantly increased in ovarian and peritoneal endometriosis compared to eutopic endometrium (Table 5). In contrast, the HSCORE of ZEB1 was nearly 2-fold and thus significantly higher in deep infiltrating endometriotic lesions (Table 5). Of note, immunolabeling of ZEB1 showed an exclusive nuclear localization in 88% of epithelial cells of the eutopic endometrium compared to 87% in ovarian lesions, 57% in DIE, and 37% in peritoneal lesions. The colocalization of ZEB1 in the nucleus and cytoplasm was identified in 10% of the epithelial cells in the eutopic endometrium compared to 7% in ovarian lesions, 38% in DIE, and 59% in peritoneal lesions.

Discussion

In our IHC study, we mainly analyzed epithelial cells of eutopic endometrial glands and endometriotic lesions in the ovary, peritoneum, and DIE, using 3 different epithelial markers, K18, K19, and MUC1. In contrast, vimentin and ZEB1 were used as mesenchymal markers. Because we did not analyze S100A4, Snail, Twist, N-cadherin as mesenchymal markers, the conclusions of our study are limited. However, the strength of our study is the simultaneous analysis of epithelial and mesenchymal markers. Our results demonstrate convincingly that nearly all epithelial cells of nearly all endometrial glands as well as nearly all ectopic endometriotic lesions express the 3 epithelial biomarkers.

Keratin filaments constitute type I and type II intermediate filaments with at least 20 subtypes. For instance, the keratins K7, K8, K18, and K19 are generally expressed in simple epithelia[23,24] such as the human endometrium. Since keratin expression varies considerably among different epithelia, they have been widely used to fingerprint various carcinomas because the keratin profile usually remains constant even if an epithelium undergoes malignant transformation.[23,24]

Our quantification of K18, K19, and MUC1 protein expression by the HSCORE demonstrated for K18 highly similar values for eutopic endometrium as well as for the 3 endometriotic entities. In contrast, the presence of K19 and MUC1 in the endometriotic entities was modestly reduced compared to eutopic endometrium. However, all 3 proteins are still localized in nearly all epithelial cells of the endometrium and in nearly all glands or cysts of peritoneal, ovarian, and DIE suggesting that the epithelial phenotype of the cells is retained in endometriosis. Similarly, 2 other reports also showed a very high identity between endometrial and endometriotic lesions in the expression of keratins.[14,18] However, in one of them[18] and in contrast to our study, they did not quantify the number of keratin-positive glands and lesions and only analyzed very few endometriotic lesions. In the other study,[14] keratin protein expression was quantified with IHC scores. It showed less epithelial keratin marker expression in peritoneal, ovarian, and deep infiltrating lesions compared to menstrual endometrium. However, the number of keratin-positive and keratin-negative glands again was not quantified.[14]

In line with our findings that the epithelial phenotype of the cells is retained in endometriotic lesions, in menstrual effluents of 15 of 16 patients, no keratin-negative epithelial cells were found,[25] therefore suggesting no transient EMT of endometrial epithelial cells. Thus, the loss of the epithelial phenotype as evidenced by a loss of keratin expression was never convincingly demonstrated for endometriosis, neither in tissues nor in biological fluids.

Mucin-1, which is normally expressed on polarized epithelial cells of normal glandular epithelia, is a member of the mucin family[26] and is also a component of glandular secretions.[27] Abnormal expression of MUC1 is observed in over 80% of some cancers and is associated with a poor prognosis.[26] Additionally, during EMT, the polarized organization of the epithelial cells is lost, and MUC1 is either redistributed or downmodulated.[28]

In the female genital tract, MUC1 is found on the endometrial cell surface[29,30] and is expressed at similar levels in proliferative and secretory endometrium.[22] Furthermore, MUC1 is also expressed in epithelial cells of the fallopian tubes and ovarian endometriosis[31] which was confirmed in our study.

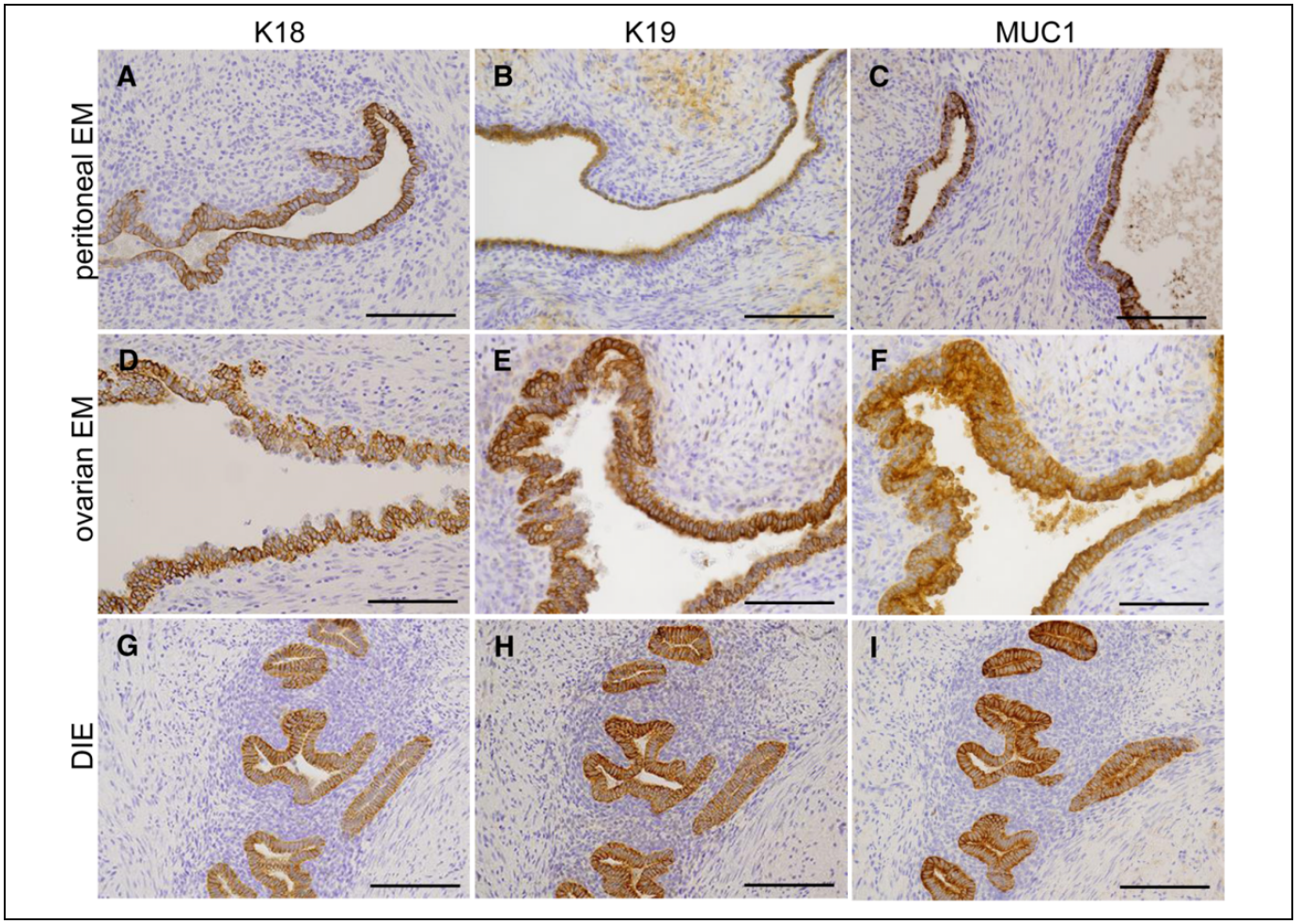

Figure 2. Representative microphotographs of K18 (A, D, and G), K19 (B, E, and H), and MUC1 (C, F, and I) in peritoneal endometriosis (A-C, ovarian fossa), ovarian endometriosis (D-F), and DIE (G-I, rectovaginal septum). EM indicates endometriosis; DIE, deep infiltrating endometriosis. Scale bars 100 μm.

Remarkably, we found MUC1 to be expressed by nearly all glands, cysts, and epithelial cells in all endometrial and endometriotic lesions studied, thus suggesting no loss of the epithelial cell organization. Similarly, MUC1 mRNA expression has been shown to be equal in eutopic endometrium and ectopic endometriotic lesions.[22]

Despite a multitude of epithelial and mesenchymal markers, there are 2 undisputed hallmarks of EMT, downregulation of epithelial markers coupled with an upregulation of mesenchymal markers.[5-10] In the past, upregulation of EMT markers such as vimentin, S100A4, Snail, Twist, N-cadherin, or ZEB1[13,14,16] was reported in endometriotic lesions. However, in these studies, the loss of epithelial markers was not or only rarely investigated.[14]

To further assess the possible mesenchymal character of the epithelial cells, we analyzed vimentin, which is primarily a marker for mesenchymal cells and for EMT.[32] In contrast to epithelial markers, mesenchymal markers normally show a higher fidelity.[33] As clearly shown in our study, vimentin expression was highest in the epithelial cells in the endometrial epithelium and decreased significantly in ovarian lesions, agreeing with previous findings.[14,34,35] Furthermore, we could show that vimentin expression was also decreased moderately in peritoneal lesions but not in DIE. In contrast, Matsuzaki and Darcha[14] claimed that vimentin expression was higher in peritoneal lesions compared to menstrual endometrium. However, they only analyzed a limited set of superficial lesions and only used menstrual endometrium for comparison, which because of the high content of blood cells, might cause problems in IHC studies. Additionally, we found an increased stromal expression of vimentin in ectopic endometrium compared to eutopic endometrium. We suppose that the higher stromal vimentin expression, especially in ovarian endometriosis, might be due to the role of vimentin in providing mechanical integrity and shape to cells,[36] because in ovarian cysts, cells are exposed to a high pressure caused by the blood inside in contrast to glands.

Recently, it could be demonstrated that ZEB1 was not expressed in glandular epithelium, very slightly in ovarian endometriosis, but was strongly upregulated in DIE,[16] similar to our results. Also consistent with our study, Furuya et al[16]

Table 5. HSCORE of All Markers in The Endometrium and The 3 Entities of Endometriosis.

Proteins	Endometrium (a)	Ov. EM (b)	DIE (c)	Perit. EM (d)
MUC1				
Mean HSCORE	289	257	265	276
SEM	3.2	7.8	9	6.1
N (median age)	67 (41)	24/26 (33)	15/20 (33)	19/32 (32)
P value		(a, b) 0.001	(a, c) 0.05	NS
K18				
Mean HSCORE	225	211	204	210
SEM	8.3	9.9	15.8	9.1
N (median age)	67 (41)	24/26 (33)	15/20 (33)	19/32 (32)
P Value		NS	NS	NS
K19				
Mean HSCORE	285	264	264	253
SEM	4	6.4	7.0	6.1
N (median age)	67 (41)	24/26 (33)	15/20 (33)	19/32 (32)
P value		(a, b) 0.01	(a, c) 0.01	(a, d) 0.001
Vimentin				
Mean HSCORE	72	15	94	46
SEM	11.0	6.1	19.1	13.1
N (median age)	50 (41)	29/30 (33)	21/21 (31)	25/27 (31)
P value		(a, b) 0.001	(b, c) 0.001	NS
Vimentin (Stroma)				
Mean HSCORE	8.5	84.8	49.1	38.2
SEM	2.1	15.7	13.9	10.4
N (median age)	50 (41)	29/29 (33.5)	21/21	25/27 (31)
P value		(a, b) 0.001	NS	NS
ZEB1				
Mean HSCORE	84	145	202	138
SEM	9.6	18.2	21.2	20.5
N (median age)	50 (41)	29/30 (33)	21/21 (31)	25/27 (31)
P value		NS	(a, c) 0.001	NS

Abbreviations: OV EM, ovarian endometriosis; perit., peritoneal; NS, not significant; ZEB1, zinc finger E-box-binding homeobox 1.
[a]24/26 means 26 ovarian lesions from 24 patients; for example, (b, d) means b compared to d.

Table 6. Number of Glands and Cysts in The Endometrium and The 3 Entities of Endometriosis Showing Expression of MUC1, K18, and K19.

Proteins	Endometrium	OV EM	DIE	Peritoneal EM
MUC1				
Mean (%)	99.8	99.2	100	100
SEM	0.07	0.77	0	0
N (median age)	67 (41)	24/26 (33)	15/20 (33)	19/32 (32)
P value		NS	NS	NS
K18				
Mean (%)	94.9	100	98.9	99.5
SEM	1.51	0	1.15	0.33
N (median age)	67 (41)	24/26 (33)	15/20 (33)	19/32 (32)
P value		NS	NS	NS
K19				
Mean (%)	99.9	100	100	100
SEM	0.15	0	0	0
N (median age)	67 (41)	24/26 (33)	15/20 (33)	19/32 (32)
P value		NS	NS	NS

Abbreviations: OV EM, ovarian endometriosis; MUC1, mucin-1; NS, not significant; SEM, standard error of the mean.
[a]24/26 means 26 ovarian lesions from 24 patients.

identified ZEB1 in endometrial stroma but in contrast to our study not in stromal cells of endometriotic lesions. Although we also found in 16 of 50 endometrial samples a very low or no HSCORE, we identified ZEB1 in majority of endometrial glandular epithelial cells. The difference is possibly due to the fact that Furuya et al[16] only analyzed a limited number of samples (n = 10) from eutopic endometrium. Furthermore, we used the EnVision system which is superior in sensitivity and background staining compared to the Avidin-Biotin Complex (ABC) system.[37]

We suppose that the abundance of ZEB1 in the endometrial stromal and epithelial cells suggests that both cell types exhibit a high cellular plasticity because the human endometrium is reconstructed approximately once per month. Similarly, ZEB1 was suggested to be a marker for cellular plasticity in pancreatic cancer.[38] Recently, we could furthermore demonstrate that endometrial stroma showed a marker signature highly similar to multipotent stromal cells.[39] Consistent with our study, in squamous and small cell carcinoma of the esophagus[40] and colorectal cancer[41] specimen, a cytoplasmic localization of ZEB1 was described and needs further investigation.

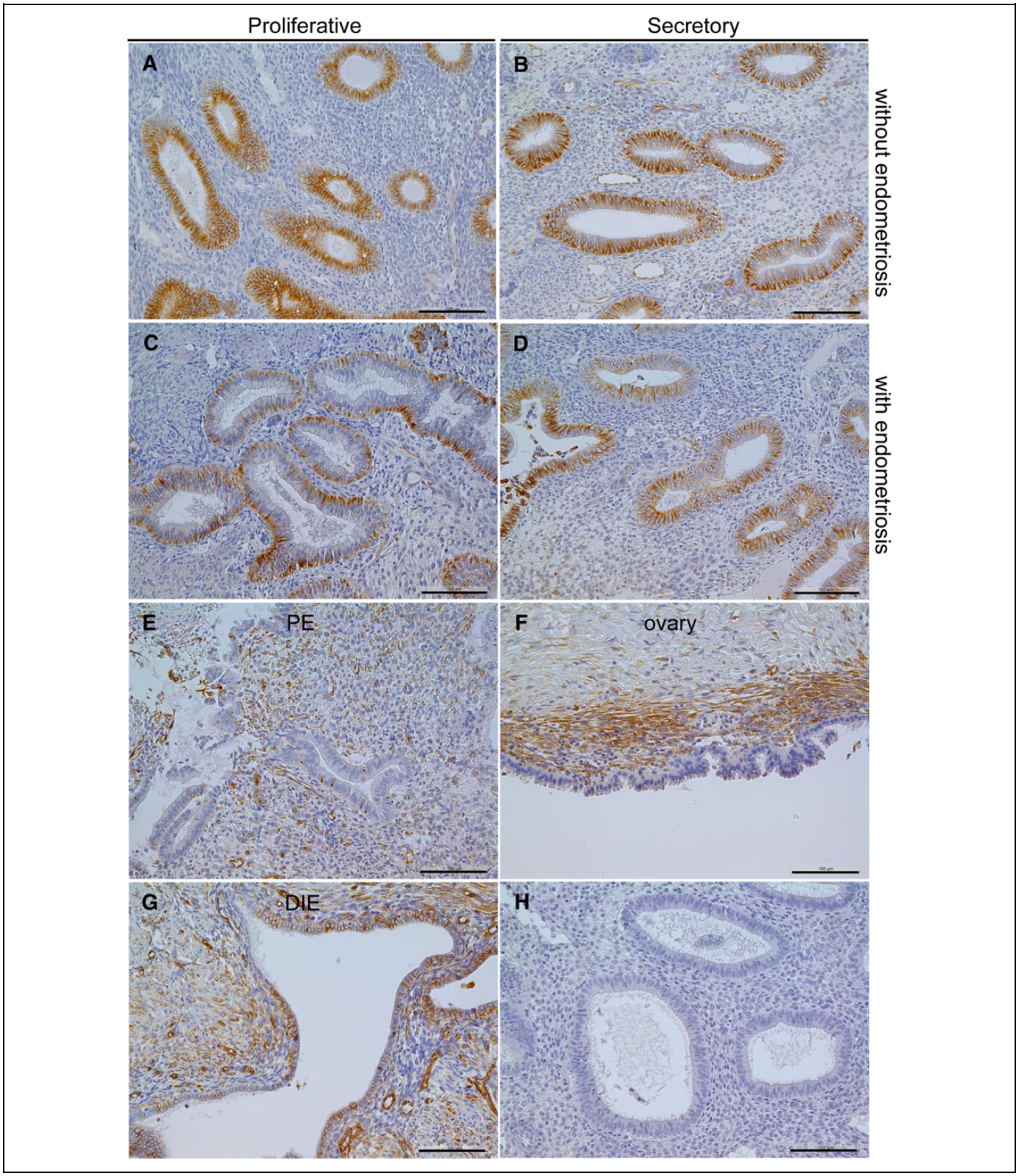

Figure 3. Representative microphotographs of vimentin in proliferative (A) and secretory (B) endometrium without endometriosis and in proliferative (C) and secretory (D) endometrium with endometriosis. One patient also showed endometriosis at the bladder, intestine, and pouch of Douglas (C) and the other at both uterosacral ligaments (D). The patients showed peritoneal endometriosis (E, bladder), ovarian endometriosis (F), and DIE (G, uterosacral ligament). A representative negative control is shown (H). DIE indicates deep infiltrating endometriosis; PE, peritoneal endometriosis. Scale bars 100 μm.

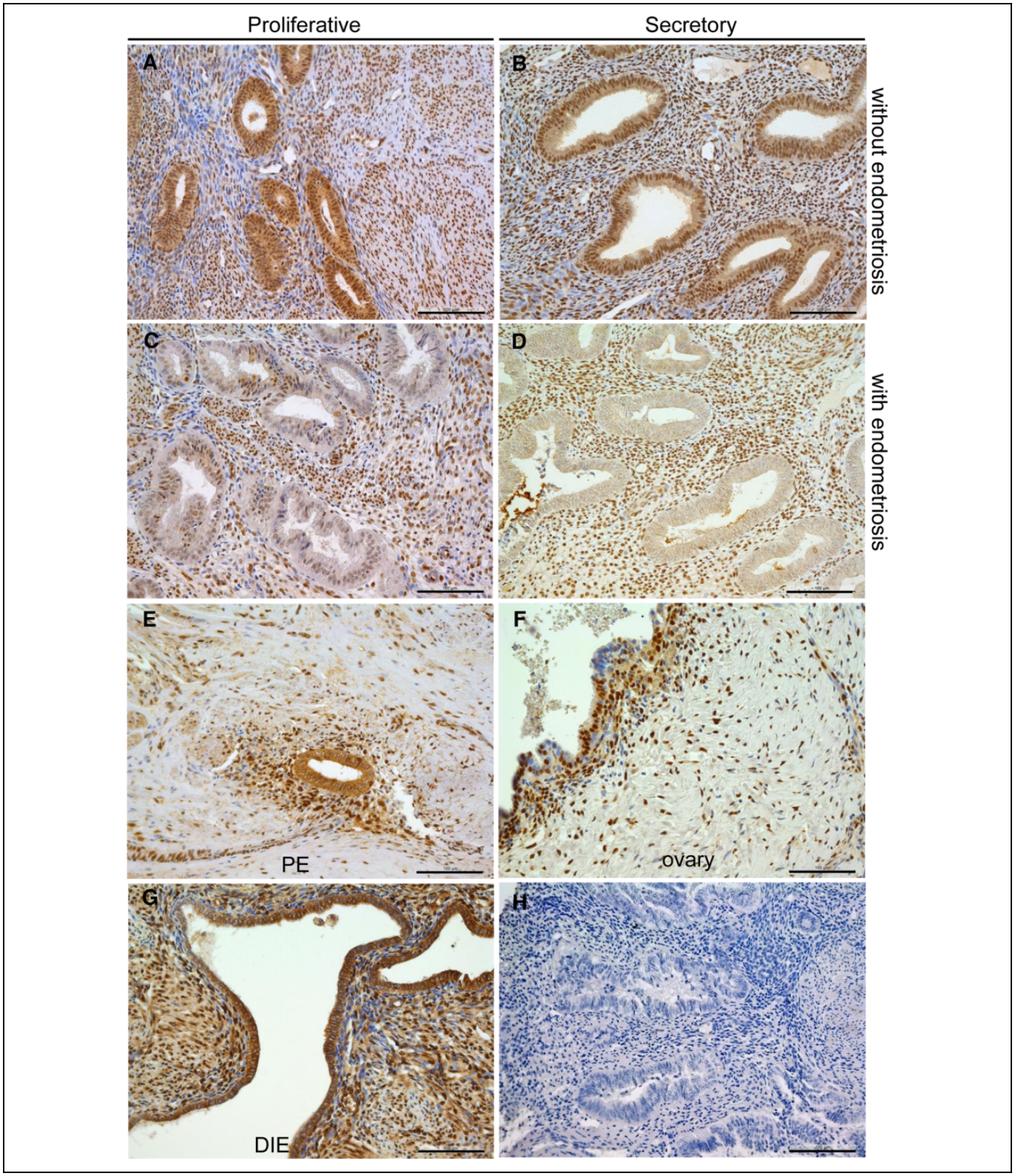

Figure 4. Representative microphotographs of ZEB1 in proliferative (A) and secretory (B) endometrium without endometriosis and in proliferative (C) and secretory (D) endometrium with endometriosis. One patient also showed endometriosis at the bladder, intestine, and pouch of Douglas (C) and the other at both uterosacral ligaments (D). The patients showed peritoneal endometriosis (E, bladder), ovarian endometriosis (F), and DIE (G, uterosacral ligament). A representative negative control is shown (H). The same patients as in Figure 3 are shown. DIE indicates deep infiltrating endometriosis; PE, peritoneal endometriosis; ZEB1, zinc finger E-box-binding homeobox 1. Scale bars 100 μm.

Taken together, although our data does not show a gain of the mesenchymal marker vimentin, we found an increased abundance of ZEB1 in endometriotic lesions. We suppose that this might indicate a partial EMT[42] which is not accompanied by a loss of the epithelial phenotype as evidenced by a stable keratin and MUC1 expression. Thus, we suggest that EMT is probably not a main factor in the pathogenesis of endometriosis.

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Declaration of Conflicting Interests

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