MMAC tumor supressor gene expression in ovarian endometriosis and ovarian adenocarcinoma

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Summary

Objective: The aim of this study was to investigate the role of MMAC1 protein in the relationship between ovarian endometriosis and clear cell and endometrioid-type ovarian adenocarcinomas.

Methods: A total of 63 subjects who underwent surgery for a pelvic tumoral mass, 30 of whom were diagnosed with grade 1 to 3 ovarian adenocarcinoma and 33 of whom were diagnosed with grade 1 to 4 endometriosis during histopathological examination were included in this study. The mean age for subjects with ovarian endometrioid type adenocarcinoma was 51.8 ± 12.4, whereas the mean age for subjects with ovarian clear cell type adenocarcinoma was 59.5 ± 13.7. Ovarian carcinomas were graded in accordance with the FIGO 1989 grading system. The mean age for subjects with endometriosis was 37 ± 11.9. New sections were obtained from paraffin blocks in the archives of Ege University, School of Medicine, Department of Pathology onto lysinated slides and immunohistochemical staining by using mouse monoclonal antibody (MMAC1, 28H6 clone, Novocastra, UK) as MMAC antibody was applied in order to determine MMAC1 protein. Brown staining on the nucleus was considered as positive immunoreactivity. Immunoreactive staining was evaluated as percentage staining over the whole preparative.

Results: Of the 63 subjects included in the immunohistochemical study, ovarian endometrioid adenocarcinoma was identified in 18 subjects, while 12 subjects were diagnosed with ovarian clear cell adenocarcinoma and 33 subjects with ovarian endometriosis. No significant relationships were observed between age and MMAC immune staining in the ovarian endometrioid adenocarcinoma ($r = -0.41$, $p = 0.08$) and ovarian endometriosis ($r = 0.12$, $p = 0.50$) groups, whereas a significant relationship was observed in the ovarian clear cell adenocarcinoma group ($r = 0.631$, $p = 0.02$). No significant relationships were observed between CA125 levels and MMAC immune staining in the ovarian endometrioide adenocarcinoma ($r = 0.056$, $p = 0.82$), ovarian endometriosis ($r = 0.21$, $p = 0.36$) and ovarian clear cell adenocarcinoma ($r=0.363$, $p=0.24$) groups. No correlations were observed between endometriosis stages and the MMAC immune staining ($r = -0.17$, $p = 0.92$).

There was no correlation between mean diameter of endometrioma and MMAC immune staining ($r= -0.230$, $p = 198$). Mean endometrioma diameter was 5.7 ± 3.5 (1-15.5). No correlations were detected between MMAC immune staining and ovarian endometrioide adenocarcinoma or ovarian clear cell adenocarcinoma stage ($r = -0.22$, $p = 0.37$; $r = 0.44$, $p = 0.14$, respectively).

No significant relationships with respect to MMAC immune staining were detected between the endometriosis and ovarian clear cell adenocarcinoma groups ($p = 0.05$) and between the ovarian clear cell adenocarcinoma and ovarian endometrioid adenocarcinoma groups ($p = 0.27$). A significant relationship with respect to MMAC immune staining was observed between ovarian endometrioide adenocarcinoma and endometriosis groups ($p = 0.001$).

Conclusion: Immunohistochemical determination of MMAC defective protein expressions could be considered for utilization as a new, simple and useful technique in determination of endometriosis patients with increased risk of malignant transformation, patients where early surgical treatment would be necessary and patients that should be subjected to follow-up controls with a higher frequency.

Key words: Endometriosis; MMAC protein; Ovarian adenocarcinoma.

Introduction

Ovarian endometriosis is one of the disorders frequently observed during female life which is commonly associated with complaints of infertility and pelvic pain [1]. Hereditary, environmental, immunological, angiogenical and endocrinal factors act together in the etiology of endometriosis. In treatment of patients with advanced pelvic endometriosis who wish to have child, it is recommended that fertility be protected through conservative surgical treatments or that sound ovarian tissues be protected for possible assisted reproductive techniques. Although endometriosis has been considered as a 'benign' disorder, it has been suggested by recent studies that there could be a 'neoplastic' process in endometriosis [2, 3]. According to this hypothesis, there is a risk that endomeriosis could transform into ovarian clear cell and endometrioid adenocarcinomas, and there are some molecular similarities between ovarian cancer and endometriosis [4]. MMAC (mutated in multiple advanced cancers) is a tumor suppressor gene, which is also known as PTEN (phosphatase and tensine homologous exposed to deletion from the tenth chromosome). Being a product of the MMAC gene, MMAC1 is a protein whose cellular functions have recently been demonstrated. MMAC1 protein regulates the relation between its host cell and other cells, and it plays a role in critical apoptosis tracts [5-8].

The aim of this study was to demonstrate the role of MMAC1 protein in the relationship between ovarian endometriosis with clear cell and endometrioid-type ovarian adenocarcinomas.

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Materials and Methods

A total of 63 subjects who underwent surgery for a pelvic tumoral mass, 30 of whom were diagnosed with grade 1 to 3 ovarian adenocarcinoma and 33 of whom were diagnosed with grade 1 to 4 endometriosis during histopathological examination were included in this study at Ege University, School of Medicine, Department of Obstetrics and Gynecology.

The mean age of subjects with ovarian endometrioid adenocarcinoma was 51.8 ± 12.4, while the mean age of subjects with ovarian clear cell adenocarcinoma was 59.5 ± 13.7. Ovarian cancers were graded in accordance with the FIGO 1988 gradation system [9].

Eighteen subjects with endometriosis were referred due to pelvic pain, while seven subjects were referred for irregular menstrual patterns, four subjects for primary infertility and four subjects for follow-up controls. Mean age of the subjects with endometriosis was 37 ± 11.9.

Diameter of the endometrioma was calculated as the mean of dimensions measured in two different axes. Taking intraoperative inspection into consideration, endometriosis subjects were graded in accordance with the classification of the American Fertility Society [10].

New cross-sections from paraffin blocks obtained from the archives of Ege University School of Medicine, Department of Pathology were prepared on lysine lams. Deparaffinized cross-sections were rehydrated with distilled water. The cross-sections were immersed in 0.5% hydrogen peroxide/methanol solution for 10 min and then rinsed under running water. In order to reveal antigenic characteristics of the tissues, they were heated under pressure in a citric acid buffer solution for 25 min. The specimens were treated with primer antibodies following incubation in normal goat serum for 30 min. Mouse monoclonal antibody (MMAC1, 28H6 clone, Novocastra, UK) was used as a MMAC antibody. Brown staining on the nucleus was considered as positive immunoreactivity. The immunoreactive staining was evaluated as percentage staining over the whole preparative.

SPSS 11.0 statistics software was used for the analysis of study data. The data were expressed in the form of mean ± standard deviation with $p < 0.05$ considered as statistically significant. The endometriosis group was compared with ovarian endometrioid and clear cell adenocarcinoma types, with respect to MMAC1 immunoreactivity by using non-parametric analysis of variance, Kruskal Wallis and post-hoc Mann-Whitney U tests.

Results

A total of 63 subjects were included in the immunohistochemical study. Eighteen subjects were considered as ovarian endometrioid-type adenocarcinoma, while 12 subjects were considered as ovarian clear cell-type adenocarcinoma and 33 subjects as ovarian endometriosis. Demographic data of the groups are presented in Table 1.

Correlations between age and MMAC immune staining were investigated but no significant relationship was found in the ovarian endometrioid adenocarcinoma (r = -0.41, p = 0.08) and ovarian endometriosis groups (r = 0.12, p = 0.50). However a significant relationship was found in the ovarian clear cell adenocarcinoma group (r = 0.631, p = 0.02) (Figure 1). Correlations between MMAC immune staining and serum CA125 levels were also investigated and no significant relationship was found in the ovarian endometrioid adenocarcinoma (r = 0.056, p = 0.82), ovarian endometriosis (r = 0.21, p = 0.36) and ovarian clear cell adenocarcinoma groups (r = 0.363, p = 0.24). In addition relationships between MMAC immune staining and grades of endometriosis were investigated and no correlation was observed between grades of endometriosis and MMAC immune staining (r = -0.17, p = 0.92) (Figure 2).

No correlations were detected between mean endometriosis diameter and MMAC immune staining (r = -0.230, p = 198). Mean diameter for endometrioma was

Table 1. — *Demographic data of subject groups.*

	Ovarian endometrioid adenocarcinoma (no. = 18)	Ovarian clear cell adenocarcinoma (no. = 12)	Ovarian endometriosis (no. = 33)
Age	51.8 ± 12.4	59.5 ± 13.7	37.4 ± 11.9
Parturition	1.8 ± 1.4	2.4 ± 2.1	1 ± 1.1
Abortion	1.2 ± 1.6	0.2 ± 0.4	0.6 ± 0.9
CA125	764 ± 1,324	543 ± 627	82 ± 98

Table 2. — *Relationship between grades of ovarian endometrioid adenocarcinoma and MMAC immune staining.*

Grade	No.	Immune staining
1	10	94 ± 10
2	1	90
3	7	89 ± 10

Table 3. — *Relationship between grades of ovarian clear cell adenocarcinoma and MMAC immune staining.*

Grade	No.	Immune staining
1	6	78 ± 20
2	1	70
3	5	94 ± 8

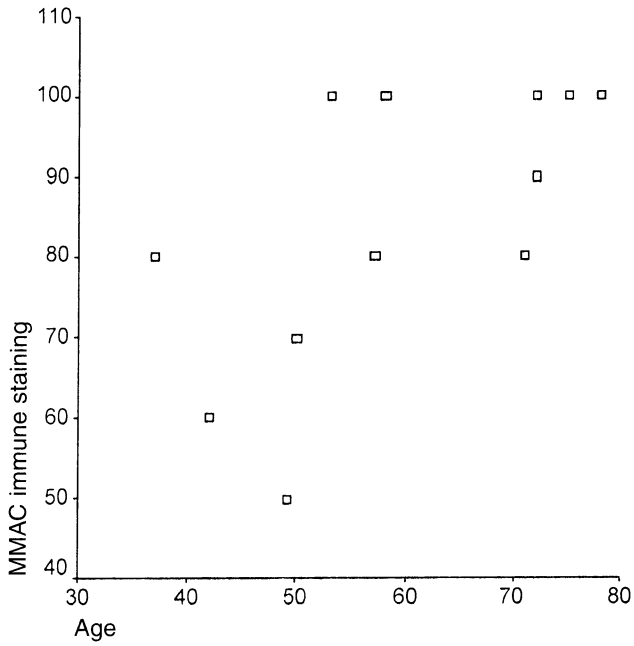

Figure 1. — Relationship between MMAC immune staining percentage and age in ovarian clear cell adenocarcinoma.

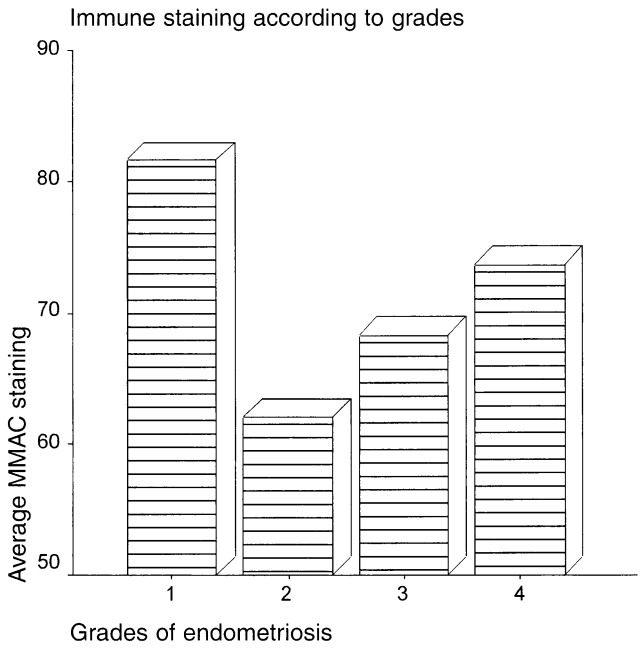

Figure 2. — Relationship between MMAC immune staining percentage and age in ovarian clear cell adenocarcinoma.

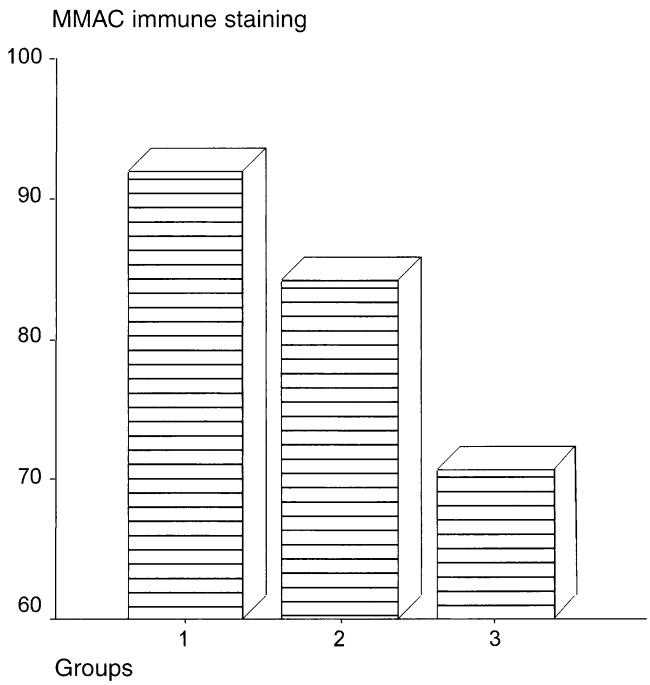

Figure 3. — MMAC immune staining according to groups.

5.7 ± 3.5. Two subjects with endometrioma diameters of 1 cm were operated on due to acute abdomen. No correlations were observed between grades of ovarian endometrioid-type adenocarcinoma and MMAC immune staining (r = -0.22, p = 0.37) (Table 2). There was also no correlation between grades of ovarian clear cell-type adenocarcinoma and MMAC immune staining (r = 0.44, p = 0.14) (Table 3).

Correlations among groups with respect to MMAC immune staining were investigated and no significant relationship was observed between the endometriosis and ovarian clear cell adenocarcinoma groups (p = 0.05) or the ovarian clear cell adenocarcinoma and ovarian endometrioid adenocarcinoma groups (p = 0.27). However, a significant relationship was found with respect to MMAC immune staining between the ovarian endometrioid adenocarcinoma and endometriosis groups (p = 0.001) (Figure 3).

Discussion

In 1925, Sampson was investigating whether endometriotic lesions might transform into tumors [11]. The hypothesis was established over the findings that endometriosis and carcinoma often existed together and particularly, endometrioid and clear cell-type carcinomas were present within the same specimen.

Although endometriosis could be clinically classified in accordance with various systems, it is still not a uniform disorder. Recently, the most popular classification system is the one of the American Fertility Society, which is based on weight of the disorder [10]. According to this classification, grades 3-4 are the levels in which the reproductive system is most severely affected by the disorder. All these gradations in which endometriosis is considered as a benign disorder, are partially related to the experience of the operator and are focused on assessment of the potential future effects of this disorder on fertility [10].

It has been reported by many clinical and histological studies (consisting of molecular data) that endometriosis could be a precursor for the sporadic endometrioid and clear cell-type carcinomas, which develop from an extrauterine focal point. Taking these results into consideration, it would not be possible to characterize endometriosis as a benign disorder. According to the estimations, ovarian endometriosis can transform into carcinoma in one out of 100 females [12]. In a study where PTEN expression was investigated in 46 subjects with grade 3-4 endometriosis, it was suggested that PTEN expression played a role in the malignant transformation of endometriosis [13]. These findings could be used as neoplastic transformation markers in subjects with aggressive endometriosis and increased tumor markers. Therefore, the most significant achievement would be the determination of a small subgroup of endometriosis, which has a high risk of malign transformation. It supports the idea that a more careful approach to these data and analysis of prognostic symptoms would be necessary if the patient has advanced levels of endometriosis, significant change in serum tumor marker levels, severe pain and unfavorable radiodiagnostic findings prior to surgical interventions.

It has been reported in numerous studies that MMAC mutations have a role in endometrial carcinoma, development of endometrial cysts and ovarian endometrioid and clear cell-type carcinomas [14]. It has been stated that MMAC mutations exist in 20.6% of endometrial cysts, 20% of ovarian endometrioid-type carcinomas and

8.3% of ovarian clear cell-type carcinomas [15]. The majority of the tumors in which MMAC mutations have been detected are grade 1 tumors. This gives the idea that this gene might have become inactivated during early phases of ovarian tumor development [15-18]. This hypothesis has been confirmed by Mutter *et al.*, who discovered MMAC mutations showing decreased protein expressions in 55% of endometrial precancerous lesions [19]. According to Sato *et al.*, MMAC mutations are early occurrences in development of ovarian carcinomas [14].

The purpose of this study was to investigate whether decreased expressions of MMAC proteins were an early indicator for malignant transformation in endometriosis, as shown in endometrial and ovarian carcinomas. Thirty-three subjects with grade 1-4 endometriosis, 18 subjects with grade 1-3 ovarian endometrioid adenocarcinoma and 12 subjects with ovarian clear cell adenocarcinoma were included in the study.

In contrast with studies [14-19] which were carried out on a similar number of subjects, decreased protein expressions due to increased MMAC gene mutation in advanced endometriosis and ovarian cancers (endometrioid and clear cell-type) were not observed in this study. However, a significant difference in MMAC immune staining between endometriosis (70% ± 21) and ovarian endometrioid adenocarcinoma (91% ± 10) was observed ($p = 0.001$), which was again in contrast with the literature.

Together with the determination of other proteins, expressed by MMAC genes in research studies, it is believed that different mutations and consequently different proteins in MMAC genes could explain these differences in staining. It has been shown by these findings that prospective studies on a higher number of subjects are necessary.

Immunohistochemical determination of MMAC defective protein expressions could be considered as a new, simple and useful technique to determine endometriosis patients with increased risk of malignant transformation, patients where early surgical treatment would be necessary and patients that should be subjected to follow-up controls with a higher frequency.

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