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Immunoexpression of the PTEN protein and matrix metalloproteinase-2 in endometrial cysts, endometrioid and clear cell ovarian cancer

Immunoekspresja białka PTEN i metaloproteinazy-2 w torbielach endometrialnych, raku endometrioidalnym i jasnokomórkowym jajnika

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Abstract

Objectives: *Endometrioid and clear cell ovarian adenocarcinomas are suspected to derive from ectopic endometrial foci. The aim of the study was to determine PTEN and MMP-2 immunoexpression in endometrial ovarian cysts, endometrioid and clear cell ovarian carcinomas and to assess the relationship between the abovementioned values and clinical data of patients in order to find the marker of increased risk of malignant proliferation based on ovarian endometriotic lesions. Detailed analysis of the collected data was conducted to investigate the correlation between immunohistochemical expression of the examined antigens, histopathological diagnosis and clinical condition of patients.*

Material and methods: *20 endometrial adenocarcinomas, 21 clear cell ovarian cancers and 26 endometrial cysts were included in the study. The control group consisted of 29 specimens of physiological endometrium: 16 samples of the proliferative phase and 13 samples of the secretory phase.*

Protein expression of PTEN and MMP-2 was evaluated by immunohistochemistry. Protein immunoexpression in the collected specimens was estimated with the use of light microscope and MultiScan software. Immunoreactivity of the PTEN antigen was assessed by the quantitative method, whereas MMP-2 immunoexpression was evaluated by the semi-quantitative method.

Two-sided tests were used for statistical inference. Generalized linear models were used to compare the studied groups. Error distributions were selected using the Akaike criterion (AIC). Statistical analysis was conducted with the use of the R Statistical Package.

Results: *MMP-2 immunoreactivity differed significantly between the study groups and controls ($p<0.001$). PTEN immunoexpression was the strongest in endometrial cysts (53.7 %), lower in clear cell cancers (50.2%) and the lowest in endometrioid adenocarcinomas (43.88%), but the differences were not statistically significant ($p=0.17$). PTEN reactivity in the group of endometrioid carcinomas was significantly higher ($p=0.02$), while MMP-2 expression had a falling tendency ($p=0.076$) in obese women.*

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Conclusions: *Increased MMP-2 expression in the successive groups may imply a rising invasive potential of the epithelial cells in endometrial cysts, endometrioid and clear cell adenocarcinomas. Strong immunoreactivity for PTEN in proliferative endometrium implies its role in the regulation of endometrial proliferation. PTEN activity may reduce MMP-2 expression in insulin resistant women suffering from endometrial ovarian cancer. Simultaneous evaluation of PTEN and MMP-2 immunoexpression in ectopic endometrial foci cannot be used to identify women with an increased risk of neoplastic transformation.*

Key words: **endometriosis** / **endometrioid ovarian cancer** / **clear cell ovarian cancer** / / **PTEN** / **MMP-2** /

Streszczenie

Cele: *Istnieje hipoteza o zwiększonym ryzyku powstawania ognisk raka endometrioidalnego i jasnokomórkowego jajnika na podłożu endometriozy jajnikowej. Celem pracy była analiza immunoreaktywności białka PTEN i metaloproteinazy-2 (MMP-2) w torbielach endometrialnych, raku endometrioidalnym i jasnokomórkowym jajnika w kontekście poszukiwania markera zwiększonego ryzyka proliferacji nowotworowej na podłożu istniejących ognisk endometriozy jajnikowej a także ocena zależności pomiędzy immunoekspresją białka PTEN i metaloproteinazy-2 a stanem klinicznym kobiet z gruczolistością zewnętrzną oraz rakami endometrioidalnym i jasnokomórkowym jajnika.*

Materiał i metody: *grupy badane tworzył tkankowy materiał pooperacyjny pochodzący z torbieli endometrialnych (N1=26), raków endometrioidalnych jajnika (N2=20), raków jasnokomórkowych jajnika (N3=21). Grupę porównawczą stanowiły preparaty niezmienionego endometrium (N4=29): fazy proliferacyjnej (n1=16) i wydzielniczej (n2=13).*

Komórkową ekspresję białka PTEN oraz MMP-2 oceniano przy użyciu metod immunohistochemicznych. Immunoreaktywność badanych antygenów oceniano używając komputerowego systemu analizy obrazu. Immunoreaktywność antygenu PTEN oceniano metodą ilościową, natomiast antygenu MMP-przy użyciu metody półilościowej.

Wnioskowanie statystyczne przeprowadzono na poziomie 0,05 przy użyciu testów dwustronnych. Do porównania wartości średnich w badanych grupach zastosowano model regresji liniowej dla parametru PTEN oraz uogólniony model regresji linowej z rozkładem Gamma dla błędu losowego. Obliczeń statystycznych dokonano przy pomocy pakietu statystycznego R.

Wyniki: *stwierdzono znamienne statystycznie różnice w immunoekspresji MMP-2 pomiędzy grupami badanymi oraz porównawczą (p<0,001). Najsilniejsza immunoekspresja PTEN występowała w materiale z torbieli endometrialnych (53,7 %), kolejno słabsza była w materiale z raków jasnokomórkowych jajnika (50,2%), najmniej intensywna wśród raków endometrioidalnych jajnika (43,88%), jednakże obserwacje nie były istotne statystycznie (p=0,17). W grupie chorych z rakiem endometrioidalnym jajnika immunoreaktywność PTEN była znamiennie wyższa u kobiet z nadwagą i otyłością (p=0,02), natomiast immunoekspresja MMP-2 w tej grupie wykazała tendencję spadkową (p=0,076).*

Wnioski: *Silniejsza immunoekspresja MMP-2 w kolejnych badanych grupach może wskazywać na wzrastający potencjał inwazyjny komórek nabłonkowych torbieli endometrialnych, raka endometrioidalnego i jasnokomórkowego jajnika. Silna immunoreaktywność białka PTEN w endometrium fazy proliferacyjnej przemawia za jego udziałem w kontroli proliferacji nabłonka endometrium.*

Aktywność PTEN może hamować ekspresję MMP-2 wśród kobiet z insulinoopornością chorujących na raka endometrioidalnego jajnika. Oznaczanie immunoreaktywności białka PTEN i MMP-2 w materiale tkankowym jaki wykorzystano w badaniu nie może być użyte jako marker zwiększonego ryzyka proliferacji nowotworowej na podłożu endometriozy jajnika.

Słowa kluczowe: **endometrioza** / **rak jajnika endometrioidalny** / / **rak jajnika jasnokomórkowy** / **PTEN/ MMP-2** /

Introduction

Ovarian cancer has the worst prognosis of all gynecological malignancies [1]. Although no cause-and-effect relationship between the presence of endometriosis and ovarian cancer has been evidenced thus far, numerous data suggesting the possibility of cancer proliferation associated with ovarian endometriosis have been reported [2, 3]. Due to similarity of ovarian endometrioid and clear cell cancer morphology to that of uterine mucinous carcinomas, the former are suspected to derive from ectopic endometrial foci.

PTEN protein is responsible for a multistage control of a cell cycle transformation process [4, 5]. Due to its structural homology to tensin, PTEN protein reacts with the cell membrane proteins and elements of the extracellular matrix. Therefore, its involvement in malignant transformation at the stage of cell proliferation, as well as at the time of acquiring the ability of the cell to invade tissues, has been implied [6]. Koul et al., observed a correlation between the activity of matrix metalloproteinase-2 (MMP-2) and PTEN protein activity in cancer cell lines derived from the outside of reproductive organs [6]. A similar analysis

has not been performed either in in vitro or in vivo conditions in tissue samples from reproductive organs. Moreover, no data on the relationship of the MMP-2 expression and activity with PTEN protein activity in the pathomechanism of ovarian cancerogenesis have been published to date.

There are some reports on the prevalence of errors in the expression of genes responsible for the synthesis of enzymes decomposing the extracellular matrix (ECM) among patients with endometriosis (7-10). So far, twenty matrix metalloproteinases have been described. MMP-2 and MMP-9 are regarded as the most important metalloproteinases for invasive processes observed in endometriosis and carcinogenesis [11-13]. Coincidence of ovarian cancer and endometriosis remains an issue [2, 14-21].

Similar polymorphisms and mutations in the suppressor gene region predominate among DNA disorders of ectopic endometrial and cancer cells. One of the most frequent genetic dysfunctions is the impairment of PTEN/MMAC1 (phosphatase and tensin homolog deleted from chromosome 10/mutated in multiple advanced cancers) suppressor gene [7, 14, 15, 22-24].

The aims of the study were to determine immunoreactivity of the PTEN protein and metalloproteinase-2 in the proliferative and secretory endometrium, endometrial cyst ependyma, and ovarian endometrioid and clear cell carcinoma; to analyze the relationship between immunoexpression of the PTEN protein and matrix MMP-2 and the clinical state of the studied women and to verify the PTEN protein and matrix MMP-2 immunoreactivity as markers of an increased risk for cancer proliferation associated with ovarian endometriotic lesions.

Material and methods

Study material

Tissue samples were gathered during surgical treatment of 96 patients hospitalized at various departments of the Polish Mother's Health Centre Research Institute between 1999 and 2009. The control samples were collected during routine curettage of uteri of women hospitalized for static disorders of the pelvic organs.

The following study groups were distinguished: patients with ovarian endometrial cysts (N_1=26), with ovarian endometrioid adenocarcinoma (N_2=20) and with ovarian clear cell carcinoma (N_3=21). The control group consisted of women with physiological endometrium (N_4=29) in the proliferative (n_1=16) and secretory (n_2=13) phases.

Histopathological diagnoses were established at the Department of Pathomorphology in accordance with the World Health Organization classification.

Immunohistochemistry

Tissue sections were embedded in paraffin according to standard procedures. Four-micrometer sections were cut and mounted on slides. The sections were deparaffinized by xylene and rehydrated in graded ethanol baths.

In order to depict the needed antigens, the sections were incubated in a water bath at 97°C for 40 min with a 0.001M versenate buffer (EDTA) of pH 8.0 for MMP-2 and a Target Retrieval Solution buffer at pH 9.0 for the PTEN protein. After cooling, the sections were rinsed in 0.05M TRIS buffer (Tris-Buffered Saline, DAKO) and incubated in a humidity chamber at room temperature for 60 min with appropriately diluted antibodies: Monoclonal-Mouse Anti-Human PTEN Clone 6H2.1 (DAKO) 1:100 and Lyophilized Mouse Monoclonal Antibody MMP-2 (Novocastra) 1:60. After the incubation, the sections were rinsed twice in TRIS buffer and the two-stage visualization system DAKO EnVision was used to disclose the antigen-antibody reaction. The first stage of the reaction included 30 min incubation with a peroxidase-labeled polymer linked to secondary goat antibodies directed against the monoclonal antibodies used. The last stage of detection demonstrated an enzymatic reaction in which staining was elicited by using a peroxidase substrate, 3,3'-diaminobenzidine (DAB). After receiving a color immunohistochemical reaction, cellular nuclei were stained with Meyer's hematoxylin and dehydrated by increasing concentrations of ethanol and passing through a series of xylene. Finally, the specimens were mounted on sialinized slides and covered with Canadian balm. Positive and negative controls were provided.

Evaluation of immunoreactivity

Analysis was performed without the knowledge of clinical and histopathological parameters.

The PTEN antigen immunoreactivity was evaluated using the image analysis computer system. A stained (dark brown) product of immunohistochemical reaction inside the nucleus or cytoplasm was considered a positive response.

The PTEN antigen immunoreactivity was established using the quantitative method. The percentage value of positively stained cells per 1000 cells was calculated. The antigen immunoreactivity index was expressed as percentages.

MMP-2 antigen immunoreactivity was evaluated by observing 10 adjacent vision fields in the light microscope at 200x magnification. The first field was selected randomly. The intracytoplasmic presence of the stained product (slight brown) of immunohistochemical reaction signified a positive response.

Due to the presence of the MMP-2 antigen in the cell cytoplasm and the extracellular environment, the semi-quantitative method was used to evaluate immunoreactivity of this antigen: 0 – no reaction; 1 – mild reaction; 2 – moderate reaction; 3 – strong reaction.

Two independent examiners performed the assessment in order to objectively verify immunoexpression of the studied antigens.

Statistical analysis of the results

Two-sided tests were used at a significance level of 0.05 to analyze the obtained data. A linear regression model for the PTEN parameter and a generalized linear regression model with a gamma distribution for a random error were used to compare mean values of the studied parameters. The model was chosen on the basis of the information criterion Akaike (AIC). First, the differences between the groups were compared. The second stage of the analysis included an effect of other factors (age, BMI, hypothyroidism, etc.) on the parameters. Each group as well as the mean difference in all groups were compared. The interaction test was used to assess differences in the mean effect of all parameters studied in each group. At this stage of the analysis, groups which showed no differentiation in regard to the characteristic features or where only one observation was present (e.g. in the group with the secretory stage endometrium

Table I. Clinical characteristics of the patients. Part 1.

Histopathological diagnosis	Age [years]				BMI [kg/m^2]				Number of deliveries			
	mean	median	min	max	mean	median	min	max	mean	median	min	max
Proliferative stage	41.3	42	31	53	23.3	22.3	18.6	33.2	1.4	1.5	0	3
Secretory stage	39.9	40	31	49	24.9	23.1	18.0	39.7	1.5	2	0	2
Endometrial cysts	36.1	37	22	55	22.8	22.1	17.0	32.8	0.9	0.5	0	3
Endometrioid adenocarcinoma	52.7	52.5	35	69	28.6	27.0	23.4	46.1	1.3	1.5	0	3
Clear cell adenocarcinoma	57.3	57	39	78	26.0	25.4	21.1	33.2	1.7	1.5	0	7

Table II. Clinical characteristics of the patients. Part 2.

Histopathological diagnosis	Deliveries		Menopause		Type II diabetes		Hypothyroidism		Cholelithiasis	
	no (nulliparae)	yes (multiparae)	no	Yes	no	yes	no	yes	no	Yes
Proliferative stage	5	11	16	0	16	0	12	4	14	2
Secretory stage	1	12	12	0	13	0	12	1	13	0
Endometrial cysts	13	13	26	0	25	1	24	2	25	1
Endometrioid adenocarcinoma	6	14	8	11	19	1	18	2	19	1

there were no patients with cholelithiasis) were excluded from the study. Statistical calculations were performed using the statistical package R.

Results

Correlations between immunoexpression intensity of particular antigens and clinical features of the patients qualified for the study (Table I and II) were analyzed.

All cases of ovarian clear cell carcinoma are treated as poorly differentiated (G-3). Therefore, only samples derived from patients with ovarian endometrioid adenocarcinoma were subjected to grading analysis (Table III and IV). No statistical differences were observed.

Ninety-six paraffin sections underwent the procedure of immunohistochemical staining to detect the presence of the MMP-2 and PTEN protein antigens. A positive reaction to MMP-2 was achieved in 92 sections. No reaction to MMP-2 was observed in 1 case of endometrial cyst, ovarian endometrial and clear cell carcinoma and proliferative endometrium. A positive reaction to PTEN was achieved in 93 cases. No immunohistochemical reaction was noted in 3 sections: 1 of ovarian endometrial cancer, 1 of clear cell cancer and 1 of proliferative endometrium.

After evaluating the MMP-2 immunoreactivity according to its intensity, the lowest values were found in the proliferative endometrium, higher ones in the secretory endometrium, endometrial cysts, and ovarian endometrioid adenocarcinoma, and the highest in clear cell ovarian carcinoma. The differences were statistically significant ($p<0.001$) (Table V).

The immunohistochemical reaction to PTEN was the strongest in the proliferative endometrium and the weakest in the secretory endometrium (Table VI).

Table III. Mean values of MMP-2 immunoexpression in ovarian endometrioid adenocarcinoma.

Group	Grading		p
	1 or 2	3	
Endometrioid adenocarcinoma	1.07	1.73	0.202

Table IV. Mean values of PTEN protein immunoexpression in ovarian endometrioid adenocarcinoma.

Group	Grading		p
	1 or 2	3	
Endometrioid adenocarcinoma	47.8	29.0	0.187

Table V. MMP-2 immunoexpression in tissue samples.

Group	MMP-2			
	Mean	95% CI	p	
Proliferative stage	0.68	0.53-0.87	ref.	ref.
Secretory phase	0.67	0.51-0.88	0.93	
Endometrial cysts	0.86	0.71-1.05	0.14	0.072
Endometrioid adenocarcinoma	1.21	0.96-1.51	0.001	0.001
Clear cell adenocarcinoma	1.27	1.02-1.58	<0.001	<0.001
	p<0.001			

Table VI. PTEN protein immunoexpression in tissue samples.

Group	PTEN			
	Mean	95% CI	p	
Proliferative stage	61.1	47.3-75.0	ref.	0.033
Secretory stage	37.8	23.0-52.7	0.033	ref.
Endometrial cysts	53.7	43.0-64.4	0.5	0.084
Endometrioid adenocarcinoma	43.8	31.5-56.1	0.1	0.49
Clear cell adenocarcinoma	50.2	38.2-62.1	0.32	0.18
	p=0.17			

Table VII. MMP-2 immunoexpression in the tissue samples in regard to BMI.

Group	**BMI** [kg/m^2]		p
	[17-25]	(25-46]	
Proliferative stage	0.70	0.60	0.624
Secretory stage	0.76	0.57	0.286
Endometrial cysts	0.92	0.68	0.193
Endometrioid adenocarcinoma	1.61	1.05	0.076
Clear cell adenocarcinoma	1.31	1.15	0.568
Total	interaction p=0.91		0.017

Table VIII. PTEN immunoexpression in regard to BMI.

Group	**BMI** [kg/m^2]		p
	[17;25]	(25;46]	
Proliferative stage	63.2	53.1	0.648
Secretory stage	33.0	43.5	0.397
Endometrial cysts	53.4	54.6	0.939
Endometrioid adenocarcinoma	26.6	52.7	0.020
Clear cell adenocarcinoma	49.0	52.5	0.804
Total	interaction p=0.367		0.152

Due to the fact that the extreme values of this parameter were reached in two control subgroups, further stages of the statistical analysis evaluating PTEN immunoreactivity were performed by dividing the control group into two subgroups. Next, the intensity of the immunohistochemical reaction was again compared, with the material from the endometrium of the proliferative and secretory phase treated as one group. As mentioned above in the first case, the difference in immunoexpression between the proliferative and secretory endometrium was distinct, while in the subsequent case the difference was observed between the control group and the material from endometrial cysts (p=0.084).

Significantly higher immunoreactivity of MMP-2 was recorded in the tissue material derived from the group of patients up to 45 years of age. PTEN protein immunoreactivity did not correlate with patient age in any of the groups.

In each group, MMP-2 immunoreactivity values were found to be higher in women whose BMI values were within the normal range (17-25 kg/m^2), however the differences between groups formed according to the BMI were not statistically significant (Table VII). Only in the group of patients with endometrioid adenocarcinoma of the ovary and proper BMI values, MMP-2 immunoexpression indicated a strong growing tendency as compared to overweight or obese patients (p=0.076).

The PTEN protein was characterized by significantly different immunoreactivity in regard to BMI only in ovarian endometrial cancer (Table VIII). PTEN immunoexpression was considerably higher in overweight and obese patients than in patients without similar metabolic disorders (p=0.02).

Analysis of the influence of parity on MMP-2 and PTEN immunoreactivity did not reveal any relationship.

The evaluation of MMP-2 immunoreactivity in the samples from endometrioid adenocarcinoma related to the menopause effect on intensity of immunohistochemical reaction did not indicate statistically significant correlations. In the material from patients with clear cell cancer after menopause, considerably weaker MMP-2 immunoexpression was observed as compared to the results of women before menopause, nonetheless the differences were not significant. PTEN immunoreactivity did not correlate with menopause.

A detailed analysis of MMP-2 and PTEN immunoreactivity did not prove any relationship between the immunoexpression of the examined antigens in the study and the control groups. (Figure 1).

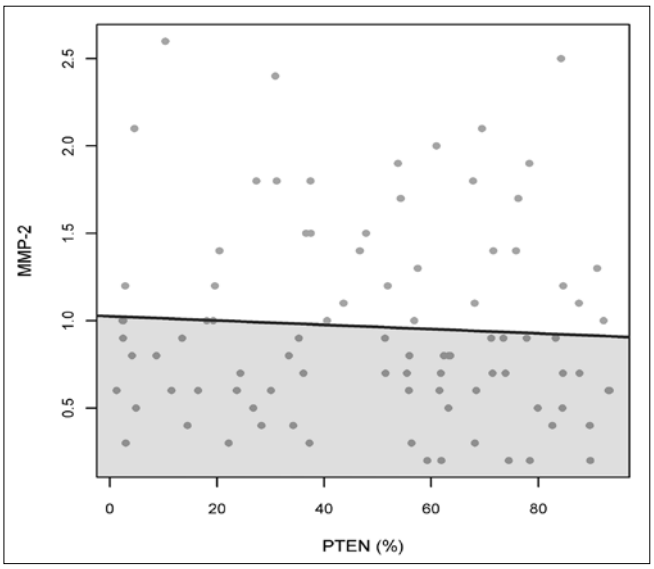

Figure 1. MMP-2 and PTEN immunoexpression.

Discussion

Neoplastic transformation of ectopic endometrial foci seems to affect 1% of cases (14,16,25-27).

Melin et al., using the data from the National Swedish Cancer Registry, studied the incidence rate of ovarian cancer and other malignancies among patients with endometriosis hospitalized between 1969-2000 (28). The index of risk for developing malignancies generally equaled that for the entire population and was 1.04, whereas it was considerably higher for ovarian adenocarcinoma (1.43), and even reached 2.23 among women with persisting endometriosis.

Up to 10% of endometrial ovarian cancer cases arise from ectopic endometrial foci, the remaining 90% derive from the epithelium primarily covering the ovary. Association of clear cell cancer with endometriosis is stronger and concerns about 35% of cases [29]. In 20-25% of clear cell adenocarcinoma cases, a component of endometrioid adenocarcinoma is observed histopathologically, providing indirect evidence for a mutual origin of these two neoplasms.

In 3 of 20 cases of ovarian endometrioid adenocarcinoma in our study, the presence of texture signifying neoplastic transformation on the basis of the previous endometrial focus (15%) was microscopically detected. Tissue material from clear cell carcinoma of 21 women revealed concomitant endometrioid adenocarcinoma in 4.8% cases.

Wu et al., confirmed a monoclonal character of the cells in 75% of endometrial cyst cases simultaneous with ovarian endometrioid adenocarcinoma foci, and in 28% of cyst cases without cancer foci [30]. This information indirectly presents the evidence of changes undergoing in the endometrial cells, which in effect enable cellular transformation and proliferation. There are regions 1p, 5q (25%), 6p, 6q (27%), 9p (21%), 10q, 11q, 22q (15-31%) among DNA fragments, common for endometrial and malignant cells, which most often get damaged [14, 15, 29, 31-33]. Loss of heterozygoticity with the 10q localization is associated with inactivity of the PTEN suppressor gene.

Obata et al., were among the first who proved the incidence of LOH and somatic mutations of the PTEN gene, both in endometrial cysts and foci of concomitant cancer [31]. The authors suspect that lack of heterozygoticity signifies an initial phase of cellular independence, while the occurrence of the PTEN gene somatic mutation is a trigger element for carcinogenesis.

Similarity between the structure of the PTEN protein and tensin inspired Tamura et al., to search for an interaction with extracellular matrix (ECM) elements [34]. There is evidence that the mechanisms of cell survival and apoptosis are dependent not only upon intracellular proteins but also upon the contact with extracellular elements [29].

Goffin et al., investigated metalloproteinase expression in the human endometrium during the entire menstrual cycle [35]. They observed that some protenases from the family exhibit expression during the entire menstrual cycle (MMP-2, MMP-19, MT-MMP-1,

MT-MMP-2). The other group is synthesized only at the time of menstruation (MMP-1, MMP-3, MMP-8, MMP-9, MMP-12). The maintenance of the matrix metalloproteinase production during the entire menstrual cycle may provide evidence for the involvement of these endopeptidases in the proper course of endometrial cell metabolism. We detected immunohistochemically the presence of MMP-2 both in the proliferative and secretory stages of the physiological endometrium. MMP-2 immunoexpression appeared to be significantly higher in the endometrial secretory stage as compared to the proliferative stage ($p<0.001$).

Gaetje et al., observed an evidently increased production of the membrane-type MT-MMP-5 in the cells of the ectopic endometrium, which they believed to be the reason for the increased ability of these cells to adhere and invade other cells [12]. MT-MMP-5 is a well known activator of numerous metalloproteinases, including MMP-2.

We evaluated MMP-2 immunoreactivity not only in the physiological epithelium of the proliferative ($n_1=16$) and secretory ($n_2=13$) endometrium, but also in endometrial cysts of the ovary ($N_1=26$). It differed significantly in intensity among the groups and was the strongest ($p<0.001$) in endometrial cysts. The results demonstrate the MMP-2 involvement in the pathogenesis of forming ectopic endometrial foci.

Ovarian cancers are characterized by a special proliferation process in which the moment of malignant cell penetration to the peritoneal cavity is a key element in the prognosis. We know that secondary ovarian carcinomas do not disseminate intraperitoneally in a similar way, thus we would like to understand which properties of the cells determine such course. One of the theories considers its higher capacity to synthesize metalloproteinases [36].

Davidson and Schmalfeldt evaluated the activity of MMP-2, MMP-9 and their tissue activators [37, 38]. They observed that MMP-2 and MMP-9 capacity for invasion grew along with the increase in their expression in cancerous cells, without considering histopathological grading of the cancer.

According to Sillanpää, however, an increase in MMP-2 and MMP-9 tissue expression is accompanied by a better prognosis and longer survival rates ($p<0.0057$) [39]. In our analysis the relationship between MMP-2 antigen immunoexpression, microscopic diagnosis and clinical features was investigated. MMP-2 immunoreactivity differed significantly between the study groups and controls ($p<0.001$). A higher MMP-2 expression in successive groups may imply a growing invasive potential of the epithelial cells in endometrial cysts, endometrioid and clear cell adenocarcinoma (Table VII).

In the present study, MMP-2 immunoexpression was significantly higher ($p<0.05$) in the group of women below 46 years of age than in older patients. This correlation was strongly expressed in women with diagnosed ovarian endometriosis and endometrioid ovarian cancer. The immunoreaction to MMP-2 in the tissue samples collected from the older patients, irrespective of primary histopathological diagnosis, displayed a weaker intensity.

Kenny et al., are of the opinion that the interaction of potentially malignant or malignant cells with such a specific environment as the mesothelium covering the abdominal cavity is the key to understand ovarian cancer proliferation process [36]. The authors observed that the process of adhesion and invasion was accompanied by intensified MMP-2 expression in the cancerous as well as mesothelial cells.

A hypothetical effect of PTEN activity loss on the pathogenesis of endometriosis, endometrioid and clear cell ovarian cancer, as well as metalloproteinase involvement in the early stage of adhesion and proliferation of ectopic endometrial foci, encouraged us to interpret these phenomena together.

Tumor suppressor properties of PTEN are relatively well-known [8]. However, the relationship of PTEN expression and cellular activity with cell invasiveness remains obscure. The connection of PTEN and metalloproteinase activity with pathogenesis of endometriosis and neoplastic transformation requires extensive investigation. Koul et al., carried out crucial studies to understand the role of PTEN in regulation of metalloproteinase activity [6]. They reported that cells with active PTEN/MMAC1 were characterized by limited growth and significantly more frequent apoptosis via anoikiosis (the process of programmed cell death that starts with degeneration of the nucleus). Moreover, properly functioning PTEN showed a three-fold decrease in cell migration and invasion capacity. These authors indicated that PTEN correlates not only with pro-MMP-2 inactivation but also directly inhibits expression and transcription of genes responsible for MMP-2 synthesis.

Relative availability of immunohistochemical methods was the reason why they were selected for our research. The analysis of immunoreactivity of the preferred antigens is a method enabling the evaluation of the final product of frequently complex multistage pathways. Taking into account endometriosis incidence and its potential of neoplastic transformation, we decided to seek the marker of increased risk of cancerogenesis based on ectopic endometrium. We made an attempt to assess the importance of PTEN and MMP-2 activity in the pathogenesis of endometriosis, endometrial and clear cell ovarian carcinoma.

PTEN is a potential regulator of MMP-2 activity, acting on different levels. Its inactivation might be a trigger factor for neoplastic proliferation induced on the basis of ectopic endometrium.

The PTEN protein immunoexpression in the endometrium depended on the stage of the menstrual cycle. A stronger PTEN antigen immunoreactivity was observed in the proliferative rather than in the secretory stage. The difference was statistically significant (<0.05). This condition might reflect an enhanced activity of suppressive PTEN in the intensive physiological stage of cell proliferation. PTEN immunoreactivity reached the lowest value in the secretory endometrium, at the stage of cellular stability and was in compliance with the observations of Mutter and Guzelgloglu-Kayisli [40, 41].

Loss of the PTEN gene activity is a characteristic phenomenon of an early stage of ovarian carcenogenesis induced on the basis of ectopic endometrial foci [14, 15, 24, 29, 31-33].

In our study, the analysis showed the highest PTEN immunoexpression in endometrial cysts, followed by a lower immunoexpression in the clear cell adenocarcinoma cells, and the lowest in ovarian endometrioid adenocarcinoma. The differences in immunoreactivity between the groups were not statistically significant although, in compliance with findings of other authors, they may correspond to a more frequent incidence of PTEN gene dysfunction in ovarian endometrioid adenocarcinomas as compared to clear cell adenocarcinomas of the ovary [32, 42].

Similarly to Kazandi, Kolasy, and Gomes, we did not observe any differences in PTEN immunoreactivity in relation to grading of the endometrioid adenocarcinoma [32, 42, 43].

The body mass index was an additional clinical feature considered in our study. In patients with ovarian endometrioid adenocarcinoma, both PTEN and MMP-2 immunoreactivity clearly depended on the BMI values. PTEN antigen immunoexpression was significantly higher in obese women (p=0.02), while MMP-2 immunoexpression was lower in that group (statistically not significant). Considering the properties of PTEN in the regulation of cellular response to IGF-1 activity, it can be supposed that the PTEN protein reduces MMP-2 expression in women suffering from ovarian cancer with probable insulin resistance.

Conclusions

The main aim of the present study was to detect the marker of an increased risk of neoplastic transformation deriving from ectopic endometrium. We did not find any relationship between PTEN immunoreactivity and MMP-2 immunoexpression among the examined patients. According to the results mentioned above, simultaneous determination of PTEN and metalloproteinase-2 immunoexpression in ectopic endometrial foci in patients with endometriosis cannot be used to identify women with a higher risk of neoplastic transformation.

To the best of our knowledge, our research is the first complex study on the importance of the PTEN protein and MMP-2 in the pathogenesis of endometriosis and ovarian cancer.

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