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MMAC/PTEN gene expression in endometrial cancer: RT-PCR studies

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Mutations in the *MMAC/PTEN* (phosphatase and tensin homologue deleted on chromosome 10) gene are documented in cancers of the breast, prostate, ovary, colon, melanoma, glioblastoma, lymphoma and endometrium.

In the present work *MMAC/PTEN* gene expression in women with endometrial adenocarcinoma (n=70) in RNA samples obtained from cancer tissue were investigated. Control DNA was obtained from 68 normal endometrial tissue. The *MMAC/PTEN* expression was determined by RT-PCR analysis.

The expression of *MMAC/PTEN* gene in endometrial adenocarcinoma cases was significantly reduced compared to the expression in the normal samples (P<0.05). Furthermore the significant difference (P<0.05) was observed between the expression of *MMAC/PTEN* in stage III versus lower stages of endometrial cancer.

The results support the hypothesis that the *MMAC/PTEN* gene expression may be associated with the incidence of endometrial cancer.

Introduction

Endometrial cancer (EC) is the most common gynaecological malignancy in the developed world. The majority of cases can be divided into two broad categories based on clinico-pathological and molecular characteristics; type I oestrogen-dependent with endometrioid morphology and type II non-oestrogen-dependent with serous papillary or clear cell morphology [14]. Risk factors for endometrial cancers include the following: total number of menstrual cycles, history of infertility, obesity (being very overweight), tamoxifen, estrogen replacement therapy (ERT), a diet high in animal fat, diabetes, family history and prior pelvic radiation therapy [1]. Prognostic factors commonly used to identification of EC present an incomplete picture of the tumour biology of endometrial cancer [15]. Therefore, investigation of other prognostic factors is of special clinical relevance, particularly in view of the unexpectedly progressive course of the disease and frequent relapses in some cases.

MMAC/PTEN, a tumor suppressor gene located on chromosome 10q, has recently been shown to act as a phosphatidylinositol 3, 4, 5-triphosphate phosphatase and to modulate cell growth and apoptosis. Somatic mutations of MMAC/PTEN have been reported in a number of human cancers. Somatic mutation or deletion of the PTEN tumor suppressor gene has been reported in approximately 40% and 40%–76%, respectively, of endometrial adenocarcinomas [6, 12, 13, 16]. In particular, the regions 10q23 (where PTEN is located) and 10q25–q26 have been strongly correlated to endometrial tumorigenesis [4, 11]. Disease-causing mutation of the MMAC/PTEN have been found in 10–38% of endometrial cancers [13]. That the inactivation of the MMAC/PTEN gene is an early event in endometrial carcinogenesis is also supported by data from two independent studies showing a 22% frequency of MMAC/PTEN mutations in premalignant lesions of endometrial hyperplasia both with and without atypia [7, 10].

As mentioned above the *MMAC/PTEN* tumor suppressor gene is frequently mutated and homozygously deleted in endometrial cancer, but there is only sparse information about *MMAC/PTEN* expression in hormone-dependent female tumors. Kappes et al, show significantly decreased *MMAC/PTEN* expression in endometrial carcinomas compared with normal endometrial tissue samples, especially in the endometrioid histological subtype [3].

For further understanding of the involvement of *MMAC/PTEN* in endometrial carcinogenesis, we analysed the expressions of *MMAC/PTEN* in patients with endometrial adenocarcinomas.

Materials and Methods

Endometrial cancer samples

Tumour tissues were obtained from postmenopausal women with endometrial adenocarcinoma treated at Department of Obstetrics and Gynaecology at the Institute of Polish Mother's Memorial Hospital in Lodz between 2000–2004. Clinical data for the patients and histological data were registered. There were 70 women and their mean age was 63 years (range: 58–71 years). The cancer tissue samples were fixed routinely in formalin and embedded in paraffin. Archival paraffin-embedded tumour sections on slides were deparaffinized in xylene and rehydrated in ethanol and distilled water. All tumours were staged according to the criteria of the International Federation of Gynaecology and Obstetrics (FIGO). There were 15 tumours of I stage, 43 of II stage and 12 of III stage in total. DNA from normal endometrial tissue (n=68) served as control.

RNA isolation and RT-PCR assay of MMAC/PTEN expression

mRNAs were extracted from tissue using commercially available TRIzol^{R}Reagents (Invitrogen^{TM}, San Diego, CA) according to manufacturer's instruction and converted to cDNA with the use of random primers and reverse transcriptase (Invitrogen^{TM}, San Diego, CA). The relative quality and quantity of mRNA/cDNA were assessed by the examining the expression of enolase, and then the relative expression of *MMAC/PTEN* was depicted as a ratio of *MMAC/PTEN* to enolase. PCR primer pairs for *MMAC/PTEN* were designed to amplify a region from 117 to 741 bp (F1- 5' CAGAAAGACTTGAAGGCGTAT 3' and B1- 5' AACGGCTGAGGGAACTC 3'), which also contained a *Nsi*I restriction site in the *MMAC/PTEN* pseudo-gene, but not in the 10q gene. The primers designed to amplify α-enolase comprised a region from 77 to 532 bp of the coding region of the gene (EF1- 5' TGGCAGGATGACTTCAGA 3' and EB1- 5' AGTGGCTAGAAGTTCACC 3'). The PCR was carried out in a DNA Thermal Cycler (GeneAmp PCR System 2400; Perkin-Elmer, Norwalk, CT, U.S.A.). The thermal cycling conditions were 45 s at 94°C, 45 s at 54°C, 1 min at 72°C, repeated for 40 step cycles. 25 μl of PCR reaction contained 100 ng cDNA, 1 μl of each appropriate primer (ARK Scientific GmbH Biosystems, Darmstad, Germany), 1.5 mM $MgCl_2$, 0.2 mM dNTPs (Qiagen GmbH, Hilden, Germany) and 1 unit of Taq Polymerase (Qiagen GmbH, Hilden, Germany). PCR products were treated with *Nsi*I and then electrophoresed in a 2% agarose gel and visualised by ethidium bromide staining (Figure 1).

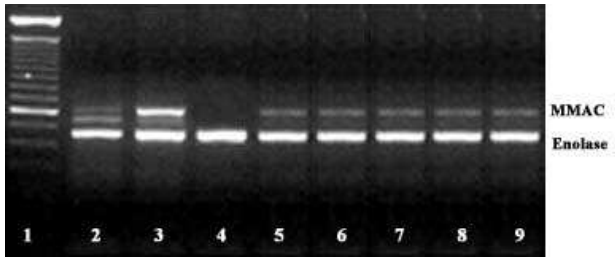

Fig. 1. Illustration of relative *MMAC/PTEN* RT-PCR expression. Endometrial cancer cases and control were subjected to 40 cycles of PCR amplification in the presence of primer pairs for *MMAC/PTEN* and enolase. PCR products were separated in a 2% agarose gel and stained with ethidium bromide. Lanes 1 – molecular weight marker (SIGMA-ALDRICH, St Louis, USA), 2–5 – normal samples, lanes 6–9 – endometrial cancer samples.

Statistical analysis

None of the parameters recorded in tumour material passed tests for being normally distributed (Smirnow-Kolmogorov test) and therefore nonparametric statistical tests were used for analysing the results. *P*-values < 0.05 were considered to be significant.

Results

The expression status of *MMAC/PTEN* gene was determined in 70 endometrial adenocarcinoma samples and in control (n=68) by RT-PCR and compared with the expression of enolase, a constitutively but low expressed gene product.

MMAC/PTEN expression was identified in 46% of carcinoma samples (32/70) and in 62% of control samples (42/68). The expression of *MMAC/PTEN* in cancer samples was significantly reduced compared to the expression in the normal tissues (P<0.05). Dependencies of the pres-

TABLE 1
MMAC/PTEN gene expression in endometrial cancer women and in control samples

	Expression of *MMAC/PTEN*	
	yes	no
Control (n = 68)	42 (0.62)	26 (0.38)
Patients		
Total (n = 70)	32 (0.46)[a]	38 (0.54)
stage I[b] (n = 15)	12 (0.80)	3 (0.20)
stage II (n = 43)	18 (0.40)	25 (0.60)
stage III (n = 12)	2 (0.17)	10 (0.83)

[a]*P*<0.05 as compared with controls, [b]according to FIGO criteria

ence of *MMAC/PTEN* expression on the tumour stage of patients with endometrial adenocarcinoma are displayed in Table 1. *MMAC/PTEN* expression in stage III was significantly reduced compared with that observed in lower grade carcinoma.

Discussion

Endometrial tumorigenesis is still poorly understood. The development of endometrial cancer is associated with an accumulation of specific genetic alterations. The activation of oncogenes, the loss or inactivation of repressor genes and impaired mismatch-repair function are known to be involved in the development of tumours. MMAC/PTEN mutated in multiple advanced tumors encodes a 54 kDa protein which acts as a dual specificity phosphatase and is capable of dephosphorylating both phospho-Ser/Thr and phospho-Tyr proteins. MMAC/PTEN was initially characterized as a tumor suppressor gene in Cowden syndrome (CS) and has since been found to be defective in many other human cancers including those from brain, breast, kidney, prostate and endometrium [8, 9]. Inactivation of the two alleles of *MMAC/PTEN* is required, because it is a tumor suppressor gene.

MMAC/PTEN is the most frequently mutated gene identified yet in endometrial cancers. *MMAC/PTEN* mutations were detected in 33%–55% of the endometrial cancers [5, 6, 13, 16, 18, 19] Furthermore, *MMAC/PTEN* mutations occur in early well-differentiated lesions (stage I/grade 1) as well as in very advanced and invasive tumors (stage IV/grade 3), suggesting their involvement in the initiation of endometrial tumorigenesis.

In light of substantial evidence that the progression of endometrial cancer can be associated with *MMAC/PTEN*, it seems reasonable to check a possible correlation between the expression of this gene and clinical status of endometrial cancer patients. In this work conducted on 70 endometrial adenocarcinoma patients we observed that the expression of *MMAC/PTEN* was significantly suppressed in endometrial adenocarcinoma when compared with normal tissues. Similar to the results described herein, decreased expression of *MMAC/PTEN* was reported previously in a series of prostate cancer [21], anaplastic thyroid cancer [2], and breast cancer [3]. The lack observed *MMAC/PTEN* expression may be accounted by several different mechanisms. One possibility is altered methylation of the transcriptional regulatory region of the gene [12, 21]. This is known to occur for several tumor suppressor genes, although direct evidence for this has been lacking for *MMAC/PTEN*. Another possibility is that the *MMAC/PTEN* gene may be targeted for homozygous deletion. In support of this possibility Teng et al shown that the frequency of homozygous deletion in cancer cell lines is significantly higher than in tumor specimens, suggesting that homozygous deletions may be underrecognized in some tumor specimens [20]. A third possibility arises from a study of Li et al, who described the identification of *MMAC/PTEN* based on its similarity to dual specificity phosphatases [8]. In their study, they also observed that *MMAC/PTEN* expression was regulated in part by transforming growth factor β. Because TGFβ and its altered signaling pathway has been implicated in a number of human cancers, the possible relationship between these two interesting pathways needs to be further examined.

Recent studies have suggested those genetic alterations, including *p53, p21, p16* mutations, amplifications of oncogenes *K-ras* and *HER2/neu*, and microsatellite instability, can be detected in endometrial cancer [15]. Our findings provide additional evidence that genetic alterations, including *MMAC/PTEN* expression, may occur as relatively early events in the development of endometrial cancer.

Our study implies that it is possible that the expression of the *MMAC/PTEN* gene may be involved in the development and/or progression of endometrial adenocarcinoma. Further studies, conducted on a larger population, are required to clarify this point.

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