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Original Article

Mismatch repair system in endometriotic tissue and eutopic endometrium of unaffected women

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Abstract: Objective: To test the immunohistochemical staining pattern of some mismatch repair (MMR) system proteins in endometriotic tissue (ET) and eutopic endometrium. Methods: This was a retrospective study conducted at the Pathology and Obstetrics and Gynecology Departments of the Udine University Hospital. We analyzed 528 samples obtained from 246 patients affected by endometriosis and 71 samples from 71 patients with normal endometrium. A tissue microarray model was used to analyze the immunohistochemical expression of MMR system proteins. Results: Significant loss of MMR proteins was found in the stromal component of ETs. We found MSH2 to be expressed at a higher level than any other MMR system proteins in eutopic endometrium and ETs, to be significantly correlated to Ki-67 expression in both stromal and glandular components of ETs, and to be expressed at a significantly higher level in ETs than in eutopic endometrium. When considering the subgroup of endometriosis with high recurrence rate and glandular cytoplasmic staining for aurora A kinase, we found MMR proteins expressed at a significantly higher level in these ETs than in other ETs and eutopic endometrium of unaffected women. Conclusions: We found significant loss of MMR proteins (known to be associated with microsatellite instability) in the stromal component of ETs. The group of ETs with glandular cytoplasmic staining for aurora A kinase had higher MMR protein expression, suggesting an increased activity of this system. Our result suggests a novel role of increased MSH2 expression in cellular proliferation of endometriosis.

Keywords: Endometriosis, mismatch repair system proteins, MLH1, PMS2, MSH2, MSH6, microsatellite instability, aurora A kinase

Introduction

Endometriosis is a gynecological disorder, classically defined as the presence of endometrial-like glands and stroma outside the uterus [1, 2]. The precise etiology of endometriosis is unknown but some theories involving cell implantation or metaplasia describe the possible mechanisms of initiation of endometriotic lesions [3, 4].

Evidence in the literature suggests that oxidative stress is a component of the inflammatory reaction associated with endometriosis and in the long term it can induce genetic damage, such as mutations to DNA single base pairs [5, 6]. In chronically inflamed epithelium, these processes have been shown to lead to cancer development [7].

The DNA mismatch repair (MMR) system is important for promoting the genetic stability of eukaryotes. MMR proteins recognize and fix DNA single-base mismatches and small insertion/deletion loops occurring during DNA replication, homologous recombination, or other forms of DNA damage such as oxidative stress [8, 9]. The MMR system is composed of different proteins, six MutS homologues (MSH1 to MSH6) and four MutL homologues (MLH1, PMS1, PMS2, and MLH3). As part of a complex mechanism, MutS homologues 2 (MSH2) and MutS homologues 6 (MSH6) heterodimers bind to single base-pair mismatches. Successively, MutL homologues 1 (MLH1) and Post-meiotic segregation 2 (PMS2) heterodimers are recruited to this complex for completing the DNA repair process [10].

One of the results of defective function of the MMR system is microsatellite instability (MSI). Microsatellites are noncoding areas of mononuclear or dinuclear acid repeats, which are chemically unstable and prone to mutations [11]. MSI can be identified either using molecular diagnostics (by the resultant instability in length of microsatellite repeat tracts) or by immunohistochemical staining (for loss of mismatch repair proteins) [12]. Both methods are effective and widely-accepted for identifying MMR defects [13].

Although MSI has traditionally been implicated in the molecular pathogenesis of epithelial cell carcinogenesis [5], new data suggest that such dysfunction is also found in mesenchymal cells undergoing tissue remodeling in chronic inflammatory disorders [11, 14]. It is now known that DNA alterations occur in atherosclerosis, asthma, chronic obstructive pulmonary disease, rheumatoid arthritis, and Crohn's disease (CD) [11, 15-17]. These studies hypothesized that the pathophysiology of the altered behavior of mesenchymal cells could be linked to an accumulation in DNA damage, which may be directly related to an altered DNA-MMR function. This could further lead to uncontrolled cell proliferation associated with either benign pathology or, more drastically, neoplastic transformation. Few studies have linked loss of expression of MMR proteins and MSI in ETs with the subsequent development of endometriosis related cancer [6, 18].

Aurora A kinase (AAK) is an important mitotic regulator implicated in the assembly of the mitotic spindle and chromatin segregation [19]. Recent studies suggest that AAK is overexpressed in cancer and is associated with genomic instability [20]. In our previous publication we found high glandular cytoplasmic expression of AAK in a subgroup of endometriotic lesions with higher prevalence of relapse [21].

The main aim of our current study was to test the positivity of MMR system proteins in endometriotic tissue (ET) and eutopic endometrium; further, we wanted to analyze also the immunohistochemical staining pattern of ET with high glandular cytoplasmic AAK staining. To our knowledge, this is the first study investigating a potential link between endometriosis, MMR proteins and AAK expression.

Materials and methods

From the registry of the Pathology Department of the Udine University Hospital, a total of 277 women who had a histopathological diagnosis of endometriosis between January 2000 and December 2010 were identified; 246 of them met the following inclusion criteria. We included all Caucasian women of reproductive age at the time of intervention who had not taken any hormone therapy during the year before surgery. A total of 528 paraffin blocks containing ET were available from these 246 women and we obtained for every block one core biopsy from formalin-fixed paraffin-embedded ET with representative stromal and glandular components. The majority of the population affected by endometriosis and the methods used to prepare our tissue microarray model (TMA) were the same as already described in our two previous publications, with the exception of an increased number of cases analysed in the current study [21, 22]. Among the included cases there were neither cases with cytologic/architectural atypia of ET nor cases affected by known germline mutation in MMR proteins.

Eutopic endometrial tissue was obtained from formalin-fixed paraffin-embedded uterine tissues from all women who underwent hysterectomy for leiomyomas during the period from 2006 to 2010 and met the previously described inclusion criteria [21]. All Caucasian women with regular menses and without endometrial pathology, histological diagnosis of adenomyosis, or use of hormonal medication during the year before surgery were included [21]. We obtained 71 endometrial core biopsies from 71 women. According to the date of menses and histological confirmation based on Noyes criteria [23], among these women, 29 were in the follicular phase (proliferative endometrium) and 42 in the luteal phase (secretory endometrium).

The clinical and surgical data were collected as previously explained [21]. Staging was determined according to the American Society of Reproductive Medicine (ASRM) classification of endometriosis [24]. This study was conducted according to the declaration of Helsinki, following internal review board approval.

All immunohistochemistry stains were performed on 4 μm thick sections, which were

Table 1. Miss-match repair proteins expression. The negative staining of at least one miss-match repair protein will suggest the presence of microsatellite instability as previous described in the literature. The *p*-values reported in the table refers to chi-square or Fisher exact test

A. All samples				
Gland	ETs (528)	Proliferative E. (29)	Secretory E. (42)	*P* (*)
≥ 1 absent proteins of 4	14%	7%	17%	NS
Stroma				
≥ 1 absent proteins of 4	24%	7%	19%	(1)
B. Aurora A kinase glandular cytoplasm expression in ETs				
Gland	ETs (29)	Proliferative E. (29)	Secretory E. (42)	*P* (*)
≥ 1 absent proteins of 4	7%	7%	17%	NS
Stroma				
≥ 1 absent proteins of 4	21%	7%	19%	NS

(*) Significant differences ($P < 0.05$) between: (1) ETs and proliferative endometrium. NS = non significant.

dewaxed in automatic PTlink station (Dako) in retrieval solution pH6 (S1700, Dako). Immunohistochemistry was preceded by a peroxidase blocking step [3% hydrogen peroxide (Sigma) for 5 min]. The sections were rinsed in PBS and then incubated in a wet chamber at 4°C for 60 minutes with the following primary antibodies: rabbit monoclonal MLH1 (2786-1, Epitomics, Burlingame, CA, diluted 1:200), rabbit monoclonal MSH2 (2848-1, Epitomics, Burlingame, CA diluted 1:200), rabbit monoclonal anti-Human MSH6 (AC-0047, Epitomics, Burlingame, CA diluted 1:200), and rabbit monoclonal PMS2 (2858-1, Epitomics, Burlingame, CA diluted 1:100) [25-28]. A Dako REAL™ EnVision™ Rabbit/Mouse antibody (Dako, K5007, Glostrup, DK) was used as second antibody. HRP activity was detected using Dako REAL™ DAB+Chromogen (Dako, K5007, Glostrup, DK) as substrate for 3 min in accordance with the manufacturer's instructions. Before mounting, sections were counterstained with hematoxylin. The added samples were also stained with monoclonal Aurora A (NCL-L-AK2, Novocastra) at a dilution of 1:50 and the proliferation marker Ki-67 (M7240, Dako), as previously described [21].

Microscopic analysis of the immunohistochemical staining was done by two independent pathologists (M.O, L.M.) as described earlier [21, 22]. In case of disagreement, the two previous pathologists reviewed the specimen with a third pathologist (C.A.B.). Lymphocyte, normal myometrium, and tissue surrounding the lesions served as positive internal controlwhenanalyzingMLH1, PMS2, MSH2, and MSH6 expression. Expression of MLH1, PMS2, MSH2, and MSH6 was scored as negative when cells were negative with a positive internal control (0/+), positive when both lesions and internal control where positive (+/+) [29]. Moreover, in the positive cases the staining was semiquantitatively evaluated as H-score (product of percentage of positive cells and intensity of staining). Interpretation of staining results was performed as previously recommended [29, 30].

Data were analyzed using R (version 3.0.1) and $P < 0.05$ was considered significant. For all proteins analysed the staining was investigated in terms of H-score or percentage of positive cells when Ki-67 was considered. The normality of variables was tested using the Kolmogorov-Smirnov test. Non-parametric data were presented with the median value and the interquartile range (IQR), whereas parametric data were described as the mean value and the standard deviation. For bivariate analysis, the following statistical tests were applied: the Wilcoxon test, T-test, and Kendall's tau for continuous variables and the Chi-square and Fisher exact test for categorical variables. Because of the statistically significant age difference between cases and controls, it was necessary to evaluate the results by multivariate logistic regression analysis with age adjustment considering as dependent variable the immunohistochemical score over the median of the distribution.

Results

Population description

Women with normal eutopic endometrium were older and had higher parity rates compared to women affected by endometriosis ($P < 0.05$). The surgical indication was in the majority of

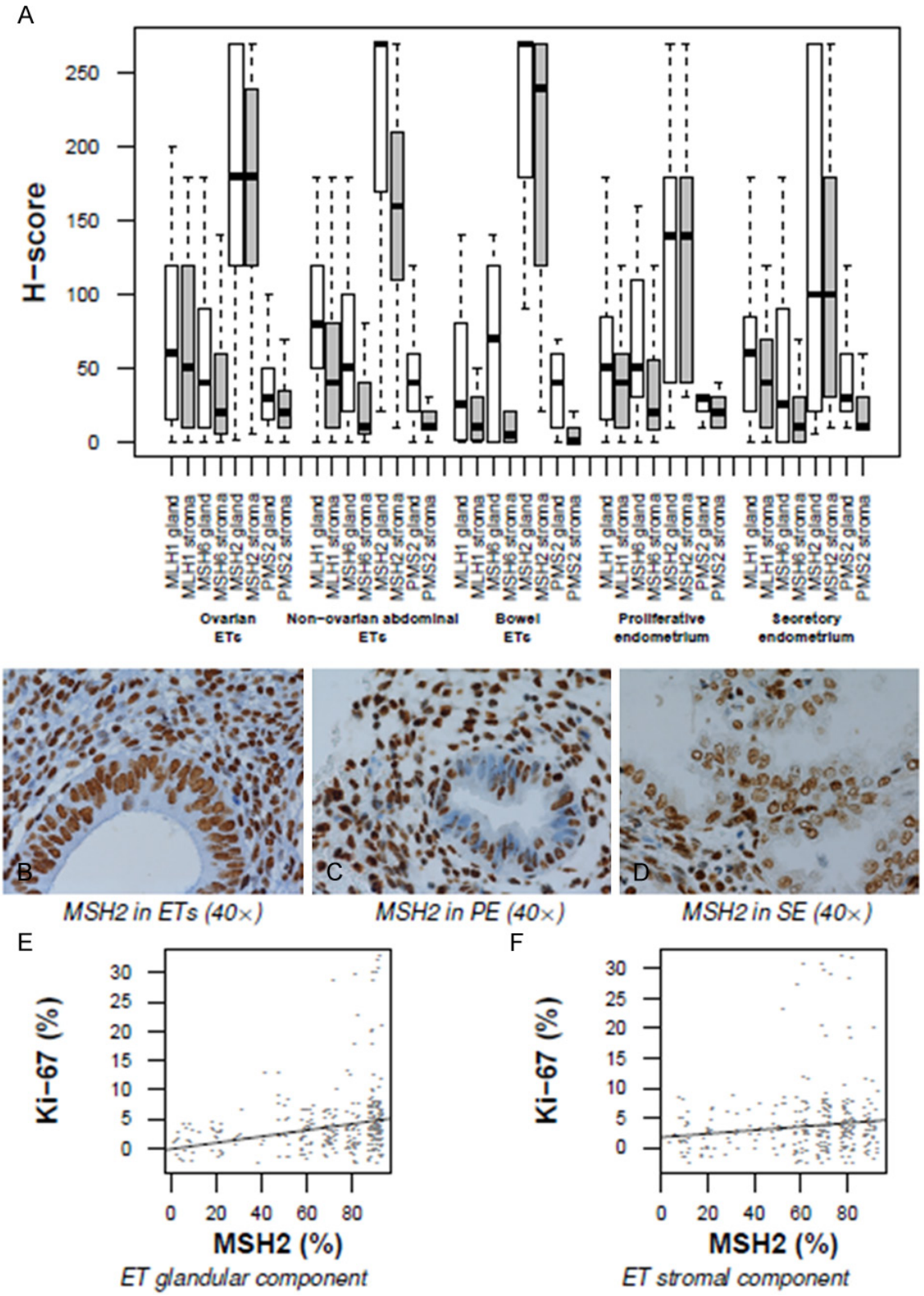

Figure 1. A: Overview of H-score values of MMR complex proteins among ETs and eutopic endometrium of unaffected women. B-D: show immunostaining of MSH2 in ovarian ETs and in proliferative and secretory endometrium. E and F: show the correlation of Ki-67, with MSH2 considering the percentage of positive cells per slide. We found that cell positivity for MSH2 had a significant direct correlation with cell positivity for Ki-67 in glandular ($P < 0.05$) and stromal ($P < 0.05$) components of ETs.

cases the presence of a pelvic mass (76%, 187/246) or of chronic pelvic disease (21%, 52/246). The most common location of ET was the ovary (80%), followed by other abdominopelvic locations (18%) and the bowel (2%). The majority of cases were affected by stage III or IV disease (83%, 204/246) (ASRM classification). The 55% of women (135/246) affected by endometriosis were operated during follicular phase and 45% (111/246) during luteal phase. No significant differences were found in the studied proteins expression between ETs obtained during follicular or luteal phases.

MMR system proteins in endometriotic tissues

We analyzed our TMA model to find possible defects in the expression of MMR system proteins and we found a significant higher prevalence of MMR protein loss in the stromal component of ET than in the stroma of proliferative endometrium ($P < 0.05$, **Table 1**). In addition, the most interesting finding was that in the glandular and stromal components of ETs, MSH2 H-score values were higher than any other MMR system proteins (**Figure 1A-D**) and MSH2 expression correlated positively with Ki-67 expression ($P < 0.05$, **Figure 1E** and **1F**). Moreover, Ki-67 was more highly expressed in proliferative endometrium than in other locations ($P < 0.05$) and in non-ovarian than other ET locations ($P < 0.05$).

Table 2 reports the semiquantitative score of immunostaining (H-score) for the studied MMR system proteins. Bivariate analysis showed no significant differences between proliferative and secretory eutopic endometrium. Significant differences in the whole population of our TMA model were found among stromal and glandular MSH2 staining in bivariate and multivariate analysis (**Table 2A**). We further analyzed the MMR expression subdivided by ET location. Glandular MSH2 H-score values were higher in non-ovarian than in ovarian ETs and eutopic endometrium (**Table 2C**, (§§) difference n° 1, 6, and 7). Moreover, glandular MSH2 H-score values were lower in proliferative endometrium than in ovarian and bowel ET (**Table 2C**, (§§) n° 3 and 8). Meanwhile, stromal MSH2 H-score was higher in ovarian, non-ovarian, or bowel ETs than eutopic endometrium (**Table 2C**, (§§) n° 3, 4, 7, 8, and 9). Furthermore, glandular MLH1 H-score values were higher in non-ovarian ET than in other ET and eutopic endometrium (**Table 2C**, (§§) n° 1, 5, 6, and 7). Stromal MLH1 and PMS2 H-score values in ovarian ET were higher than in bowel and non-ovarian ETs respectively.

MMR system protein pattern in endometriotic tissue expressing positive glandular cytoplasmic staining for AAK

Only 5.5% (29/528) of samples had glandular cytoplasmic expression of AAK with a median H-score of 35 (10-50). Among these samples expressing glandular cytoplasmic AAK we found higher expression of Ki-67, glandular and stromal expression of MSH6 and MSH2, and glandular expression of MLH1 and PMS2 than samples non-expressing glandular cytoplasmic AAK (**Table 3** and **Figure 2**). **Table 2B** shows the analysis of H-score values of MMR proteins in ET specimens with glandular cytoplasmic expression of AAK and normal endometrium. MLH1 values in the glandular component of ETs were expressed at higher levels than in eutopic endometrium; however the difference was significant only when compared to secretory endometrium. MSH2 glandular and stromal staining values were significantly higher in ETs than in proliferative and secretory endometrium. PMS2 values in the glandular component of ETs were expressed at a higher level than in eutopic endometrium, but the difference was statistically significant only when in comparison to proliferative endometrium (**Table 2B**). Furthermore, among the considered ET locations in this subgroup expressing AAK, no significant differences in MMR protein expression were found.

Discussion

In this study we found significant loss of MMR protein expression in the stromal component of ETs. Despite this, we discovered MSH2 to be significantly correlated with Ki-67 expression in both stromal and glandular components of ETs and to be expressed at a significantly higher level in ETs than in eutopic endometrium. When analyzing only ET specimens staining positively for glandular cytoplasmic expression of AAK, we found MMR proteins to be expressed at significantly higher levels compared to other ETs and eutopic endometrium.

To our knowledge, there is only one other study in the literature investigating the role of MMR proteins in ET. Fuseya et al. found MSH2 and

Table 2. H-score values of miss-match repair proteins immunostaining among ETs and eutopic endometrium of unaffected women. (A) All population (immunostaining among all ETs and eutopic endometrium of unaffected women); (B) Only aurora A kinase glandular cytoplasm expression endometriosis (immunostaining among all ET expressing aurora A kinase and eutopic endometrium of unaffected women); (C) All population (immunostaining among different ET locations and eutopic endometrium of unaffected women)

A	ETs (528 specimens)			Proliferative E. (29)	Secretory E. (42)	*P* (*)	*P* (**)
MLH1 gland	70 (20-120)			50 (15-85)	60 (20-85)	NS	NS
MLH1 stroma	50 (10-100)			40 (10-60)	40 (10-68)	NS	NS
MSH6 gland	40 (10-90)			50 (30-105)	25 (0-90)	NS	NS
MSH6 stroma	20 (5-58)			20 (8-55)	10 (0-30)	NS	NS
MSH2 gland	210 (120-270)			140 (40-180)	100 (20-270)	(1, 2)	(1, 2)
MSH2 stroma	180 (120-240)			140 (40-180)	100 (30-180)	(1, 2)	(1, 2)
PMS2 gland	30 (20-60)			30 (20-30)	30 (20-58)	NS	NS
PMS2 stroma	20 (10-30)			20 (10-30)	10 (10-30)	NS	NS
B	ETs (29)			Proliferative E. (29)	Secretory E. (42)	p (*)	p (**)
MLH1 gland	80 (52.5-155)			50 (15-85)	60 (20-85)	(2)	(2)
MLH1 stroma	50 (10-120)			40 (10-60)	40 (10-68)	NS	NS
MSH6 gland	50 (20-80)			50 (30-105)	25 (0-90)	NS	NS
MSH6 stroma	20 (5-60)			20 (8-55)	10 (0-30)	NS	NS
MSH2 gland	180 (140-270)			140 (40-180)	100 (20-270)	(1, 2)	(1, 2)
MSH2 stroma	180 (140-240)			140 (40-180)	100 (30-180)	(1, 2)	(1, 2)
PMS2 gland	50 (30-60)			30 (20-30)	30 (20-58)	(1)	(1)
PMS2 stroma	20 (10-40)			20 (10-30)	10 (10-30)	NS	
C	Ovarian ETs (421)	Non-ovarian ETs (94)	Bowel ETs (13)	Proliferative E. (29)	Secretory E. (42)	*P* (§)	*P* (§§)
MLH1 gland	60 (15-120)	80 (50-120)	25 (1-80)	50 (15-85)	60 (20-85)	(1, 5, 6, 7)	(1, 5, 6, 7)
MLH1 stroma	50 (10-120)	40 (10-80)	10 (1-25)	40 (10-60)	40 (10-68)	(2, 5, 8)	(2)
MSH6 gland	40 (10-90)	50 (20-98)	70 (0-120)	50 (30-105)	25 (0-90)	NS	NS
MSH6 stroma	20 (5-60)	10 (5-40)	5 (0-20)	20 (8-55)	10 (0-30)	NS	NS
MSH2 gland	180 (120-270)	270 (170-270)	270 (180-270)	140 (40-180)	100 (20-270)	(1, 3, 4, 6, 7, 8, 9)	(1, 3, 6, 7, 8)
MSH2 stroma	180 (120-240)	160 (115-210)	240 (120-270)	140 (40-180)	100 (30-180)	(3, 4, 7, 8, 9)	(3, 4, 7, 8, 9)
PMS2 gland	30 (15-50)	40 (20-60)	40 (10-60)	30 (20-30)	30 (20-58)	(6)	NS
PMS2 stroma	20 (10-35)	10 (10-20)	0 (0-10)	20 (10-30)	10 (10-30)	(1, 2, 5, 6, 8, 9)	(1)

The *p*-value refers to Wilcoxon test (median and interquartile range) (§ or *) and multivariate logistic regression with woman age correction (§§ or **). (* or **) Significant differences ($P < 0.05$) between: (1) ETs and proliferative endometrium; (2) ETs and secretory endometrium; and (3) proliferative and secretory endometrium. (§ or §§) significant differences ($P < 0.05$) between: (1) ovarian and non-ovarian ETs; (2) ovarian and bowel ETs; (3) ovarian ETs and proliferative endometrium; (4) Ovarian ETs and secretory endometrium; (5) non-ovarian and bowel ETs; (6) non-ovarian ETs and proliferative endometrium; (7) non-ovarian ETs and secretory endometrium; (8) bowel ETs and proliferative endometrium; (9) bowel ETs and secretory endometrium; (10) proliferative and secretory endometrium. NS = non significant.

Table 3. Woman's age and expression of studied proteins between samples expressing or not glandular cytoplasmic aurora A kinase (AAK). The p-value refers to Wilcoxon test

	Samples non-expressing glandular cytoplasmic AAK	Samples expressing glandular cytoplasmic AAK	*P*
Woman's age (years)	38 (31-46)	39 (33-47)	0.301
MLH1 gland (H-score)	60 (10-120)	80 (52-155)	< 0.05
MLH1 stroma (H-score)	50 (10-100)	50 (10-120)	0.838
MSH6 gland (H-score)	20 (5-60)	50 (20-80)	< 0.05
MSH6 stroma (H-score)	10 (0-40)	20 (5-60)	< 0.05
MSH2 gland (H-score)	160 (90-240)	180 (140-270)	0.079
MSH2 stroma (H-score)	140 (90-210)	180 (140-240)	< 0.05
PMS2 gland (H-score)	30 (10-60)	50 (30-60)	< 0.05
PMS2 stroma (H-score)	20 (10-30)	20 (10-40)	0.643
Ki-67 positivity (%)	0 (0-3)	3 (1-6)	< 0.05

MLH1 to be significantly more expressed in normal endometrium than ovarian ET; they also noted these values to be significantly higher in proliferative than in secretory eutopic endometrium [6]. We found no significant differences between proliferative and secretory endometrium and, in contrast with previous findings; we observed significantly higher MSH2 levels in ovarian ET compared to normal endometrium. Our study also included information on additional MMR proteins (MSH6 and PMS2) in different ET locations -ovarian, non-ovarian and bowel- and in stromal and glandular components of ET and eutopic endometrium. Finally, in contrast with our results, Fuseya et al. found a significant correlation between MLH1 and MSH2 proteins and Ki-67 expression only in eutopic endometrium and not in ET [6].

Loss of MMR protein expression is known to be associated with MSI. Fuseya et al. demonstrated a significant correlation between loss of MMR protein expression and MSI in ET [6]. In our study, we looked for the absence of any of the studied MMR system proteins among ET locations and eutopic endometrium. Considering the low incidence of ovarian cancer (3.1% of female cancers in our region) and the young mean age of our patients with endometriosis (39 years old), no cases of cancers were identified in our sample.

We found a prevalence of MMR protein expression loss of 7% (gland) and 7% (stroma) in proliferative endometrium, 17% (gland) and 19% (stroma) in secretory endometrium, and 14% (gland) and 24% (stroma) in ETs (**Table 1**). Studies in the literature report 0% MSI in normal endometrium, 5% in hyperplastic endometrium [6, 31], 15% in endometriosis and 30% in ovarian cancer [6]. When further analyzing our results, loss of MMR protein expression in stromal endometriosis (24%) was significantly higher than in the stroma of proliferative endometrium (7%) ($P < 0.05$, **Table 1**). Moreover, we noticed an unexpected high prevalence of MMR protein expression loss in secretory endometrium. These results are in contrast with previous literature; this could be due to other studies having smaller sample sizes and considering less than four immunohistochemical markers.

Recently, Fuseya et al found immunohistochemical expression of MLH1 and MSH2 to decrease and MSI to respectively increase stepwise in ovarian ETs, ovarian carcinoma accompanied by endometriosis, and solitary ovarian carcinoma [6]. Moreover, they found increased MSI in endometriosis compared to eutopic endometrium, suggesting that inflammation in endometriosis induces MMR abnormalities, leading to the malignant transformation of ovarian endometriosis [6].

In our previous publication we showed a higher recurrence rate in endometriotic lesions with high cytoplasmic expression of AAK [21]. In this current study, when analyzing only this subgroup of ET specimens, overexpression of several MMR proteins was found in comparison to eutopic endometrium. Glandular and stromal staining of MSH2 and glandular staining of MLH1 and PMS2 were expressed at a higher level in ETs than in eutopic endometrium. Furthermore, we found in this subgroup of patients with endometriosis a higher expression of MLH1, MSH6, MSH2, and PMS2 than in other patients affected by endometriosis.

Patients with biochemical recurrence after radical prostatectomy for prostate cancer had significantly higher levels of PMS2 than patients

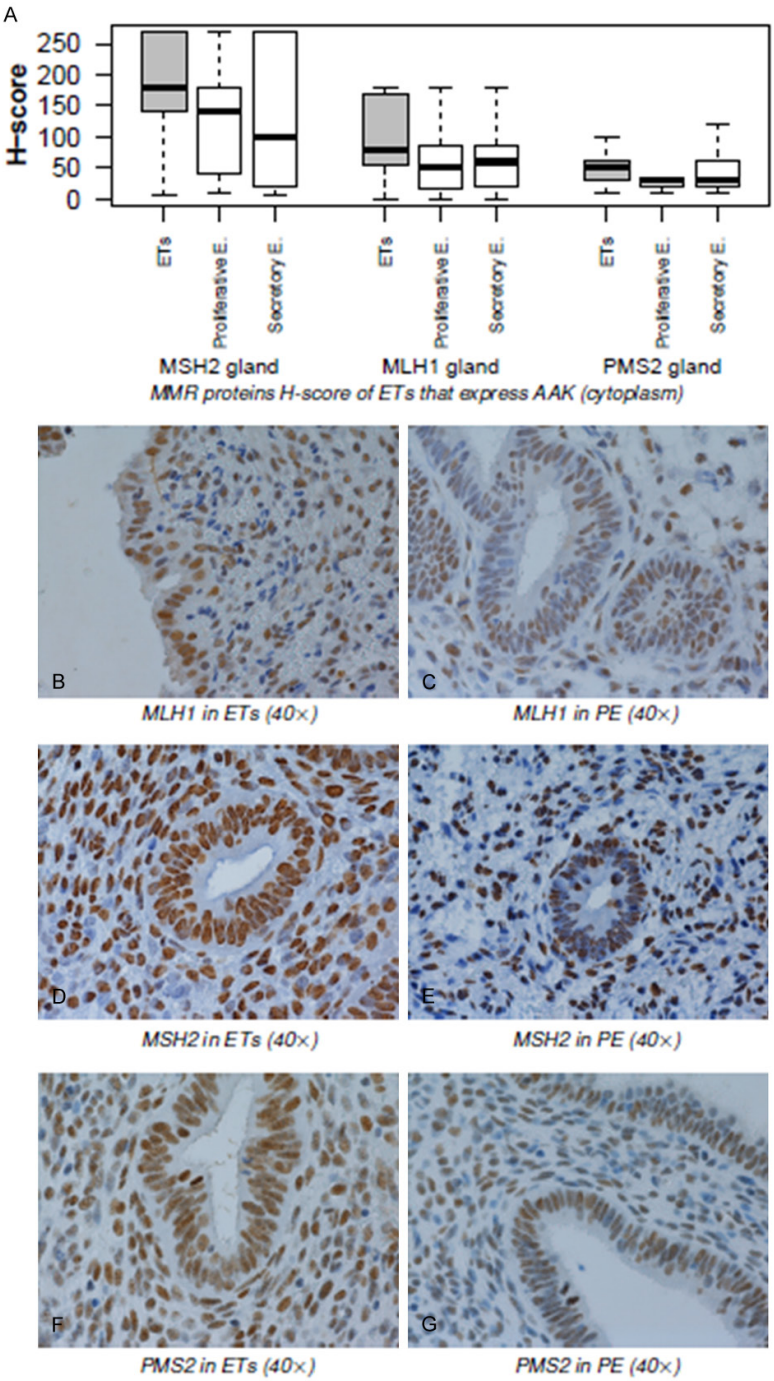

Figure 2. A: H-score values of MMR complex proteins in a selected sub-gruop of ETs expressing aurora A kinase at higher levels than eutopic endometrium. B-G: show immunostaining of MLH1, MSH2, and PMS2 in ovarian ETs and in proliferative endometrium among a selected sub-gruop of ETs expressing high glandular cytoplasmic aurora A kinase.

with no recurrences [32]. Moreover, high MLH1-expression has been proposed as a marker for the prediction of high risk of distant metastases in colon cancer [33].

The exact role of MMR protein elevation is still speculative but may involve aberrant and improper binding of these proteins, leading to inactivation of the MMR system [34]. This could result from uncommon mutations in the MMR system not leading to an alteration of protein expression, but of protein function [33]. In addition to elevated AAK values, as previously discovered in our study [21], MMR protein elevation may be a predictor of biochemical recurrence of endometriotic lesions following surgery. This finding may identify a potential new marker for recurrent endometriosis and uncover a potentially novel pathway for targeted therapeutics.

In our analysis we demonstrated that MSH2 H-score values in glandular and stromal components of ETs were higher than any other MMR proteins. In addition, MSH2 was significantly correlated to ki-67 and was significantly more expressed in ETs than eutopic endometrium suggesting a possible role of MSH2 in endometriosis proliferation. In support of this hypothesis, newer literature suggests that chronic inflammation may alter the MMR system [6, 11, 14]. Using immunohistochemistry, Floer and colleagues demonstrated an isolated increased expression of MSH2, but no MSI, in tissues and myofibroblasts affected by CD [11]. In fact, MSH2 modulates both cell cycle regulation and apoptosis [35].

Moreover, it is known that in ETs the oxidative environment, characteristic of the inflammatory reaction, promotes cellular proliferation [36]. In our study we found MSH2 positivity to be sig-

nificantly correlated to Ki-67 positivity in both stromal and glandular components of ETs. Also in CD affected specimens, increased expression of MSH2 was associated with increased cell proliferation [11]. These similar results let us assume a role of MSH2 alike that hypothesized for CD. In the presence of oxidative stress, MSH2 could play a role in promoting cellular proliferation.

This is the first study demonstrating increased expression of MSH2 and a positive correlation between MSH2 and Ki-67 levels in endometriotic lesions. Increased expression of MSH2 in endometriotic cells appeared to be linked to their increased proliferative capacity, proposing a new pathophysiological mechanism underlying cell proliferation and scar formation in ETs.

Conclusions

Despite two weaknesses -the retrospective nature of the study and the use of immunohistochemical analysis alone- a major strength of this study is the great number of cases analyzed. Our purposes, hypothesis and conclusions were based on data obtained from this TMA study; future in vitro and in vivo studies are planned to validate our hypothesis.

In our study, we found loss of expression of MMR proteins in the stromal component of ETs, using immunohistochemistry. The group of ETs with glandular cytoplasmic staining for AAK, previously shown to be associated with higher recurrence rates [21], had higher MMR protein expression suggesting an increased activity of this system in the subset of endometriotic lesions with high recurrence rate. Furthermore, higher MSH2 expression in ETs than in eutopic endometrium suggests a novel function of MSH2 in promoting cellular proliferation in the presence of oxidative stress.

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Disclosure of conflict of interest

None.

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