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 ($P=0.008$) 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 estrogen 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...
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. Adjunct radiation therapy was postoperatively administered in patients with ≥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 IIc and IVb). In all patients with endometrial carcinoma, the following histopathological 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 χ² 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
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; ρ=−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

| 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)                                                     |
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, 4 (14.8%) cases had a depth of myometrial invasion equal to two thirds 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 equal to half the thickness of the myometrium, 3 (23.1%) cases had a depth of myometrial invasion equal to three quarters of the thickness of the myometrium, 1 (7.7%) case had a depth of myometrial invasion 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,
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 (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 ρ=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 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)           |
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 (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

| Characteristics       | Cases, n (%) | IHC results of p53, N (%) |
|-----------------------|-------------|--------------------------|
| Age (years)           |             |                          |
| <60                   | 23 (23.2)   | 0 (0.0)                  |
| ≥60                   | 76 (76.8)   | 4 (33.3)                 |
| Histological type     |             |                          |
| Endometroid           | 86 (86.9)   | 0 (0.0)                  |
| Clear cell and papillary serous | 13 (13.1)   | 12 (100.0)               |
| Clinical stage        |             |                          |
| I                     | 68 (68.7)   | 0 (0.0)                  |
| II                    | 15 (15.2)   | 0 (0.0)                  |
| III                   | 5 (5.1)     | 0 (0.0)                  |
| Histological differentiation |         |                          |
| G1                    | 20 (20.2)   | 0 (0.0)                  |
| G2                    | 49 (49.5)   | 0 (0.0)                  |
| G3                    | 30 (30.3)   | 0 (0.0)                  |
| Myometrial invasion   |             |                          |
| <1/2                  | 34 (34.3)   | 0 (0.0)                  |
| ≥1/2                  | 65 (65.7)   | 5 (41.7)                 |
| Lymph-vascular space invasion |        |                          |
| Positive              | 14 (14.1)   | 0 (0.0)                  |
| Negative              | 51 (51.5)   | 10 (83.3)                |
| Fallopian tube and/or ovarian invasion | |                          |
| Positive              | 19 (19.2)   | 0 (0.0)                  |
| Negative              | 27 (27.3)   | 7 (58.3)                 |
| Tumoral necrosis      |             |                          |
| Yes                   | 7 (7.1)     | 0 (0.0)                  |
| No                    | 52 (52.5)   | 9 (75.0)                 |

| Characteristics       | IHC results of PTEN, N (%) |
|-----------------------|---------------------------|
| Age (years)           |                          |
| <60                   | 0 (0.0)                  |
| ≥60                   | 8 (66.7)                 |
| Histological type     |                          |
| Endometroid           | 12 (100.0)               |
| Clear cell and papillary serous | 0 (0.0)   |
| Clinical stage        |                          |
| I                     | 8 (66.7)                 |
| II                    | 1 (8.3)                  |
| III                   | 0 (0.0)                  |
| Histological differentiation |         |                          |
| G1                    | 3 (25.0)                 |
| G2                    | 8 (66.7)                 |
| G3                    | 1 (8.3)                  |
| Myometrial invasion   |                          |
| <1/2                  | 5 (41.7)                 |
| ≥1/2                  | 7 (58.3)                 |
| Lymph-vascular space invasion |        |                          |
| Positive              | 6 (14.3)                 |
| Negative              | 22 (52.4)                |
| Fallopian tube and/or ovarian invasion | |                          |
| Positive              | 4 (9.5)                  |
| Negative              | 15 (35.7)                |
| Tumoral necrosis      |                          |
| Yes                   | 2 (4.8)                  |
| No                    | 22 (52.4)                |

P<0.05: Statistically significant results.

Table III. Correlations between clinicopathological characteristics and sum of stain intensity and scores of p53 expression.
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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 immunohistochemical 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 (p=0.282; P=0.025). Furthermore, the sum of stain intensity and immunohistochemical scores of p53 was positively correlated with PTEN expression (p=0.256; P=0.044). The findings indicate an intrinsic association between the overexpression of the two major tumor 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 IV. Correlations between clinicopathological characteristics and sum of stain intensity and scores of PTEN expression.

| Characteristics                  | Cases (N) | 0   | +   | ++  | +++ | P-value |
|----------------------------------|-----------|-----|-----|-----|-----|---------|
| 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)  |         |
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 immunopositive cells in relation to clinopathological 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

| 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.
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

| 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.
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 endometrioid 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

| 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.
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 immunexpression 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.

References

1. Liu FS: Molecular carcinogenesis of endometrial cancer. Taiwan J Obstet Gynecol 46: 26-32, 2007.
2. Sasański A, Jonašienė V, Krikštopioniene A, Butkūtytė S, Dabkevičienė D, Kanopišienė D, Kazbariene B and Didžiapetrenienė J: NOTCH1, NOTCH3, NOTCH4, and JAG2 protein levels in human endometrial cancer. Medicina (Kaunas) 50: 14-18, 2014.
3. Elbasatemy SS, Salem AA, Abdelsalam WA and Salem R: Immunohistochemical expression of cancer stem cell related markers CD44 and CD133 in endometrial cancer. Pathol Res Pract 212: 10-16, 2016.
4. Li Y, Zhang X, Ge J, Liu X, Xu S, Zhu Z, Fang G, Liu J, Zhang H and Sun X: Can Nup88 expression be associated with atypical endometrial hyperplasia and endometrial cancer? A preliminary study. Pathol Res Pract 212: 274-278, 2016.
5. Agopianz M, Forgez P, Casse JM, Lacomme S, Charras-Brunaud C, Clerc-Urmès I, Morèl O, Bonnet C, Guéant JL, Vignaud JM, et al: Expression of neurotensin receptor 1 in endometrial adenocarcinoma is correlated with histological grade and clinical outcome. Virchows Arch 471: 521-530, 2017.
6. Khabaz MN, Abdelrahman AS, Butt NS, Al-Maghribi B and Al-Maghribi J: Cyclin D1 is significantly associated with stage of tumor and predicts poor survival in endometrial carcinoma patients. Ann Diagn Pathol 30: 47-51, 2017.
7. Mittal P, Klingler-Hoffmann M, Arentz G, Winderbaum L, Kaur G, Anderson L, Scurry J, Leung Y, Stewart CJ, Carter J, et al: Annexin A2 and alpha actinin 4 expression correlates with metastatic potential of primary endometrial cancer. Biochim Biophys Acta Proteins Proteom 1865: 846-857, 2017.
8. Qu M, Bao W, Wang J, Yang T, He X, Liao Y and Wan X: FOXA1 promotes tumor cell proliferation through AR involving the Notch pathway in endometrial cancer. BMC Cancer 14: 78, 2014.
Significantly decreased P27 expression, et al., Khabele D, Liang SX, Zheng W, Mohammed K, et al.: PTEN loss is a marker of progression to endometrial carcinoma. Cancer Res 68: 25-30, 2008.

Possible insights on pathogenesis discerned from immunohistochemical analysis of PTEN in endometrial carcinoma of the endometrium with nodal metastases: Fadare O and Parksh V: p53 aberrations in low grade endometrial cancer. Cancer 17: 697-704, 2007.

Immunohistochemical expression of PTEN in normal, hyperplastic, and carcinomatous endometrium. Int J Gynecol Cancer 14: 938-946, 2004.

Marks of the p53 pathway further refine molecular profiling in high-risk endometrial cancer: A TransPORTEC initiative. Gynecol Oncol 146: 327-333, 2017.

Stabilic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtoscs C, Sasaki T, Ruland J, Penninger JM, Sidorov DP and Mak TW: Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell 95: 29-39, 1998.

Scully MR and Helgeson LK, Wu LS and Jackson TA: Rapid estrogen signaling negatively regulates PTEN activity through phosphorylation in endometrial cancer cells. Horm Cancer 5: 218-231, 2014.

Erlkanl S, Kayaselu F, Kucsu E, Bagis T, Bolat F, Haberal A and Donihan B: Expression of survivin, PTEN and p27 in normal, hyperplastic, and carcinomatous endometrium. Int J Gynecol Cancer 16: 1412-1418, 2006.

Assessment of the quality and frequency of mutations in PTEN gene in endometrial carcinomas and hyperplasias. Cancer Lett 207: 47-51, 2004.

Kumura F, Watanahe J, Hata H, Fujisawa T, Kamata Y, Nishimura Y, Joho T and Kuramoto H: PTEN immunohistochemical expression is suppressed in GI endometrioid adenocarcinoma of the uterus corpus. J Cancer Res Clin Oncol 130: 161-168, 2004.

Maioli DP and Monaghan H: PTEN, C-erbB-2 and differentiative uterine serous papillary carcinoma from endometrioid endometrial carcinoma. Int J Gynecol Cancer 14: 938-946, 2004.

Kapucuoglu N, Aktepe F, Kaya H, Bircan S, Karahan N and Ciric M: Immunohistochemical expression of PTEN in normal, hyperplastic and malignant endometrium and its correlation with hormone receptors, bcl-2, bax, and apoptotic index. Pathol Res Pract 203: 153-162, 2007.

Kanamori Y, Kigawa J, Itamochi H, Sultana H, Suzuki M, Ohwada M, Kamura T, Sugiyama T, Kikuchi Y, Kita T, et al.: PTEN expression is associated with prognosis for patients with advanced endometrial carcinoma undergoing postoperative chemotherapy. Int J Cancer 100: 686-689, 2002.

Mayo LD, Dixon JE, Durden DL, Tonks DK and Donner DB: PTEN protects p53 from MDM2 and sensitizes cancer cells to chemotherapy. J Biol Chem 277: 5484-5489, 2002.

Garg K, Broadus RR, Soslow RA, Ubrauer DL, Levine DA and Dowsett M: Immunohistochemical expression of PTEN in normal, hyperplastic and malignant endometrium and its correlation with clinicopathological factors and patient outcome. Clin Exp Metastasis 24: 503-517, 2007.

Pallares J, Bussaglia E, Martinez-Guitarte JL, Dolcet X, Llobet D, Pallares J, Bussaglia E, Martínez-Guitarte JL, Dolcet X, Llobet D, Ciriş M, Bussaglia E, Martínez-Guitarte JL, Dolcet X, Llobet D, Ciriş M and Djordjevic B: Pathologic scoring of PTEN immunohistochemistry in different types of endometrial lesions. Med Sci Monit 22: 1705-1710, 2016.

Gu X, Liu Q, Yang N, Shen JF, Zhang XG, Cao F and Ding HZ: Clinicopathological significance of increased ZIC1 expression in human endometrial cancer. J Huazhong Univ Sci Technol Med Sci 30: 890-903, 2010.

Bansal N, Yendluri V and Wenham RM: The molecular biology of endometrial cancers and the implications for pathogenesis, classification, and targeted therapies. Cancer Control 16: 8-13, 2009.

Dohi S, Ohno S, Ohno Y, Kyo S, Soma G, Sugiyama H and Inoue M: WT1 expression correlates with angiogenesis in endometrial carcinomas. Cancer Lett 160: 1-8, 2001.
89. Peiffer SL, Herzog TJ, Tribune DJ, Mutch DG, Gersell DJ and Goodfellow PF: Allelic loss of sequences from the long arm of chromosome 10 and replication errors in endometrial cancers. Cancer Res 55: 1922-1926, 1995.

90. Nagase S, Sato S, Tezuka F, Wada Y, Yajima A and Horii A: Deletion mapping on chromosome 10q25-q26 in human endometrial cancer. Br J Cancer 74: 1979-1983, 1996.

91. Akiyama-Abe A, Minaguchi T, Nakamura Y, Michikami H, Shikama A, Nakao S, Sakurai M, Ochi H, Onuki M, Matsumoto K, et al.: Loss of PTEN expression is an independent predictor of favourable survival in endometrial carcinomas. Br J Cancer 109: 1703-1710, 2013.

92. Bussaglia E, del Rio E, Matias-Guiu X and Prat J: PTEN mutations in endometrial carcinomas: A molecular and clinicopathologic analysis of 38 cases. Hum Pathol 31: 312-317, 2000.

93. Kanomori Y, Kigawa J, Itamochi H, Shimada M, Takahashi M, Kamazawa S, Sato S, Akeshima R and Terakawa N: Correlation between loss of PTEN expression and Akt phosphorylation in endometrial carcinoma. Clin Cancer Res 7: 892-895, 2001.

94. Minaguchi T, Yoshikawa H, Oda K, Ishino T, Yasugi T, Onda T, Nakagawa S, Matsumoto K, Kawana K and Taketani Y: PTEN mutation located only outside exons 5, 6, and 7 is an independent predictor of favorable survival in endometrial carcinomas. Clin Cancer Res 7: 2636-2642, 2001.

95. Dellas A, Jundt G, Sartorius G, Schneider M and Moch H: Combined PTEN and p27kip1 protein expression patterns are associated with obesity and prognosis in endometrial carcinomas. Clin Cancer Res 15: 2456-2462, 2009.

96. Mackay HJ, Gallinger S, Tsao MS, McLachlin CM, Tu D, Keiser K, Eisenhauer EA and Oza AM: Prognostic value of microsatellite instability (MSI) and PTEN expression in women with endometrial cancer: Results from studies of the NCIC Clinical Trials Group (NCIC CTG). Eur J Cancer 46: 1365-1373, 2010.

97. Terakawa N, Kanomori Y and Yoshida S: Loss of PTEN expression followed by Akt phosphorylation is poor prognostic factor for patients with endometrial cancer. Endoerc Relat Cancer 10: 203-208, 2003.

98. Salvesen HB, Stefansson I, Kalvenes MB, Das S and Akslen LA: Loss of PTEN expression is associated with metastatic disease in patients with endometrial carcinoma. Cancer 94: 2185-2191, 2002.

99. Catasus L, Gallardo A, Cuatrecasas M and Prat J: Concomitant PDK-AKT and p53 alterations in endometrial carcinomas are associated with poor prognosis. Mod Pathol 22: 522-529, 2009.

100. Nout RA, Bosse T, Creutzberg CL, Jürgenliemk-Schulz IM, Jobsen JJ, Lutgens LC, van der Steen-Banasik EM, van Eijk R, Ter Haar NT and Smit VT: Improved risk assessment of endometrial cancer by combined analysis of MSI, PI3k-AKT, Wnt/-β-catenin and P53 pathway activation. Gynecol Oncol 126: 466-473, 2012.

101. Uegaki K, Kanomori Y, Kigawa J, Kawaguchi W, Kaneko R, Naniwa J, Takahashi M, Shimada M, Oishi T, Itamochi H and Terakawa N: PTEN-positive and phosphorylated-Akt-negative expression is a predictor of survival for patients with advanced endometrial carcinoma. Oncol Rep 14: 389-392, 2005.