

Expression of p53 and PTEN in human primary endometrial carcinomas: Clinicopathological and immunohistochemical analysis and study of their concomitant expression

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Abstract. Endometrial carcinoma is a common malignancy of the female genital tract. Alterations in the expression levels of various oncogenes and tumor suppressor genes serve important roles in the carcinogenesis and biological behavior of endometrial carcinoma. The aim of the present study was to evaluate the combination and individual expression of p53 and phosphatase and tensin homolog (PTEN) protein in human endometrial carcinoma. In addition, the correlation of these proteins with clinicopathological parameters was also assessed. Retrospective immunohistochemical analysis of the expression of p53 and PTEN tumor suppressor proteins was conducted in 99 women with endometrial carcinoma. The overall rate of p53 and PTEN positivity was 89 and 77%, respectively, according to the sum of stain intensity and scores of immunopositive cells. The sum of p53 positivity correlated strongly with PTEN expression ($\rho=0.256$; $P=0.044$). The concomitant sum of p53 and PTEN expression was identified in 45% of patients with endometrial adenocarcinoma. Notably, the sum of the immunohistochemical expression of p53 was significantly correlated with patient age ($P=0.037$), histologic type ($P=0.008$), histologic grade ($P=0.002$) and fallopian and/or ovarian invasion ($P=0.014$). Furthermore, PTEN expression was associated with myometrial invasion ($\rho=-0.377$; $P=0.002$) and clinical stage ($P=0.019$). In addition, concomitant p53 and PTEN expression was correlated with

patient age ($P=0.008$) and histologic differentiation ($P=0.028$). The findings indicated a correlation between the expression of p53 and PTEN in endometrial adenocarcinoma, which suggested an intrinsic association between expression levels of these tumor suppressor genes. The study also suggested that concomitant p53 and PTEN expression contributed in characterizing the tumor behavior of endometrial carcinoma. Taken together, the present study suggested the combined expression of p53 and PTEN in the development of high-grade endometrial carcinoma in older patients. In addition, the findings indicated activation of different molecular pathways in the tumor progression between low-grade and high-grade endometrial carcinomas.

Introduction

Endometrial carcinoma is the most common invasive neoplasm of the female genital tract in the Western world, with a rising incidence. Furthermore, endometrial carcinoma is a significant contributor to gynecological mortality and the fourth most common cancer in women after breast, colon and lung cancer. Endometrial carcinoma primarily affects perimenopausal and postmenopausal women at a median age of diagnosis of 60 years old. Likely risk factors for this disease include diabetes, thyroid disease, hypertension, postmenopausal status, nulliparity, increased obesity, polycystic ovarian syndrome, early menarche and late menopause, radiation exposure, long-term use of unopposed exogenous estrogenic stimulation, a personal history of endometrial hyperplasia or breast cancer, and a family history of endometrial cancer (1-7).

Endometrial carcinoma is classified into two clinicopathological types (type I and type II). Type I endometrial carcinoma is the most common subtype, accounting for >80% of endometrial tumors, and typically has a favorable prognosis. They are usually low-grade, well-differentiated endometrioid adenocarcinomas. These tumors are pathogenetically linked to an excess of unopposed estrogen, arise from endometrial

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hyperplasia and have hormone-receptor positivity. However, type II endometrial carcinoma is a less common type of serous or clear cell adenocarcinoma, accounting for only ~10% of endometrial tumors. They are poorly differentiated, estrogen-independent tumors, which are associated with atrophic endometrium and have poorer outcomes (8,9). Endometrial carcinoma is believed to arise from a variety of genetic alterations involving signaling pathways, activation of proto-oncogenes and inactivation of tumor suppressor genes. The development and progression of each group of endometrial carcinoma follows distinct molecular mechanisms of oncogenesis, reflecting the presence of type-specific genetic alterations. Although there are well-established surgical, radio- and chemotherapeutic treatments, the identification and characterization of biomarkers is necessary for improving the understanding of molecular pathways of the disease and for the development of specific novel molecular targeted therapies, with the aim to achieve greater specificity in tumor progression and metastatic processes, and to accurately evaluate the prognosis, particularly for recurrent and unfavorable disease course (3,5,10,11).

Phosphatase and tensin homolog (PTEN) was identified in 1997, and is a tumor suppressor gene located on chromosome 10 (10q23) that suppresses cell proliferation and differentiation and is involved in the insulin signaling pathway. The protein encoded by this gene is a 55-kDa protein composed of 403 amino acids, which has protein tyrosine phosphatase activities. PTEN protein negatively regulates the phosphatidylinositol 3-kinase (PI3K) signaling pathway. A downstream effector that emanates from PI3K is the Akt protein, which is a serine-threonine kinase. Therefore, PTEN protein can act through the Akt signaling pathway (12-14). PTEN protein under normal physiological conditions has an antagonistic effect on intracellular signaling pathways induced by integrin or growth factors. Furthermore, PTEN protein inhibits intracellular signaling, cell proliferation, cell migration and cellular adhesion formation. PTEN protein can also induce apoptosis in damaged cells (15,16). Notably, PTEN protein lowers the levels of phosphatidylinositol-3,4,5-triphosphate (PIP3) in cells and down regulates cell proliferation by dephosphorylating the 3-position of PIP3, a second messenger of PI3K (14,16-18). In addition, PTEN is a proapoptotic molecule. Overexpression of wild-type PTEN is associated with increased expression of p27, which leads to suppression of cell growth through arrest of the cell cycle in G₁. Previous findings indicated that wild-type PTEN restricts murine double minute 2 (mdm2) to the cytoplasm and promotes p53 function (19,20). However, lack of functional PTEN protein contributes to tumorigenesis by preventing apoptosis and increasing growth and proliferative activity. In addition, loss of PTEN protein function leads to increased activity of mammalian target of rapamycin (mTOR) kinase, which is major downstream effector of Akt. Activation of the mTOR signaling pathway modulates angiogenesis, protein translation, growth and survival signals in neoplastic cells (21,22). PTEN loss occurs through inactivation of the two alleles of PTEN via mutations or deletions, promoter hypermethylation, loss of heterozygosity without mutation, aberrant expression of regulatory microRNA and protein degradation (18,23,24). The majority of mutations of the PTEN gene in tumors are localized in the phosphatase

domain, which influences phosphatase activity (16). Decreased expression of PTEN gene has been indicated in various types of human cancer, including glioblastoma, melanoma, prostate cancer, breast cancer, lung cancer, ovary cancer and endometrial cancer (25). Furthermore, previous studies have revealed that PTEN expression is decreased in endometrial hyperplasia and in endometrial carcinoma compared to proliferative endometrium (14,26,27).

Proapoptotic gene p53 is a tumor suppressor gene, which is located in 17p13.1 and expresses a nuclear 53-kDa phosphoprotein called p53. The p53 protein is a transcription factor that induces the expression of genes necessary for cell cycle arrest at the G₁ checkpoint and promotes the repair of damaged DNA. Additionally, the p53 protein initiates apoptosis (programmed cell death) in case of failed DNA repair (17). The p53 content of cells is maintained at low levels as the protein mdm2 binds with wild-type p53 protein and inhibits p53 transcriptional activity. The protein mdm2 acts as a negative regulator of p53. This p53-mdm2 feedback loop is vital for cell-cycle regulation (28). Mutant forms of p53 are stable and accumulate to high levels intracellularly due to inability of the p53 mutant protein to optimally transactivate its negative regulator, mdm2 (28). Mdm2 also serves an oncogene role independent of p53. Notably, mdm2 overexpression leads to excessive cell proliferation and promotes tumor formation (29). Inactivation of p53 protein provides the neoplastic cells with a higher capacity for division and proliferation, and therefore contributes to malignant change and tumor formation (17,30). Inactivation of p53 protein may occur through mutation of the p53 gene, allelic loss, expansion of its negative regulators or complex formation with other nuclear proteins that are involved in p53-mediated signaling (28). Mutations in the p53 gene can induce changes of the protein conformation and may alter the tumor suppressive function (31). It has been indicated that the PI3K-Akt signaling pathway can be deregulated by inactivation of PTEN or activation of p53, resulting in malignant transformation (32). Notably, wild-type p53 is rapidly degraded and is rarely detectable with immunohistochemistry. Mutant p53 proteins are not degraded and accumulate in the nucleus. The immunohistochemical expression of p53 in the majority of endometrial carcinoma cases results from p53 alterations or functional changes. Furthermore, complete absence of p53 protein can be result from some missense mutations (33-35). In addition, overexpression of p53 protein has been associated with endometrioid carcinoma without gene alterations. Previous findings have indicated that the overexpression of p53 protein is associated with the formation of highly stable protein complexes by the binding of p53 to other overexpressed nuclear proteins, for example mdm-2 protein (36-38). In non-endometrioid endometrial carcinoma, p53 gene mutation and the loss of p53 function are the more common genetic alterations (39-41). Notably, mutational analysis is the gold standard examination for determining p53 status (35).

The purpose of the present study was to investigate the distribution of tumor suppressor genes p53 and PTEN in primary endometrial carcinoma specimens acquired from Greek patients. In addition, the associations of p53 and PTEN as separate factors with well-established clinicopathological prognostic factors, including patient age, histologic type,

clinical stage, histologic grade, depth of myometrial invasion, lymph-vascular space invasion, presence of tumor necrosis and fallopian tube and/or ovarian invasion, were analyzed in order to understand the mechanism of endometrial carcinogenesis and clarify their prognostic significance. This was performed because results in the literature regarding this matter are contradictory (42). Also, the aim of the present study was to analyze the combination of p53 and PTEN expression with well-established clinicopathological prognostic factors and evaluate their prognostic significance by examining their potential interactions in endometrial carcinoma, as such evidence in the literature is poor.

Materials and methods

Patients. A total of 99 women with primary endometrial carcinoma and who underwent surgery were randomly selected and analyzed retrospectively. The mean age of the patients was 64 years old (range, 42-90 years old). The standard primary treatment for patients with endometrial carcinoma and localized disease was surgery, which consisted of total abdominal hysterectomy and salpingo-oophorectomy. Adjuvant radiation therapy was postoperatively administered in patients with $\geq 50\%$ invasion of the myometrium, a histologic grade of 3 or a nonendometrioid histologic type. None of the patients examined had received irradiation, hormonal therapy or chemotherapy prior to surgery. Clinical staging for all patients was performed with computerized tomography scanning and magnetic resonance imaging. Patients with metastases in the pelvic or paraaortic lymph nodes were excluded from the study (FIGO stages IIIc and IVb). In all patients with endometrial carcinoma, the following histopathologic parameters were determined: Histologic type and grade, depth of myometrial invasion, lymphovascular space invasion, fallopian tube and/or ovarian invasion and presence of tumor necrosis. Histologic grades (tumor differentiation) of endometrial carcinomas were based on the ratio of glandular or papillary structures vs. solid tumor growth (grade 1, $<5\%$ solid tumor; grade 2, 6-50% solid; and grade 3, $>50\%$ solid). The depth of myometrial invasion was defined as the percentage of the myometrium invaded by the carcinoma. Lymphovascular invasion was considered to be present when cancerous cells were within or attached to the wall of a capillary-like space.

Histopathologic analysis. For histological examination, endometrial carcinoma specimens were routinely fixed with formalin, embedded in paraffin, sliced into thin sections and stained with hematoxylin and eosin. Four-micrometers-thick sections included sufficient quantities of neoplasm mass. The sections were mounted on silane-coated glass slides.

Immunohistochemical analysis for p53 and PTEN. The following primary antibodies were used for analysis: Mouse monoclonal anti-p53 antibody (clone DO-7; Thermo Fisher Scientific Inc., Waltham, MA, USA) and monoclonal PTEN (clone MMAC; Novocastra, Newcastle, UK). Immunohistochemical staining was performed on tissue sections deparaffinized in xylene, using the standard avidin-biotin-peroxidase complex method with an automated immunostainer (Benchmark XT; Ventana Medical System,

Inc., Tuscon, AZ, USA). Sections were incubated for 45 min at room temperature with a diluted solution of primary antibodies (1:200 for p53 and 1:100 for PTEN). Visualization was performed using a DAKO EnVision immunostainer. The final stage involved dehydration and coverage of the tile.

Evaluation of immunohistochemistry. A total of 100 cells were counted in 10 random fields (with x400 objectives) and the percentage of positive cells was calculated. The semi-quantitative immunoreaction scoring system was evaluated based on the percentage of positive cells added to the stain intensity.

Regarding stain intensity, negative staining was defined as 0, weakly positive was defined as 1, moderately positive as 2 and strongly positive as 3. The scores of immunopositive positive cells were defined as follows: $<5\%$ positive cells was defined as 0 (negative); 5-25% immunopositive positive cells as 1 (low); 25-75% immunopositive cells as 2 (moderate); and $>75\%$ immunopositive positive cells as 3 (high). The sum of the stain intensity and positive cell scores was the result for each section. It was determined as -(0), + (1, 2), ++ (3, 4), and +++ (5, 6). Fig. 1A and B indicate the positive immunohistochemical expression of p53 in the nucleus. Fig. 1C-E indicate the positive immunohistochemical expression of PTEN in the nucleus.

Statistical analysis. Categorical variables were presented as absolute (n) and relative (%) frequencies, while continuous variables were presented as median (min, max). Associations between categorical variables were assessed using exact Pearson's χ^2 test. For continuous variables, differences in the median between two groups were assessed using the Mann-Whitney U test and differences between three groups were assessed with the Kruskal-Wallis test. Correlations between continuous variables were assessed with Spearman's rho (ρ). Statistical significance was set at a two-tailed P-value of <0.05 . Data were analyzed using SPSS software, version 23.0 (IBM Corporation, Armonk, NY, USA).

Results

Assessment of histologic types indicated that 86 (86.9%) cases of endometrial carcinoma were endometrioid and 13 (13.1%) cases were non-endometrioid. Assessment of histologic grades revealed that 20 (20.2%) cases were in grade 1, 49 (49.5%) cases were in grade 2 and 30 (30.3%) cases were in grade 3. According to tumor depth assessment, 34 (34.3%) cases had $<50\%$ myometrial invasion and 65 (65.7%) cases had $>50\%$. Disease clinical stage classification revealed that 68 (68.7%) cases were in stage I, 15 (15.2%) cases were in stage II and 5 (5.1%) cases were in stage III. Lymph-vascular space invasion was identified in 14 (14.1%) cases, while fallopian tube and ovarian invasion was revealed in 19 (19.1%) cases. Tumor necrosis was detected in 7 (7.1%) cases.

Table I indicates the characteristics of the 99 patients with endometrial carcinoma, whereas Table II indicates the clinicopathological parameters of the patients according to the histologic subtypes.

p53 immunohistochemistry. Scores of p53 immunohistochemical expression were not significantly associated with the

Table I. Clinicopathological characteristics of endometrial adenocarcinomas according to histological subtypes.

Clinicopathological parameters	Endometrioid adenocarcinomas (n=86) cases, n (%)	Clear cell and papillary serous adenocarcinomas (n=13) cases, n (%)
Age (years)		
<60	23 (26.7)	0 (0.0)
>60	63 (73.3)	13 (100.0)
Clinical stage		
I	62 (72.1)	6 (46.2)
II	10 (11.6)	5 (38.5)
III	4 (4.7)	1 (7.7)
IV	0 (0.0)	0 (0.0)
Histological differentiation		
G1	20 (23.3)	0 (0.0)
G2	47 (54.7)	2 (15.4)
G3	19 (22.1)	11 (84.6)
Myometrial invasion		
<1/2	32 (37.2)	2 (15.4)
≥1/2	54 (62.8)	11 (84.6)
Lymph-vascular space invasion		
Positive	10 (11.6)	4 (30.8)
Negative	44 (51.2)	7 (53.8)
Fallopian tube and/or ovarian invasion		
Positive	12 (14.0)	7 (53.8)
Negative	25 (29.1)	2 (15.4)
Tumoral necrosis		
Yes	5 (5.8)	2 (15.4)
No	43 (50.0)	9 (69.2)

mean age of the patients ($P=0.131$), histologic types ($P=0.349$), clinical stages ($P=0.100$), histologic grades ($P=0.165$), depth of myometrial invasion ($P=0.323$) or the presence of tumor necrosis ($P=0.313$). However, there was a significant association between lymph-vascular space invasion and scores of immunohistochemical p53 expression ($P=0.007$). In the presence of lymph-vascular space invasion, immunopositivity for p53 was detected in 25-75% of cells in 10 (90.9%) cases and in >75% of cells in 1 (9.1%) case. In the absence of lymph-vascular space invasion, 5-25% immunopositive cells were identified in 17 (33.3%) cases, 25-75% in 22 (43.1%) cases and >75% in 1 (2.0%) case. Patients with lymph-vascular space invasion had a larger percentage of immunopositivity for p53 compared with patients without lymph-vascular space invasion.

The intensity of p53 expression was not significantly associated with the mean age of patients ($P=0.489$), histologic grades ($P=0.539$), histologic types ($P=0.191$), depth of myometrial invasion ($P=0.696$), clinical stage ($P=0.253$), lymph-vascular space invasion ($P=0.185$), the presence of tumor necrosis ($P=0.411$) or fallopian tube invasion ($P=0.321$).

Table III reveals the sum of stain intensity and scores of p53-immunopositive cells and the association of this with the clinicopathological characteristics. There was a significant association between the sum of stain intensity and scores of

p53-immunopositive cells and the age of the patients ($P=0.037$), histologic subtypes ($P=0.008$), histologic grades ($P=0.002$) and fallopian tube and/or ovarian invasion ($P=0.014$). In addition, results implied the association between the sum of stain intensity and scores of p53-immunopositive cells with clinical stage ($P=0.089$).

PTEN immunohistochemistry. The scores of immunohistochemical expression of PTEN were not significantly associated with the mean age of the patients ($P=0.844$), histologic grade ($P=0.352$), lymph-vascular space invasion ($P=0.451$) or the presence of tumor necrosis ($P=1.000$). There was a negative statistical significance between the scores of PTEN immunohistochemical expression and the depth of myometrial invasion ($P=0.002$; $\rho=-0.377$). Among the 28 cases that demonstrated positive immunostaining for PTEN in 5-25% of cells, 6 (21.4%) cases had a depth of myometrial invasion less than half the thickness of the myometrium, 1 (3.6%) case had a depth of myometrial invasion equal to half the thickness of the myometrium, 7 (25.0%) cases had a depth of myometrial invasion equal to two thirds of the thickness of the myometrium, 7 (25.0%) cases had a depth of myometrial invasion equal to three quarters of the thickness of the myometrium and 7 (25.0%) cases had a depth of myometrial invasion equal

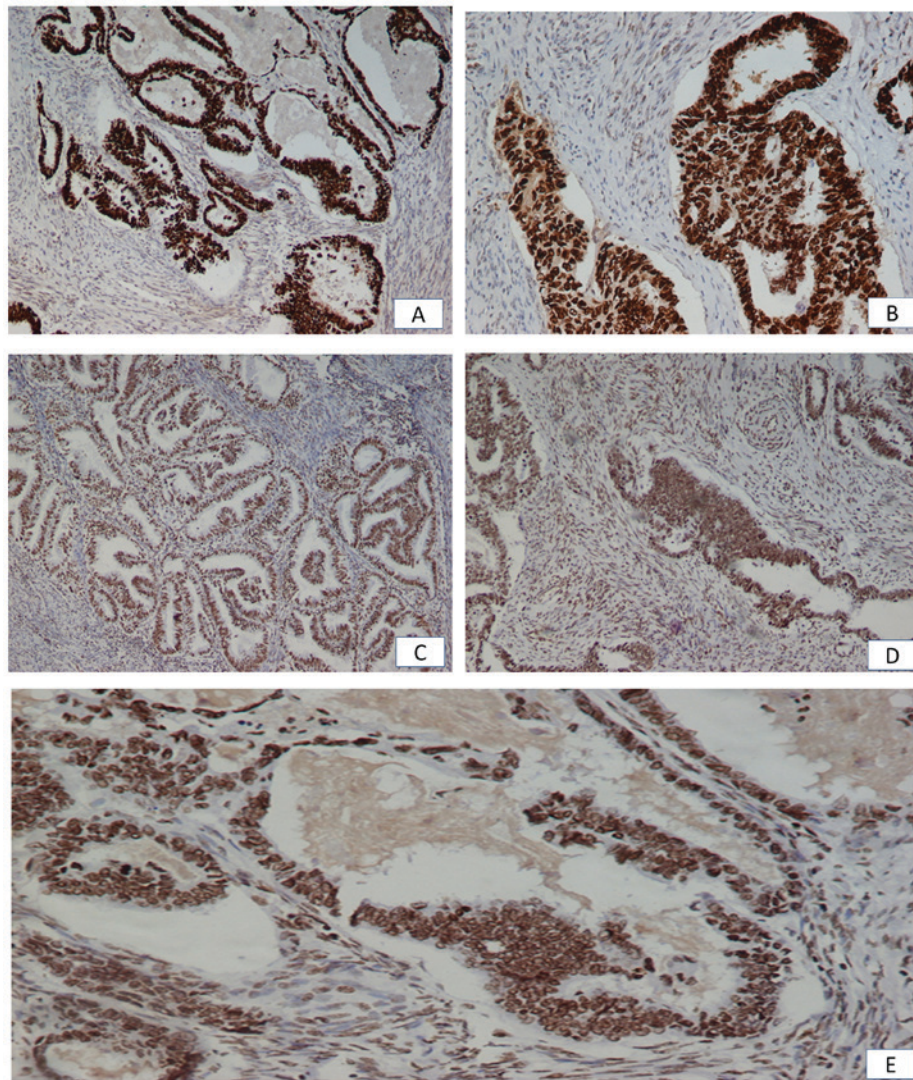


Figure 1. (A) Endometrial carcinoma: Positive immunohistochemical expression of p53 in the nucleus (magnification, x100). (B) Endometrial carcinoma: Positive immunohistochemical expression of p53 in the nucleus (magnification, x200). (C) Endometrial carcinoma: Positive immunohistochemical expression of PTEN in the nucleus (magnification, x100). (D) Endometrial carcinoma: Positive immunohistochemical expression of PTEN in the nucleus (magnification, x200). (E) Endometrial carcinoma: Positive immunohistochemical expression of PTEN in the nucleus (magnification, x400). PTEN, phosphatase and tensin homolog.

to the entire thickness of the myometrium. Regarding the 27 cases that exhibited positive immunostaining for PTEN in 25-75% of cells, 6 (22.2%) cases had a depth of myometrial invasion less than half the thickness of the myometrium, 10 (37.0%) cases had a depth of myometrial invasion equal to half the thickness of the myometrium, 1 (3.7%) case had a depth of myometrial invasion equal to two thirds of the thickness of the myometrium, 4 (14.8%) cases had a depth of myometrial invasion equal to three quarters of the thickness of the myometrium, 2 (7.4%) cases had a depth equal to the superficial lining of the myometrium and 4 (14.8%) cases had a depth of myometrial invasion equal to the entire thickness of the myometrium. Among the 13 cases that demonstrated positive immunostaining for PTEN in >75% of cells, 4 (30.8%) cases had a depth of myometrial invasion less than half the thickness of the myometrium, 3 (23.1%) cases had a depth of myometrial invasion equal to half the thickness of the myometrium, 1 (7.7%) case had a depth of myometrial invasion equal to three quarters of the thickness of the myometrium, 4 (30.8%) cases had a depth equal to the superficial lining of

the myometrium and 1 (7.7%) case had a depth of myometrial invasion equal to the entire thickness of the myometrium.

Notably, there was a significant correlation between the scores of immunohistochemical PTEN expression and the clinical stage ($P=0.019$). Among those classified as clinical stage I, 18 (26.5%) cases exhibited 5-25% PTEN-immunopositive cells, 22 (32.4%) cases exhibited 25-75% PTEN-immunopositive cells and 13 (19.1%) cases exhibited >75% PTEN-immunopositive cells. In clinical stage II, immunopositivity for PTEN was detected in 5-25% of cells in 6 (40.0%) cases, whereas there were no cases with immunopositivity for PTEN in 25-75% or in >75% of cells. Finally, in clinical stage III, 2 (40.0%) cases had 5-25% PTEN-immunopositive cells and another 2 (40.0%) cases exhibited 25-75% PTEN-immunopositive cells.

The intensity of PTEN expression was not significantly associated with the mean age of patients ($P=0.387$), histologic type of the tumor ($P=0.630$), depth of myometrial invasion ($P=0.124$), clinical stage ($P=0.621$), lymph-vascular space invasion ($P=0.442$), presence of tumor necrosis ($P=1.000$) or the presence of fallopian tube invasion ($P=0.524$). Furthermore,

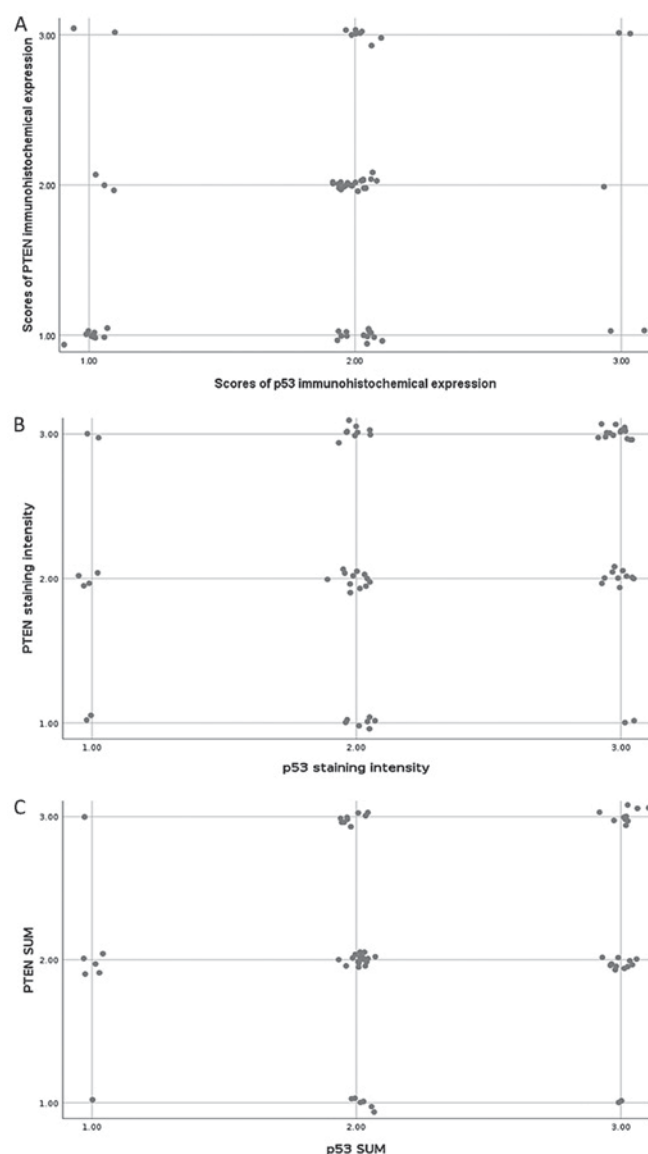


Figure 2. Scatterplot of the association between positive immunostaining scores for p53 and PTEN. (A) The scatterplot was created with jittering of the position of the data points to avoid overplotting. (B) Scatterplot of the association between staining intensity for p53 and PTEN. The scatterplot was created with jittering of the position of the data points to avoid overplotting. (C) Scatterplot of the association between the sum of stain intensity and scores of p53- and PTEN-positive cells. The scatterplots were created with jittering of the position of the data points to avoid overplotting. PTEN, phosphatase and tensin homolog.

the results suggested that there was no significant association was observed between the intensity of PTEN staining and histologic grade ($P=0.071$). Strong positive PTEN expression was observed in 4 (20.0%) cases of histologic grade G1, in 21 (42.9%) cases of grade G2 and in 5 (16.7%) cases of histologic grade G3. The corresponding frequencies for moderate PTEN expression were 9 (45.0%), 17 (34.7%) and 14 (46.7%), respectively.

Table IV indicates the sum of stain intensity and scores of PTEN-immunopositive cells and the association of this with the clinicopathological characteristics. There was no correlation between the sum of stain intensity and scores of PTEN-immunopositive cells and the age of the patients ($P=0.371$), histologic subtype ($P=1.000$), histologic grade

Table II. Characteristics of the 99 endometrial adenocarcinoma patients.

Clinicopathological parameters	No. of patients (%)
Age (years)	
<60	23 (23.2)
≥60	76 (76.8)
Clinical stage	
I	68 (68.7)
II	15 (15.2)
III	5 (5.1)
Histological differentiation	
G1	20 (20.2)
G2	49 (49.5)
G3	30 (30.3)
Myometrial invasion	
<1/2	34 (34.3)
≥1/2	65 (65.7)
Lymph-vascular space invasion	
Positive	14 (14.1)
Negative	51 (51.5)
Fallopian tube and ovarian invasion	
Positive	19 (19.2)
Negative	27 (27.3)
Tumoral necrosis	
Yes	7 (7.1)
No	52 (52.5)

($P=0.439$), myometrial invasion ($P=0.308$), clinical stage ($P=0.259$), ovarian or fallopian tube invasion ($P=0.752$) or the presence of tumor necrosis ($P=1.000$).

Concomitant expression of p53 and PTEN and the association with clinicopathological parameters. According to the scores of immunopositive endometrial carcinoma cells, p53 expression was identified in 73 (85%) cases and PTEN expression was indicated in 64 (74%) cases. According to the intensity of immunopositive cells, p53 and PTEN expression was indicated in 74 (86%) and 66 (77%) cases, respectively. According to the sum of stain intensity and scores of positive cells, endometrial carcinoma samples had a lower proportion of PTEN-positive results (77.1%) compared with p53-positive results (89.2%). Notably, 17% of patients exhibited PTEN(-)/p53(+) expression, whereas 4.8% of patients exhibited PTEN(+)/p53(-). In addition, p53 and PTEN concomitant sum expression was identified in 45% of patients with endometrial adenocarcinoma.

According to the proportion (score) of immunopositive cells, there was a coexistence of p53 and PTEN expression in 53.2% (33/62) of cases (group A) compared with 46.8% (29/62) of cases, in which there was an absence of p53 and PTEN co-expression (group B). Spearman's coefficient for co-expression of p53 and PTEN was $\rho=0.248$ ($P=0.052$), which was marginal for statistical significance. This correlation was indicated in the scatterplot (Fig. 2A). Low concomitant staining was identified in 16.1% of patients, moderate concomitant

Table III. Correlations between clinicopathological characteristics and sum of stain intensity and scores of p53 expression.

Characteristics	Cases, n (%)	IHC results of p53, N (%)				P-value
		0	+	++	+++	
Age (years)						
<60	23 (23.2)	0 (0.0)	4 (33.3)	16 (38.1)	3 (10.3)	0.037
≥60	76 (76.8)	0 (0.0)	8 (66.7)	26 (61.9)	26 (89.7)	
Histological type						
Endometrioid	86 (86.9)	0 (0.0)	12 (100.0)	40 (95.2)	21 (72.4)	0.008
Clear cell and papillary serous	13 (13.1)	0 (0.0)	0 (0.0)	2 (4.8)	8 (27.6)	
Clinical stage						
I	68 (68.7)	0 (0.0)	8 (66.7)	34 (81.0)	17 (58.6)	0.089
II	15 (15.2)	0 (0.0)	1 (8.3)	2 (4.8)	6 (20.7)	
III	5 (5.1)	0 (0.0)	0 (0.0)	1 (2.4)	3 (10.3)	
Histological differentiation						
G1	20 (20.2)	0 (0.0)	3 (25.0)	7 (16.7)	7 (24.1)	0.002
G2	49 (49.5)	0 (0.0)	8 (66.7)	26 (61.9)	6 (20.7)	
G3	30 (30.3)	0 (0.0)	1 (8.3)	9 (21.4)	16 (55.2)	
Myometrial invasion						
<1/2	34 (34.3)	0 (0.0)	5 (41.7)	16 (38.1)	9 (31.0)	0.778
≥1/2	65 (65.7)	0 (0.0)	7 (58.3)	26 (61.9)	20 (69.0)	
Lymph-vascular space invasion						
Positive	14 (14.1)	0 (0.0)	0 (0.0)	6 (14.3)	5 (17.2)	0.101
Negative	51 (51.5)	0 (0.0)	10 (83.3)	22 (52.4)	9 (31.0)	
Fallopian tube and/or ovarian invasion						
Positive	19 (19.2)	0 (0.0)	1 (8.3)	4 (9.5)	8 (27.6)	0.014
Negative	27 (27.3)	0 (0.0)	7 (58.3)	15 (35.7)	4 (13.8)	
Tumoral necrosis						
Yes	7 (7.1)	0 (0.0)	1 (8.3)	2 (4.8)	3 (10.3)	0.524
No	52 (52.5)	0 (0.0)	9 (75.0)	22 (52.4)	10 (34.5)	

P<0.05: Statistically significant results.

staining was identified in 33.9% of patients and high concomitant staining was identified in 3.2% of patients. Additionally, 40.0% of patients with high scores of p53 expression also had high scores of PTEN expression (2/5 patients), whereas 15.4% of patients with high PTEN scores exhibited high scores of p53 (2/13 patients).

According to the staining intensity, weak concomitant staining was indicated in 3.2% of patients, moderate concomitant staining was indicated in 19.0% of patients and strong concomitant staining was indicated in 23.8%. A total of 44.1% of patients with strong levels of p53 expression also exhibited strong PTEN expression (15/34 patients), whereas 50.0% of patients with strong PTEN levels exhibited strong levels of p53 expression (15/30 patients). There was a significantly positive correlation between the intensity of PTEN and p53 staining. Spearman's coefficient for the staining intensity of p53 and PTEN co-expression was $\rho=0.282$ ($P=0.025$; Fig. 2B). This suggests that strong PTEN staining was associated with strong p53 staining and vice versa.

According to the sum of stain intensity and scores of positive cells, + concomitant staining was indicated in 1.6% of

patients, ++ was indicated in 27.4% and +++ was indicated in 16.1% of patients. Notably, 34.5% of patients with +++ p53 staining also had +++ PTEN staining (10/29 patients), whereas 45.5% of patients with +++ PTEN staining levels exhibited +++ p53 staining (10/22 patients). Furthermore, it was demonstrated that the sum of stain intensity and scores of p53-immunopositive cells significantly correlated with PTEN expression ($\rho=0.256$; $P=0.044$; Fig. 2C).

According to the proportion (scores) of immunopositive cells, the age of patients was significantly different between the two groups; 33 cases with the coexistence of p53 and PTEN (group A) and the remaining 29 cases without the coexistence of p53 and PTEN (group B; $P=0.002$).

The scores of immunopositive cells between group A and group B were not significantly associated with the histologic type of the tumor ($P=0.595$), histologic grade ($P=0.259$), depth of myometrial invasion ($P=0.224$), lymph-vascular space invasion ($P=0.253$), presence of tumor necrosis ($P=0.340$) or fallopian tube invasion ($P=1.000$).

To further study the co-expression of p53 and PTEN, patients were divided into three groups that were defined as

Table IV. Correlations between clinicopathological characteristics and sum of stain intensity and scores of PTEN expression.

Characteristics	Cases (N)	Immunohistochemistry results of PTEN (N)				P-value
		0	+	++	+++	
Age (years)						
<60	19	0 (0.0)	1 (10.0)	12 (33.3)	6 (27.3)	0.371
≥60	49	0 (0.0)	9 (90.0)	24 (66.7)	16 (72.7)	
Histological type						
Endometrioid	64	0 (0.0)	9 (90.0)	34 (94.4)	22 (5.5)	1.000
Clear cell and papillary serous	4	0 (0.0)	1 (10.0)	2 (5.6)	1 (4.5)	
Clinical stage						
I	53	0 (0.0)	8 (80.0)	24 (66.7)	21 (95.5)	0.259
II	6	0 (0.0)	1 (10.0)	5 (13.9)	0 (0.0)	
III	4	0 (0.0)	0 (0.0)	3 (8.3)	1 (4.5)	
Histological differentiation						
G1	13	0 (0.0)	1 (10.0)	8 (22.2)	4 (18.2)	0.439
G2	36	0 (0.0)	4 (40.0)	18 (50.0)	14 (63.6)	
G3	19	0 (0.0)	5 (50.0)	10 (27.8)	4 (18.2)	
Myometrial invasion						
<1/2	22	0 (0.0)	3 (30.0)	9 (25.0)	10 (45.5)	0.308
≥1/2	46	0 (0.0)	7 (70.0)	27 (75.0)	12 (54.5)	
Lymph-vascular space invasion						
Positive	11	0 (0.0)	3 (30.0)	6 (16.7)	2 (9.1)	0.292
Negative	24	0 (0.0)	4 (40.0)	19 (52.8)	1 (4.5)	
Fallopian tube and ovarian invasion						
Positive	8	0 (0.0)	1 (10.0)	7 (19.4)	0 (0.0)	0.752
Negative	18	0 (0.0)	4 (40.0)	13 (36.1)	1 (4.5)	
Tumoral necrosis						
Yes	5	0 (0.0)	1 (10.0)	4 (11.1)	0 (0.0)	1.000
No	24	0 (0.0)	4 (40.0)	19 (52.8)	1 (4.5)	

follows: Patients with low p53 and PTEN expression scores; patients with moderate expression scores of either p53 or PTEN; and patients with high expression scores of p53 and PTEN. Table V summarizes the distribution of the co-expression of p53 and PTEN in endometrial carcinomas according to scores of immunopositive cells in correlation with clinicopathological characteristics. Notably, there was a correlation between the scores of p53 and PTEN co-expression and the age of the patients ($P=0.008$) and histologic grade ($P=0.028$). The findings also suggested a correlation between the scores of p53 and PTEN co-expression and lymphovascular invasion ($P=0.084$). Table VI indicates the distribution of p53 and PTEN co-expression in endometrial carcinomas according to the stain intensity in correlation with clinicopathological characteristics. Furthermore, Table VII demonstrates p53 and PTEN co-expression in endometrial carcinomas according to the sum of stain intensity and immunoexpression scores.

Discussion

The overall rate of p53 and PTEN positivity in the present study was 89 and 77%, respectively, according to sum of stain intensity and scores of immunopositive cells. In the study, the

intensity of p53 and PTEN staining was positively correlated ($\rho=0.282$; $P=0.025$). Furthermore, the sum of stain intensity and immunohistochemical scores of p53 was positively correlated with PTEN expression ($\rho=0.256$; $P=0.044$). The findings indicate an intrinsic association between the overexpression of the two major tumors suppressor genes, p53 and PTEN. This supports the previous suggestions that p53 induces PTEN expression and PTEN reduces p53-induced degradation (20). Notably, p53 and PTEN concomitant expression was demonstrated in 45% of patients with endometrial adenocarcinoma, and was considered a common event.

Previous findings have indicated that p53 alterations seem to occur at early and late phases of endometrial carcinogenesis (43,44). Early involvement of p53 alterations in endometrial carcinogenesis has been suggested because p53 has been indicated to be expressed in endometrial glands adjacent to endometrial carcinoma and it is associated with endometrial hyperplasia (30). In the present study, no correlation was indicated with the sum of stain intensity and scores of p53-immunopositive cells and clinical stage ($P=0.089$), depth of myometrial invasion ($P=0.778$) or lymph-vascular space invasion ($P=0.101$). Therefore, the findings support the hypothesis that p53 alterations occur at early and late phases of

Table V. Co-expression of p53 and PTEN in endometrial carcinomas according to scores of immunopositive cells in relation to clinicopathological parameters.

Characteristics	Patients with p53 and PTEN low scores expression cases, n (%)	Patients with either p53 or PTEN moderate scores expression cases, n (%)	Patients with p53 and PTEN high scores expression cases, n (%)	P-value
Age (years)				
<60	7 (70.0)	15 (24.6)	0 (0.0)	0.008
≥60	3 (30.0)	46 (75.4)	2 (100.0)	
Histological type				
Endometrioid	10 (100.0)	53 (86.9)	1 (50.0)	0.106
Clear cell and papillary serous	0 (0.0)	8 (13.1)	1 (50.0)	
Clinical stage				
I	9 (90.0)	44 (72.1)	2 (100.0)	0.876
II	1 (10.0)	4 (6.6)	0 (0.0)	
III	0 (0.0)	5 (8.2)	0 (0.0)	
Histological differentiation				
G1	2 (20.0)	14 (23.0)	0 (0.0)	0.028
G2	8 (80.0)	27 (44.3)	0 (0.0)	
G3	0 (0.0)	20 (32.8)	2 (100.0)	
Myometrial invasion				
<1/2	3 (30.0)	22 (36.1)	0 (0.0)	0.651
≥1/2	7 (70.0)	39 (63.9)	2 (100.0)	
Lymph-vascular space invasion				
Yes	0 (0.0)	11 (18.0)	0 (0.0)	0.084
No	9 (90.0)	23 (37.7)	0 (0.0)	
Fallopian tube and/or ovarian invasion				
Yes	1 (10.0)	8 (13.1)	0 (0.0)	0.642
No	5 (50.0)	17 (27.9)	0 (0.0)	
Tumoral necrosis				
Yes	1 (10.0)	4 (6.6)	0 (0.0)	1.000
No	8 (80.0)	24 (39.3)	0 (0.0)	

P<0.05: Statistically significant results.

the endometrial carcinoma progression. In the literature, it has been demonstrated that overexpression of p53 in endometrioid adenocarcinomas of the uterus were significantly higher in serous papillary (in 75-90% of cases) compared with endometrioid endometrial carcinomas (in 10-35% of cases) (45-70). In patients with endometrial carcinoma, overexpression of p53 has been indicated to be a significantly negative prognostic factor and associated with poor differentiation, advanced stage, increased myometrial invasion, positive lymph node involvement and distant metastases (71-81). In the present study, there was a significant association between the scores of immunohistochemical p53 expression and lymph-vascular invasion ($P=0.007$), suggesting that a larger percentage of p53-immunopositive cells in endometrial carcinoma may be involved in the metastatic process of the disease. In addition, the sum of stain intensity and scores of p53 expression were significantly correlated with patient age ($P=0.037$), histologic type ($P=0.008$), histologic grade ($P=0.002$) and fallopian and/or

ovarian invasion ($P=0.014$). The present findings indicate that p53 protein expression serves an important role in the differentiation and extension process of endometrial neoplastic cells in older patients. Daniilidou *et al* (70) revealed p53 expression, as a separate factor, was correlated with stage but not with histologic grade of endometrioid endometrial adenocarcinoma; positive p53 expression correlated with stage IIIC, while the absence of p53 expression was connected with stages IB and IC. A key difference between the present study and the study by Daniilidou *et al* (70) was that all endometrial carcinomas (including endometrioid, clear cell and serous papillary adenocarcinomas) were examined as a whole in relation to the clinicopathological factors in the present study, whereas Daniilidou *et al* (70) separately studied the clinicopathological and immunohistochemical properties for endometrioid and serous papillary adenocarcinomas. The different results probably reflect the different pathways of carcinogenesis of type I and II endometrial carcinoma. In the literature, a reduced 5-year

Table VI. Co-expression of p53 and PTEN in endometrial carcinomas according to stain intensity of immunopositive cells in relation to clinicopathological parameters.

Characteristics	Patients with p53 and PTEN weak positive expression cases, n (%)	Patients with either p53 or PTEN moderate positive expression cases, n (%)	Patients with p53 and PTEN strong positive expression cases, n (%)	P-value
Age (years)				
<60	1 (50.0)	16 (31.4)	2 (13.3)	0.261
≥60	1 (50.0)	35 (68.6)	13 (86.7)	
Histological type				
Endometrioid	2 (100)	48 (94.1)	14 (93.3)	1.000
Clear cell and papillary serous	0 (0.0)	3 (5.9)	1 (6.7)	
Clinical stage				
I	2 (100.0)	39 (76.5)	14 (93.3)	0.685
II	0 (0.0)	5 (9.8)	1 (6.7)	
III	0 (0.0)	3 (5.9)	0 (0.0)	
Histological differentiation				
G1	1 (50.0)	9 (17.6)	4 (26.7)	0.801
G2	1 (50.0)	28 (54.9)	7 (46.6)	
G3	0 (0.0)	14 (27.5)	4 (26.7)	
Myometrial invasion				
<1/2	1 (50.0)	16 (31.4)	7 (46.7)	0.513
≥1/2	1 (50.0)	35 (68.6)	8 (53.3)	
Lymph-vascular space invasion				
Yes	0 (0.0)	8 (15.7)	1 (6.7)	1.000
No	1 (50.0)	27 (52.9)	2 (13.3)	
Fallopian tube and/or ovarian invasion				
Yes	0 (0.0)	7 (13.7)	1 (6.7)	1.000
No	1 (50.0)	18 (35.3)	1 (6.7)	
Tumoral necrosis				
Yes	0 (0.0)	4 (7.8)	1 (6.7)	0.488
No	1 (50.0)	26 (51.0)	2 (13.3)	

P<0.05: Statistically significant results.

survival has been demonstrated (71,75,80). However, there is controversy regarding the independent prognostic value of p53 expression using multivariate analysis. In particular, there are studies that have indicated p53 expression as an independent prognostic factor compared with FIGO stage, tumor grade and myometrial invasion (71,75,79,82), whereas other studies have failed to demonstrate such independent prognostic value of p53 expression (42,76,81,83). As a result, there are reservations about the routine use of this marker in clinical practice. For this reason, it is very important to examine how the expression of p53 potentially interacts with other tumor suppressor genes, and the prognostic significance of their concomitant expression in endometrial carcinoma.

In endometrial carcinoma, particularly in type I, mutations of PTEN have been described to occur in 25-83% of cases; however, mutations of PTEN have also been described to occur in endometrial hyperplasia (~55%) (13,15,84-88). In a study by Lacey *et al* (26), loss of PTEN expression in

biopsies of endometrial hyperplasia was not associated with subsequent risk of endometrial carcinoma. Accordingly, inactivation of PTEN may be considered a crucial factor for early endometrial carcinogenesis. PTEN gene mutations have been revealed in more advanced stages of endometrial carcinoma (15). Loss of heterozygosity at chromosome 10q23 occurs in ~40% of endometrial carcinomas (89,90). It has been indicated that loss of PTEN expression was associated with endometrioid histology, and inversely associated with the presence of lymphovascular space invasion (91). Risinger *et al* (84) indicated that PTEN mutations were associated with low-grade and low-stage endometrial carcinomas, whereas Konopka *et al* (15) revealed a significant correlation between PTEN gene mutations and histologic grade of endometrial carcinomas, suggesting that defects in PTEN gene are associated with increased malignancy due to the loss of the ability of endometrial cells to differentiate. Other studies have indicated no correlation between PTEN expression and

Table VII. Co-expression of p53 and PTEN in endometrial carcinomas according to sum of stain intensity and scores of immunopositive cells in relation to clinopathological parameters.

Characteristics	Patients with p53 and PTEN + expression cases, n (%)	Patients with either p53 or PTEN + + expression cases, n (%)	Patients with p53 and PTEN + + + expression cases, n (%)	P-value
Age (years)				
<60	1 (100.0)	20 (32.8)	1 (10.0)	0.122
≥60	0 (0.0)	41 (67.2)	9 (90.0)	
Histological type				
Endometrioid	1 (100.0)	57 (93.4)	9 (90.0)	1.000
Clear cell and papillary serous	0 (0.0)	4 (6.6)	1 (10.0)	
Clinical stage				
I	1 (100.0)	46 (75.4)	10 (100.0)	0.548
II	0 (0.0)	6 (9.8)	0 (0.0)	
III	0 (0.0)	3 (4.9)	0 (0.0)	
Histological differentiation				
G1	0 (0.0)	11 (18.0)	4 (40.0)	0.594
G2	1 (100.0)	34 (55.7)	4 (40.0)	
G3	0 (0.0)	16 (26.2)	2 (20.0)	
Myometrial invasion				
<1/2	1 (100.0)	20 (32.8)	5 (50.0)	0.271
≥1/2	0 (0.0)	41 (67.2)	5 (50.0)	
Lymph-vascular space invasion				
Yes	0 (0.0)	10 (16.4)	0 (0.0)	0.762
No	1 (100.0)	31 (50.8)	0 (0.0)	
Fallopian tube and/or ovarian invasion				
Yes	0 (0.0)	9 (14.8)	0 (0.0)	1.000
No	1 (100.0)	21 (34.4)	0 (0.0)	
Tumoral necrosis				
Yes	0 (0.0)	5 (8.2)	0 (0.0)	1.000
No	1 (100.0)	31 (50.8)	0 (0.0)	

P<0.05: Statistically significant results.

standard prognostic factors (14,39,92-94). In the present study, the immunohistochemical scores of PTEN expression were negatively associated with myometrial invasion ($P=0.002$; $\rho=-0.377$). The lower levels of positive PTEN immunostaining scores were associated with deeper myometrial invasion and vice versa. Furthermore, an association was identified between clinical stages and the immunohistochemical scores of PTEN expression ($P=0.019$). Patients at clinical stage I had higher positive immunostaining scores, whereas patients at clinical stage II had lower scores. The findings support the hypothesis that lower PTEN expression in endometrial carcinoma occurs in later stages of endometrial carcinogenesis. However, when the sum of stain intensity and scores of PTEN expression were examined, no significant correlations between the age of patients, histologic type, clinical stage, histologic differentiation, myometrial invasion, lymph-vascular space invasion, fallopian and/or ovarian invasion or tumor necrosis were indicated. Daniilidou *et al* (70) indicated an association between PTEN expression and histologic grade of endometrioid

endometrial adenocarcinoma. Notably, the negative expression of PTEN correlated with grade 3, whereas positive PTEN expression correlated with grades I and II (70). In addition, their study revealed an association between PTEN expression and stage of endometrioid endometrial adenocarcinomas (negative expression of PTEN correlated with stages IC and IIC, while positive PTEN expression with stage IB). The findings in the literature regarding the loss PTEN protein expression and clinical outcome in endometrial carcinomas are inconsistent. Some studies have reported more favorable survival (14,28,29,91,95,96), while other studies have indicated less favorable prognosis (19,90,97,98). Terakawa *et al* (97) suggested that overexpression of PTEN is a significant prognostic indicator of improved overall survival for patients with advanced endometrial carcinoma who undergo postoperative chemotherapy, as PTEN was able to increase the chemosensitivity of neoplastic cells.

In the literature, it is apparent that concomitant genetic alterations may have a prognostic value in endometrial

carcinoma. It has been indicated that concomitant PI3K-Akt and p53 alterations were associated with poor prognosis (99). In addition, simultaneous activations of p53 and microsatellite instability were strong genetic prognostic factors for disease-free survival (100). Furthermore, Uegaki *et al* (101) demonstrated that PTEN-positive and phosphorylated-AKT-negative expression is a predictor of survival for patients with advanced endometrial carcinoma. In the present study, an association of the p53 and PTEN co-expression with well-established clinicopathological factors in patients with endometrial carcinoma was indicated, which opposed the findings of Daniilidou *et al* (70), in which there was no such correlation. The levels of concomitant p53 and PTEN expression, according to the scores of immunopositive cells, were correlated with the age of patients ($P=0.008$) and histologic differentiation ($P=0.028$) in the present study. These results suggested that p53 and PTEN co-expression may serve a role in the development of high-grade endometrial carcinoma in older patients. The present findings also suggest the involvement of different molecular pathways in the development of low-grade and high-grade endometrial carcinoma. The findings also suggested a correlation with lymphovascular invasion ($P=0.084$), whereas no correlation was identified between the co-expression of p53 and PTEN in endometrial carcinoma (according to the stain intensity or the sum of stain intensity and immunoexpression scores) or clinicopathological characteristics. Therefore, the present study indicated that concomitant p53 and PTEN expression may contribute to the characterization of tumor behavior in endometrial carcinoma. Because the findings of the present study indicated the expression of p53 was positively associated with the levels of PTEN expression in endometrial carcinoma, it was suggested that further molecular studies to estimate and determine the impact of the co-expression of these molecular factors on patient survival of the disease are required.

To conclude, the present results suggest a strong correlation between the expression of p53 and PTEN in endometrial adenocarcinoma, indicating an intrinsic association between the expression of these tumor suppressor genes. In addition, according to the scores of immunopositive cells, which were correlated with the age of patients and the histologic differentiation, concomitant p53 and PTEN expression may contribute to the characterization of tumor behavior in endometrial carcinoma. The findings suggest that combination of p53 and PTEN expression may serve a role in the development of high-grade endometrial carcinoma in older patients. Furthermore, the results imply the involvement of different molecular pathways between the progression of low-grade and high-grade endometrial carcinoma.

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Availability of data and materials

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Authors' contributions

All authors were responsible for the conception and design of the present study. TV and AT were responsible for the provision of the study materials. TV, AT, VKV and FNV were responsible for the collection and assembly of the data. AS, MV, TV, VKV, AT, FNV, AN, NK and ACL performed the data analysis and interpretation. AS, MV, TV, VKV, AT, FNV, AN, NK and ACL contributed in writing the manuscript. AS, MV, TV, VKV, AT, FNV, AN, NK and ACL read and gave the final approval of the manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Medical School of Kapodistrian University of Athens, Greece. The patient included in the case provided consent for her data to be used in this publication.

Patient consent for publication

All the patients included in this study at the time of data collection provided consent for their data to be used in this publication.

Competing interests

The authors declare that they have no competing interests.

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