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Maspin expression, subcellular localization and clinicopathological correlation in endometrial hyperplasia and endometrial adenocarcinoma

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Summary. Maspin expression in endometrial hyperplasia and endometrial endometrioid adenocarcinomas was assessed and its correlation with p53 and Ki-67 expressions and clinical outcome, as well as its potential to distinguish typical from atypical endometrial hyperplasia, were assessed in this study. Histological sections from 114 cases of endometrial endometrioid adenocarcinoma, 75 cases of endometrial hyperplasia (typical and atypical), and 23 normal endometrial tissue samples were examined. The most representative hematoxylin-eosin slides were selected and 2-3 micron-thick sections were cut for immunohistochemical staining with maspin, p53, and Ki-67 antibodies.

While there was no maspin expression in normal endometrial cells, it was present in 14.5% of the patients with endometrial hyperplasia without atypia. Staining for maspin was positive in atypical hyperplasia and endometrial adenocarcinoma in, respectively, 45% and 49.1% of the cases studied. No statistically significant correlations were found between maspin and Ki-67 antibodies or p53 expression.

Our findings showed that maspin expression, which generally correlates with a less aggressive behavior, is significantly higher in atypical hyperplasia and in endometrial endometrioid adenocarcinoma. Maspin positivity in endometrial hyperplasia could be used to identify pseudo-atypical hyperplasia and could be considered a potentially useful prognostic parameter in those cases in which adenocarcinomas are well differentiated.

Key words: Endometrium, Tumor markers, Maspin, Hyperplasia, Adenocarcinoma

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Introduction

With an overall 5-year survival rate of 80%, endometrial adenocarcinoma is the most common gynecologic neoplasia in industrialized countries (Jemal et al., 2011). Typically evolving from hyperplastic endometrium, endometrioid adenocarcinoma represents 80% of endometrial cancers. Histotype, histological grading, myometrial infiltration, lymphovascular invasion, extrauterine and lymph node metastases and the International Federation of Obstetricians and Gynaecologists (FIGO) stage are all well established prognostic factors of endometrial cancer (ACOG 2005; Pecorelli, 2009). The molecular mechanisms involved during progression from preneoplastic lesions to cancer are, nevertheless, still not entirely understood (Doll et al., 2008).

Maspin, (Mammary serin protease inhibitor), a 42kDa protein, belongs to the serpin or serine protease inhibitor superfamily. In 1994, maspin, a class II tumor suppressor gene located on chromosome 18q21.3, was identified in epithelial cells of mammary glands (Zou et al., 1994). The protein was shown to inhibit cellular migration, tissue invasion, metastasis, and angiogenesis and to increase cell adhesion (Shi et al., 2001; Schaefer and Zhang, 2003; Sheng, 2004; Bailey et al., 2006; Li

and Ming, 2010). Underexpression of maspin in prostate, mammary gland, bladder, renal, ampullary, head/neck and urothelial cancers has been associated with poor prognosis (Xia et al., 2000; Maas et al., 2001a; Machtens et al., 2001; Marioni et al., 2005, 2010; Blandamura et al., 2006, 2007, 2008). Its overexpression has recently been correlated to worse prognosis, relapse, and metastasis in other tumors such as adenocarcinomas in the colon, pancreas, stomach and ovary (Maas et al., 2001b; Song et al., 2002; Sood et al., 2002; Terashima et al., 2005). As maspin seems to act as an oncogene in those tumors, it has been hypothesized that it has tissue-specific tumorigenic effects.

While maspin has been identified in neoplastic cells of endometrial endometrioid adenocarcinoma by a few studies, the effects of its expression, subcellular localization and level in endometrial cancer remain somewhat equivocal (Murai et al., 2006; Li et al.,2007; Tsuji et al., 2007; Torres et al., 2011). No reports have as yet been published concerning maspin expression in endometrial hyperplasia. The aim of this study was to assess maspin expression in preneoplastic lesions of the endometrium and in endometrial adenocarcinoma and to evaluate its correlation with the Ki-67 proliferative index, p53, a tumor suppressor gene, and patients' outcomes.

Materials and methods

Two hundred and twelve cases referred to the Department of Women's and Children's Health, Obstetrics and Gynecology Clinic of the University of Padova Medical Center were studied retrospectively. Histological sections from 114 patients who underwent surgery for endometrial endometrioid adenocarcinoma, 75 women with histological diagnosis of endometrial hyperplasia (typical and atypical), and 23 women with normal proliferative phase endometrium were collected over a 4 year period (2002-2006).

The patients with endometrial cancer were treated surgically in accordance with the FIGO treatment recommendations for gynecologic malignancy (FIGO Committee on Gynecologic Oncology, 2000). The surgical specimens were examined and staged on the basis of pathological evidence in accordance with the 2009 Revised FIGO staging for carcinoma of the vulva, cervix and endometrium (Pecorelli, 2009); the histologic degree of differentiation of all the neoplastic lesions was also recorded. The 114 (53.8%) women with endometrial adenocarcinoma were staged in accordance with the 2009 FIGO staging system as: IA in 73 (64%) cases, IB in 31 (27.2%) and II in 10 (8.8%) cases. The histological classification of 49 (43%) of the lesions was grade 1 (Fig. 1), 57 (50%) was grade 2, and of 8 (7%) was grade 3. Twenty of the 75 women (26.6%) with endometrial hyperplasia had atypical and 55 (73.3%) simple or complex hyperplasia without atypia.

The mean age at surgery was 50.9±12.83 years in the women with normal endometrium, 54.6±12.06 years in those with endometrial hyperplasia without atypia, 56.3±8.6 years in those with endometrial atypical hyperplasia and 64.8±10.27 years in those with endometrial adenocarcinoma. Thirteen out of the 23 (56.5%) with normal endometrium, 33 out of the 55 (60%) with hyperplasia without atypia, 14 out of the 20 (70%) with atypical hyperplasia, and 103 out of the 114 (90.3%) women with endometrial endometrioid adenocarcinoma were postmenopausal.

Clinical outcome parameters, collected during the follow-up of the patients with carcinoma, were defined as follows:

- Overall survival (OS)=patients alive at the time of the latest database update.
- Event-free survival (EFS)=patients alive and free of recurrence.
- Cause-specific survival (CSS)=patients whose deaths were due to known metastatic diseases or to endometrial cancer found at autopsy. Those cases in which the cause of death was unclear were attributed to endometrial cancer whenever there were clinically evident signs of that cancer at the time of death.
- Recurrence rate (RR)=failures due to local or distant metastasis.The 5-year EFS, OS, CSS and RR were, respectively, 95.3%, 93.9%, 28.6% and 6.1% in the patients with endometrial carcinoma.

The material analyzed for all other non-neoplastic disorders (endometrial hyperplasia or normal endometrium) consisted of histological specimens collected by simple hysterectomy or endometrial biopsies performed during an outpatient diagnostic hysteroscopy. The most representative hematoxylin-eosin slides were selected and sections for immunohistochemistry were cut from the corresponding paraffin blocks.

Informed written consent was obtained from all the patients involved in this study.

Immunohistochemistry

Three micron-thick sections were cut for immunohistochemistry from paraffin tissue blocks. The antibodies used were: Maspin (mouse monoclonal antibody, clone EAW24, dilution 1:100; Novocastra Laboratories ltd, Newcastle upon Tyne, UK); p53 (mouse monoclonal antibody, clone DO-7; dilution 1:350; Dako, Glostrup, Denmark); Ki-67 (mouse monoclonal antibody, clone MIB-1, dilution 1:100; Dako).

The Automate Staining System (Bond-maX, Leica, Newcastle Upon Tyne, UK) was used. Staining was visualized using the Bond Polymer Refine Detection Kit (Leica) according to the manufacturer's protocols. The colour was developed using 3.3'-diaminobenzidine (DAB) and the slides were counterstained with Mayer's haematoxylin. All immunostained slides were analyzed and blindly scored by two pathologists who were unaware of patients' clinical data. Cells were considered Ki-67 positive or p53 positive when they demonstrated

strong, exclusive nuclear labeling. The percentage of immunopositive cells was calculated for the three antibodies (maspin, Ki-67 and p53) by counting at least 500 total cells in at least 10 high power fields (magnification 400x). Based on the percentage of positivity present in all of the positive cells, maspin's subcellular expression (cytoplasmic or nuclear) was also evaluated (Figs. 1, 2).

Statistical analysis

Data are expressed as frequencies (percentages) for categorical variables and as means ± standard deviation for continuous ones. Comparison between categorical variables (maspin) were tested with Chi square test or Fisher's exact test when necessary. Comparisons between continuous data (KI-67 and p53) were tested with Kruskal Wallis analysis of variance. The two-sample t-test with correction for unequal variances was applied after log-transforming the percentages. The two-sided P value was provided with 95% Confidence Interval (CI) for the differences between proportions.

A<0.05 p-value was considered statistically significant.

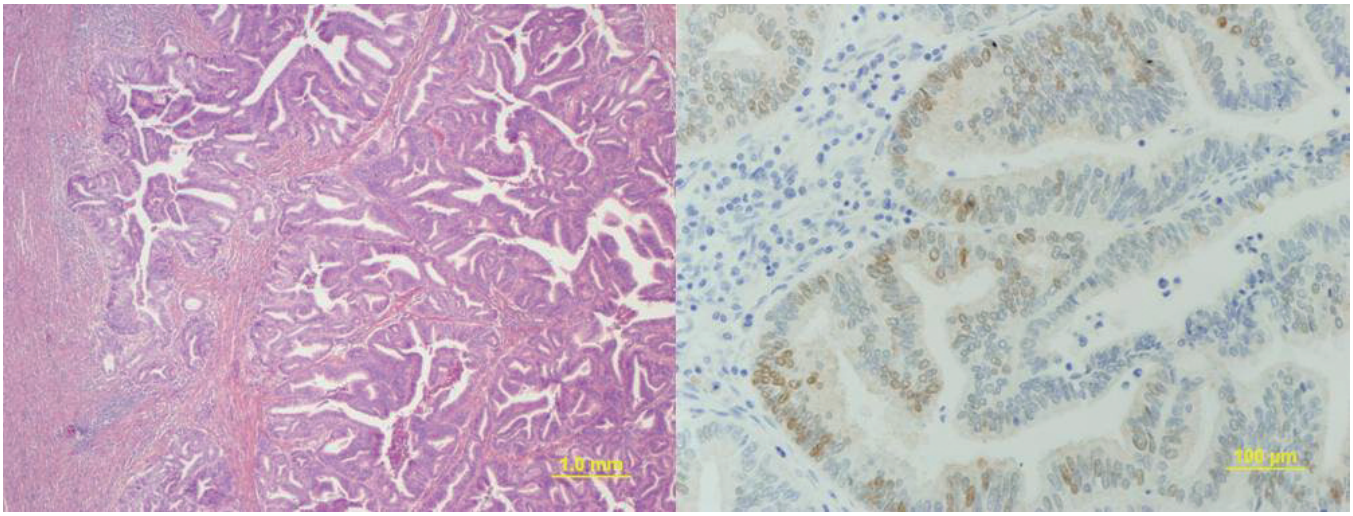

Fig 1. Endometrioid adenocarcinoma, grade I. The tumor forms well-differentiated glands with low cytologic atypia (left- Hematoxylin & Eosin, x 25). Nuclear immunoreactivity for maspin (right- Maspin immunostaining, x 200).

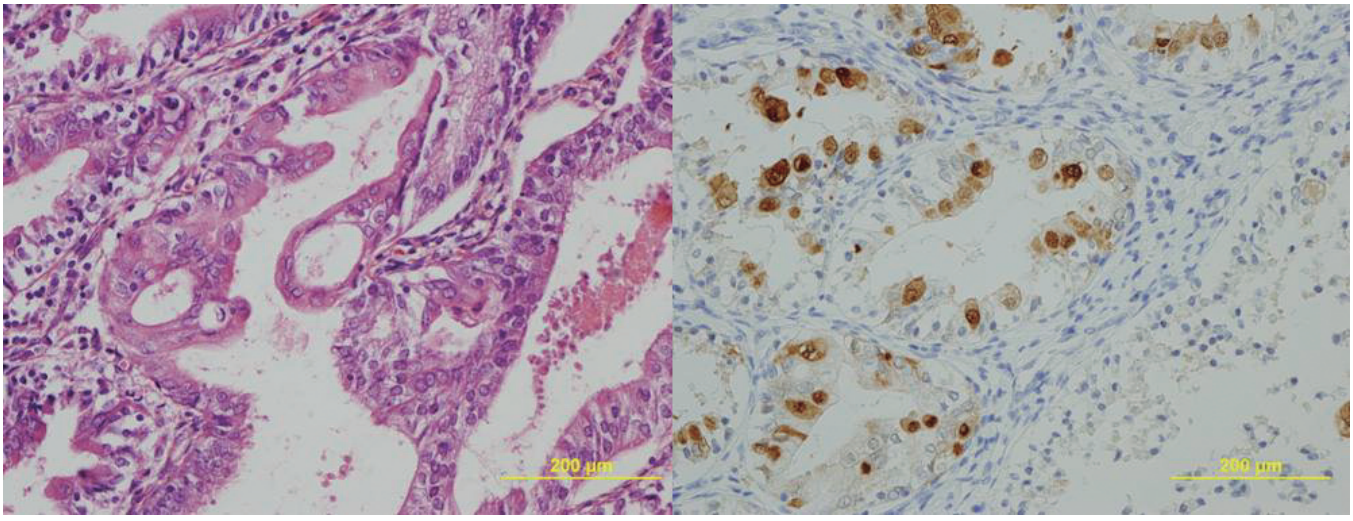

Fig. 2. Endometrioid adenocarcinoma, grade II. The tumor forms irregular glands with moderate cytologic atypia (left- Hematoxylin & Eosin, x 200). Nuclear and cytoplasmic maspin immunoreactivity (right- Maspin immunostaining, x 200).

Statistical analyses were carried out using the Statistical Package for Social Sciences 18.0 (SPSS, Chicago, IL, USA).

Results

Maspin expression was not detected in the 23 healthy endometrial tissue samples. Positive maspin immunostaining was detected, respectively, in 45% and 49.1% of the hyperplasia and endometrial adenocarcinoma samples (Table 1).

Nuclear-localized maspin was detected in only 10 (17.9%) of the endometrial endometrioid adenocarcinoma samples, all classified as FIGO stage 1A. Nuclear immunostaining was absent in higher FIGO stages. Six (60%) of the patients with positive nuclear localization had grade 1 and 4 (40%) had grade 2 .

Both typical and atypical hyperplasia showed exclusive cytoplasmic staining.

Squamous metaplasia was detected in 20 (17.5%) of the endometrial adenocarcinoma patients; maspin immunostaining was also positive in 16 of these (80%).

Statistical analysis

Expression of maspin in normal endometrial tissue, in hyperplasia with and without atypia, and in endometrial carcinoma is outlined in Table 1.

Subcellular nuclear maspin expression was significantly correlated to lower FIGO stages (p=0.0047;CI= -0.48, -0.07).

There was a statistically significant correlation between maspin expression and squamous metaplasia in the adenocarcinomas (p=0.0038; CI= -0.42, -0.05).The 5-year EFS, OS, CSS and RR in the patients with endometrial carcinoma were not correlated to maspin expression.

Although an inverse trend was noted, the correlation between maspin expression and the FIGO stage was not statistically significant. Threre was a similar, non-significant inverse trend in the correlation between maspin expression and tumor grade. Ki-67 was not correlated to the FIGO stage nor was there any correlation to tumor grade. The differences in p53 expression between stages IA and II and between stages IB and II were, instead, statistically significant. No statistically significant correlations were found between maspin and KI-67 or p53 expressions (Table 2).

Discussion

This is the first study aiming to evaluate maspin expression in typical and atypical forms of endometrial hyperplasia. Being able to formulate accurate diagnoses of these types of endometrial proliferative lesions has important clinical implications: while the risk of

Table 1. Expression of maspin in normal endometrial tissue, in endometrial hyperplasia with and without atypia, and in endometrial cancer.

	MASPIN		TOTAL
	ABSENCE	PRESENCE	
Normal endometrium	23 (100%)	-	23
Endometrial hyperplasia without atypia	47 (85.5%)	8 (14.5%)	55
Endometrial hyperplasia with atypia	11 (55%)	9 (45%)	20
Endometrial adenocarcinoma	58 (50.9%)	56 (49.1%)	114
Total	139 (65.6%)	73 (34.4%)	212

Normal endometrium vs endometrial hyperplasia without atypia p=0.097 (CI= - 0.22, 0.23); Normal endometrium vs atypical endometrial hyperplasia p<0.001 (CI= - 0.63,- 0.17); Normal endometrium vs endometrial adenocarcinoma p<0.001 (CI=- 0.57, - 0.32); Endometrial hyperplasia without atypia vs atypical endometrial hyperplasia p=0.0053 (CI= - 0.54, - 0.06); Endometrial hyperplasia without atypia vs endometrial adenocarcinoma p<0.001 (CI= -0.47, - 0.21); Atypical endometrial hyperplasia vs endometrial adenocarcinoma p=0.73 (CI= -0.27, 0.19)

Table 2. Expression of maspin, Ki 67, and p53, as well as tumor grade and staging classification according to the 2009 FIGO guidelines in the endometrial cancer patients studied.

	MASPIN (%)	KI-67	P53	Comparison between groups	MASPIN P value (CI)	KI67 P value (CI)	P53 P value (CI)
FIGO STAGE							
I A	41 (56.2%)	13.6±16.5	13.3±21.5	Stage IA vs IB	0.10 (-0.031; 0.38)	0.49 (-10.07; 4.87)	0.73 (-6.92; 9.72)
IB	12 (38.7%)	16.2±19.9	11.9±13.9	Stage IA vs II	0.12 (-0.04; 0.56)	0.57 (-13.72;7.72)	<0.001 (-57.61;-23.78)
II	3 (30%)	16.6±11	54±45	Stage IB vs II	0.61 (-0.24; 0.41)	0.95 (-13.81;13)	>0.001 (-60.35;-23.84)
GRADING							
G1	26 (53.1%)	11.6±15.9	6.7±7.1	G1 vs G2	0.68 (-0.15; 0.23)	0.10 (-11.76; 1.16)	0.002 (-18.61;-4.18)
G2	28 (49.1%)	16.9±17.4	18.1±24.6	G1 vs G3	0.14 (-0.05; 0.61)	0.21 (-20.61;4.81)	<0.001 (-32.24;-10.35)
G3	2 (15%)	19.5±21	28±35.6	G2 vs G3	0.20 (-0.08;0.56)	0.70 (-16.05;10.85)	0.31 (-29.55;9.75)

progression to malignancy is rather low in the typical hyperplasia form without atypia (1-3%), it is much higher (8-29%) in atypical hyperplasia (Kurman et al., 1985). Many studies have, nevertheless, shown high inter and intraobserver variability with regard to the histopathological diagnosis of atypical hyperplasia, which is based exclusively on non-specific, qualitatively-evaluated morphological features (Skov et al., 1997; Kendall et al., 1998; Zaino et al., 2006). The differences in maspin expression between hyperplasia without atypia and normal endometrium were not significant in our patients, but maspin was significantly higher in atypical hyperplasia. Since endometrial adenocarcinoma is now the most common gynecologic cancer and is often diagnosed in the early stages of the disease, efforts are being made to offer patients more numerous and more minimally invasive treatments, including a laparoscopic approach in the event of a demolitive intervention (Litta et al., 2003) and hysteroscopic resection of the lesion in the event of focal atypical hyperplasia (Litta et al., 2013). Prognostic factors that can help to predict tumor behavior are thus important if conservative treatment measures are to be considered.

The maspin gene was initially identified in human mammary epithelium encoding a protein that supposedly carries out tumor suppressor activities. Several studies have confirmed this hypothesis in the light of maspin's role in inhibiting cellular motility and in enhancing cellular adhesion to the extracellular matrix (Abraham et al.,2003; Bailey et al., 2006; Li and Ming, 2010). Paradoxically, according to other studies, maspin appears to function as an oncogene in some organs (Umekita et al., 2002; Blandamura et al., 2008; Marioni et al., 2010). Maspin overexpression has been found, in fact, to be linked to worse prognosis in ovarian cancer (Sood et al., 2002; Secord et al., 2011), while decreased expression has been found to be linked to disease progression in cervical cancer (Xu et al., 2005).

Only a few studies have focused on maspin expression in endometrial carcinoma and some of the findings reported are considered equivocal (Murai et al., 2006; Li et al., 2007; Tsuji et al., 2007; Torres et al., 2011).

Our findings confirm previous observations that maspin is expressed in atypical hyperplasia and in endometrioid adenocarcinoma but not in normal endometrium. Atypical hyperplasia and lower grade endometrioid adenocarcinoma could hypothetically induce maspin to block/limit tumor aggressiveness.

Murai et al. (2006) and Li et al. (2007) described an overexpression of maspin in human endometrial cancer. While Tsuji et al. (2007) reported a correlation between upregulated maspin expression and the depth of myometrial invasion + the FIGO stage + nodal metastases, they did not find a significant correlation between maspin and tumor grade. Although our results are not statistically significant, they identified a trend of higher maspin expressions in low-grade, low-stage tumors.

Two recent studies assessed maspin expression in adenocarcinomas containing areas of squamous differentiation: one including and evaluating the squamous areas (Murai et al., 2006), the other excluding them (Tsuji et al., 2007). We chose to use the latter approach to define maspin's prognostic potential in endometrial adenocarcinoma.

In the present study, 80% of the cases of squamous differentiation in endometrial carcinoma also showed positive maspin immunoreactivity. Squamous differentiation in endometrial cancer was found to be associated with increased survival according to a study by the Gynecologic Oncology Group (Zaino et al., 1991). Previously published findings (Murai et al., 2006) as well as our own confirm that maspin positivity in endometrial carcinoma with squamous differentiation is associated with increased probability of survival. The subcellular localization of maspin seems to influence the protein's effect on cancer. According to some studies, maspin exerts its role in some cancer hystotypes at the nuclear level, while the cytoplasmic component remains inactive. Thus, the nuclear localization of maspin appears to be crucial for its tumor suppressor action (Marioni et al., 2005). Some studies have reported that maspin's nuclear localization is a positive prognostic factor in tumors of some organs (Marioni et al., 2005, 2010, 2011). In agreement with previously reported findings concerning endometrial cancer (Li et al., 2007), our results found nuclear localization of maspin only in low-stage endometrial cancer. Although not significant, the decrease in nuclear expression noted with increasing cancer grade supports the hypothesis that maspin's nuclear expression is associated with a less aggressive behavior. A tumor suppressor gene, p53 is thought to be the regulator of maspin expression in several solid tumors, including breast, prostate and ovarian cancer (Zou et al., 2000; Machtens et al., 2001; Zhang and Zhang, 2002); Ki-67 is, instead, a well known protein that is strictly associated with cellular proliferation. Although the prognostic role of these markers is still unclear, endometrial tumors with elevated p53 and/or Ki-67 expressions usually show aggressive clinicopathological features (Sherman et al., 1995; Lee et al., 2010).

We noted higher levels of p53 and Ki 67 in endometrial carcinoma and, in accordance with other studies (Ferrandina et al., 2005; Zhu et al., 2009), their expressions seem to be correlated with increasing tumor grade and FIGO stage, but no statistically significant correlation emerged with regard to maspin expression. Maspin and the other two proteins seem, nevertheless, to be inversely correlated in view of the fact that with increasing tumor grade and stage the former appears to be downregulated while the latter upregulated.

In conclusion, immunohistochemical evaluation of maspin could aid pathologists in distinguishing atypical hyperplasia from hyperplasia without atypia and this could be particularly useful for the diagnostic and

clinical management of young patients.

Maspin may have a valuable prognostic value, in particular, in low-grade, low-stage endometrial adenocarcinomas.

Further studies focusing on the entire spectrum of endometrial proliferative lesions are warranted to define the protein's exact biological, diagnostic, and prognostic role.

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