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Concomitant PI3K–AKT and p53 alterations in endometrial carcinomas are associated with poor prognosis

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The status of p53 and the phosphatidylinositol 3-kinase–AKT (PI3K–AKT) signaling pathway was investigated in 132 endometrial carcinomas, including endometrioid endometrial carcinomas, non-endometrioid endometrial carcinomas, and mixed endometrioid adenocarcinomas-non-endometrioid adenocarcinomas. Results were compared with the clinicopathologic parameters associated with prognosis, patients' follow-up, and other genetic alterations found frequently in these tumors. Molecular genetic differences between low-grade and high-grade endometrioid adenocarcinomas were encountered; ie, *PIK3CA* mutations were detected in 26 and 34% of cases, respectively. We found *p53* alterations in only 17% of high-grade endometrioid adenocarcinomas. In contrast, non-endometrioid adenocarcinomas had a higher frequency of *p53* alterations (54%), *PIK3CA* mRNA overexpression (45%), and exon 20 *PIK3CA* mutations (21%). In the mixed endometrioid adenocarcinomas-non-endometrioid adenocarcinomas, the most frequent alterations were *p53* (50%) and *PIK3CA* (44%) mutations, followed by *PTEN* mutations (38%). In some cases, *p53* and *PIK3CA* alterations coexisted, but they rarely coexisted with the *PTEN* mutations. Our findings suggest that the *PIK3CA* mutations are frequent events in endometrial carcinomas of any histological type. However, location of the *PIK3CA* mutations, either in exon 9 or exon 20, varies significantly according to the histologic grade and type of carcinoma. Carcinomas with exon 20 *PIK3CA* mutations or *PIK3CA* mRNA overexpression were often high-grade carcinomas associated with myometrial invasion; in contrast, tumors that carried exon 9 mutations were more likely to be low-grade carcinomas. The Kaplan–Meier analysis suggested that *p53* alterations (strong immunoexpression or mutations) conferred a worse prognosis ($P = 0.000$). Although alterations in the PI3K–AKT signaling pathway alone did not influence overall survival, patients with deregulated PI3K–AKT pathway (*PIK3CA* and/or *PTEN* alterations) and *p53* alterations had shorter survival ($P = 0.000$) than patients with only *p53* alterations. Such a relationship was lost when we considered exon 9 *PIK3CA* mutations. Our results contribute to further characterize the molecular genetic model for endometrial carcinogenesis.
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Endometrial carcinoma is the most common malignant tumor of the female genital tract and the fourth most common female cancer in the Western world.[1] Two main types have been recognized:[2] type I are endometrioid adenocarcinomas, which represent ~80% of endometrial carcinomas.[3] They develop in peri- or post-menopausal women and are related to estrogen stimulation. They are predominantly low-grade endometrioid adenocarcinomas, often confined to the uterus and are frequently preceded by endometrial hyperplasia. Endometrioid adenocarcinomas frequently have microsatellite instability [4] and mutations of the *PTEN*,[5] *PIK3CA*,[6] *K-Ras*,[7] and β-catenin genes.[8,9] In contrast, type II tumors are high-grade non-endometrioid carcinomas which invade deeply into the myometrium and follow an aggressive clinical course. They occur in older women and are unrelated to estrogen stimulation. Non-endometrioid carcinomas are frequently associated with *p53* mutations and chromosomal instability.[10] Although this dualistic model shows some overlap, the clinical and pathologic differences are often paralleled by specific genetic alterations.[4,11]

The PI3K–AKT oncogenic signaling pathway is activated in many human epithelial cancers.[12–15] Its activation counteracts directly the action of the lipid phosphatase PTEN, a negative regulator

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of PI3K–AKT. Activated PI3K–AKT regulates the expression of several downstream target genes that inhibit apoptosis and promote cell proliferation. The p110a catalytic subunit of PI3K (*PIK3CA*) is an oncogene located on chromosome 3q26.3 and is mutated in several cancer types.[12] The *PIK3CA* mutations increase PI3K kinase activity, cell survival, motility, and cell cycle progression. Mutations are usually missense and cluster in exons 9 (helical domain) and 20 (kinase domain). We have investigated recently the *PIK3CA* status in a large series of endometrioid adenocarcinomas and compared the results with the clinical and pathologic parameters associated with prognosis.[16] In our series, the *PIK3CA* mutations occurred in 29% of endometrioid adenocarcinomas, all with myometrial invasion. We also found that histologic type, grade, and depth of myometrial invasion varied significantly depending on the location of *PIK3CA* mutations, either in exon 9 or exon 20.[16]

PTEN is a tumor-suppressor gene located in chromosome 10q23.3, a genomic region undergoing loss of heterozygosity in a wide variety of human cancers. *PTEN* encodes a phosphatase that antagonizes the PI3K–AKT pathway by dephosphorylating PIP3, a product of PI3K. In an earlier investigation, we found somatic *PTEN* mutations in 51–54% of endometrial carcinomas and they were far more frequent in endometrioid adenocarcinomas than in non-endometrioid adenocarcinomas.[5,16]

P53 mutations are the most characteristic genetic alterations of non-endometrioid adenocarcinomas. *P53* is a tumor-suppressor gene located in 17p13.1 that encodes p53 protein, a transcription factor that induces the expression of genes necessary for cell cycle arrest and apoptosis in response to DNA damage. Moreover, *P53* inactivation is observed in over 50% of all human tumors. Immunohistochemical overexpression of p53 is found in most non-endometrioid adenocarcinomas (71–85%) and may be useful in their distinction from endometrioid adenocarcinomas.[17,18]

Recently, important interactions between the PI3K–AKT and p53 signaling pathways have been described.[19–21] Studies on cell line suggest that activation of the PI3K pathway through *PTEN* or *PIK3CA* mutations causes activation of *p53*. It has been reported that activation of the PI3K–AKT pathway together with *p53* inactivation results in malignant transformation.[22,23] In this study, we have attempted to determine the status of the PI3K–AKT and p53 pathways in endometrial adenocarcinomas and compare the results with clinical and pathologic prognostic parameters and patients' follow-up.

Materials and methods

Tissue Samples and DNA Isolation

Samples from 132 endometrial carcinomas were retrieved from the Tumor Bank and the Surgical Pathology files of Hospital de la Santa Creu i Sant Pau, Barcelona, Spain. All cases were reviewed and classified using the World Health Organization (WHO) criteria. Most of the cases (102 endometrioid adenocarcinomas) have been the subject of an earlier investigation.[16] In this study, 30 high-grade carcinomas (14 non-endometrioid adenocarcinomas and 16 mixed endometrioid adenocarcinomas-non-endometrioid adenocarcinomas) were included. Genomic DNA from tumors and the corresponding non-tumor tissues was extracted using standard methods from frozen biopsies. All cases were anonymized and this study was approved by the Institutional Ethics Committee.

PIK3CA, *P53*, and *PTEN* Mutational Analysis

The *PIK3CA*, *p53*, and *PTEN* gene mutations were assessed on tumor DNA using polymerase chain reaction (PCR) amplification and subsequent sequencing analysis. Mutational analysis was performed using earlier-reported PCR conditions and primers for exons 9 and 20 of *PIK3CA*,[12] exons 5–8 of *p53*,[18] and exons 1–9 of *PTEN*[5] genes. The thermal cycling conditions included an initial 12 min at 94 °C, followed by 40 cycles of 45 s at 94 °C, 45 s at specific annealing primer temperature of 52–62 °C, 1 min at 72 °C, and a final extension of 10 min at 72 °C. The PCR conditions for exon 9 of *PIK3CA* were optimized to avoid mispriming with the *PIK3CA* pseudogene spanning exons 9–13 on chromosome 22. The PCR products were purified using the exoSAP-IT (USB, Cleveland, OH, USA) and subjected to direct sequencing using the ABI PRISM™ big Dye terminator v1.1 cycle sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequencing fragments were detected by capillary electrophoresis using an automated ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

PIK3CA Gene Expression

The mRNA levels of *PIK3CA* were evaluated by semiquantitative RT–PCR. cDNA was synthesized from 1 μg of total RNA using the HighCapacity cDNA Reverse Transcription kit (Applied Biosystems). Subsequently, products were amplified with the 7300 Real Time PCR System (Applied Biosystems) using specific primers and fluorescent TaqMan probes. The expression levels were measured in triplicate. Normal endometrium pool tissue was used as a calibrator for quantitative Real Time PCR, and *ABL-1* housekeeping gene was the reference for normalization. *PIK3CA* amplification was arbitrarily designated as two times or higher when compared with the endogenous control loci.

Immunohistochemical Analysis

Immunohistochemistry was performed on paraffin-embedded tissue sections using the EnVision system

(Dako) and diaminobenzidine as the chromogen. The reactions were carried out in a Dako Envision immunostainer. Tissue arrays were performed for an immunohistochemical analysis of pAkt, stathmin, ER, PR, β-catenin, p53, hMLH1, and hMSH2.

Statistical Analysis

Statistical analysis was performed using the statistical package SPSS/win 15.0 (SPSS, Chicago, IL, USA). The following clinicopathologic parameters were evaluated: age, tumor size, histologic type and grade, depth of myometrial invasion, lymphovascular invasion, clinicopathologic stage, hormone receptor status, p53 protein expression, *PIK3CA*, *PTEN*, and *p53* mutations, as well as patients' outcome. A value of $P \leqslant 0.05$ was considered statistically significant. Overall survival was calculated from the date of diagnosis according to the Kaplan and Meier method. Multivariate Cox regression analysis was used to investigate the relationship between prognostic parameters and survival. The following parameters were introduced: PI3K–AKT and *p53* alterations, histologic grade, stage, and vascular invasion.

Results

Clinical and Pathologic Findings

Patients' age ranged from 35 to 88 years (mean: 66.6 years). Tumor size varied from 0.4 to 11 cm (mean: 4.2 cm). Of the 132 cases, 102 (77%) were endometrioid adenocarcinomas, 14 (11%) non-endometrioid adenocarcinomas, and 16 (12%) mixed endometrioid adenocarcinomas-non-endometrioid adenocarcinomas. Of the 14 non-endometrioid adenocarcinomas, 6 were serous carcinomas, 4 clear cell carcinomas, and 4 mixed clear cell carcinomas-serous carcinomas. Of the 16 mixed endometrioid adenocarcinomas-non-endometrioid adenocarcinomas, 9 were endometrioid adenocarcinomas-clear cell carcinomas and 7 endometrioid adenocarcinomas-serous carcinomas. Thirty-four tumors were grade 1 (26%), 41 grade 2 (31%), and 57 grade 3 (43%). Most tumors were FIGO stage I (89; 67%), 16 (12%) stage II, 20 (15%) stage III, and 7 (5%) stage IV. Myometrial invasion was found in 119 cases. It involved $\leqslant 1/2$ of the myometrial thickness in 64 cases and $>1/2$ in 55. Thirty tumors (23%) had lymphovascular invasion.

Follow-up

Follow-up information was obtained in 126 cases (95.5%): 103 (78%) patients were alive without clinical evidence of tumor at a mean follow-up interval of 3.68 years (range: 2 months–10 years); tumor persisted or recurred in 5 (4%) patients at a mean follow-up interval of 1.9 years; 14 (11%) patients died of tumor between 9 months and 8.4 years (mean: 2.2 years) postoperatively; and 4 (3%) patients died of unrelated causes. Eleven patients had received tamoxifen for breast carcinoma. Patients with low-stage tumors, who lacked vascular invasion, had significantly better survival ($P = 0.001$). Patients with non-endometrioid carcinomas had shorter survival than those with endometrioid carcinomas. In fact, 93% (91/98) of patients with endometrioid adenocarcinomas survived, whereas only 39% (11/28) with non-endometrioid adenocarcinomas were alive. The difference was statistically significant ($P = 0.000$).

PIK3CA Mutations and Gene Expression

All endometrioid adenocarcinomas (102 cases) have been the subject of an earlier study, in which the *PIK3CA* mutations were identified in 19 of 73 (26%) low-grade (grades 1 and 2) endometrioid adenocarcinomas and in 10 of 29 (34%) high-grade (grade 3) endometrioid adenocarcinomas.[16] Of the 30 high-grade carcinomas included in this study, the *PIK3CA* mutations were found in 21% (3/14) non-endometrioid adenocarcinomas and in 44% (7/16) mixed endometrioid adenocarcinomas-non-endometrioid adenocarcinomas. All the *PIK3CA* mutations found in non-endometrioid adenocarcinomas and in mixed carcinomas occurred in exon 20 (Figure 1). The *PIK3CA* mutations were present in 4/13 (31%) serous carcinomas and in 6/13 (46%) clear cell carcinomas. The distribution of *PIK3CA* mutations is shown in Figure 2.

Overall 95% of mutated tumors showed myometrial invasion. Histologic type and grade varied significantly according to the distribution of the *PIK3CA* mutations between exons 9 and 20 ($P = 0.017$ and $P = 0.003$, respectively). Mutations in exon 9 were found more frequently in low-grade (grades 1 and 2 endometrioid adenocarcinomas; 13/17; 76%) than in high-grade carcinomas (grade 3 endometrioid adenocarcinomas, non-endometrioid

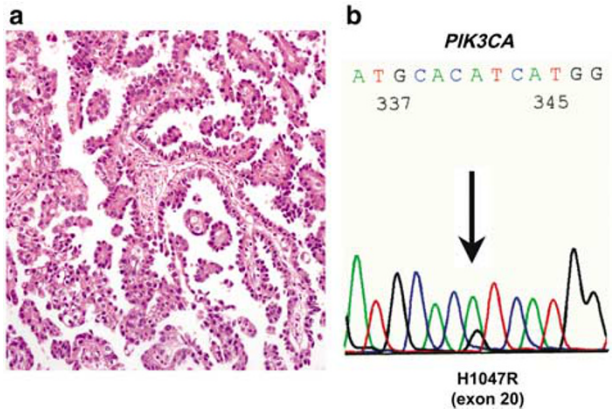
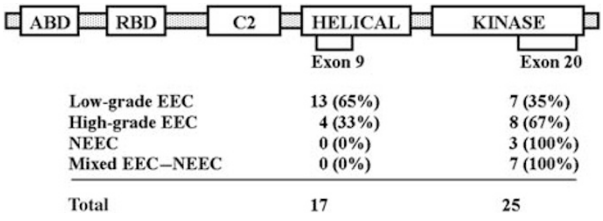


Figure 1 (**a**) Clear cell carcinoma. (**b**) Sequence analysis of exon 20 of *PIK3CA* reveals a missense mutation (H1047R).



Low-grade EEC	13 (65%)	7 (35%)
High-grade EEC	4 (33%)	8 (67%)
NEEC	0 (0%)	3 (100%)
Mixed EEC–NEEC	0 (0%)	7 (100%)
Total	17	25

Figure 2 Distribution of *PIK3CA* mutations in endometrial carcinomas. ABD, adaptor-binding domain; RBD, Ras-binding domain; EEC, endometrioid endometrial carcinoma; NEEC, non-endometrioid endometrial carcinoma.

adenocarcinomas, and mixed endometrioid adenocarcinomas-non-endometrioid adenocarcinomas; 4/17; 24%). In contrast, exon 20 mutations were more common in high-grade (18/25; 72%) than in low-grade carcinomas (7/25; 28%). Although mutated tumors had a higher frequency of lymphovascular invasion (12/39; 31%) than non-mutated tumors (18/93; 19%), the difference was not statistically significant ($P=0.153$).

Twenty-two endometrial carcinomas were evaluated for the *PIK3CA* gene expression using real-time quantitative RT–PCR. We detected a two times or greater increase in the *PIK3CA* mRNA expression in 8 of 22 (36%) adenocarcinomas compared with that in the normal control tissue. The *PIK3CA* expression was higher in non-endometrioid adenocarcinomas than in endometrioid adenocarcinomas and mixed carcinomas. No relationship was found between the *PIK3CA* overexpression and the clinical or pathologic features.

P53 Alterations

P53 alterations (strong immunoexpression or mutations) occurred more frequently in non-endometrioid adenocarcinomas (7/13; 54%) and mixed endometrioid adenocarcinomas-non-endometrioid adenocarcinomas (8/16; 50%) than in low-grade endometrioid adenocarcinomas (1/60; 2%) or in high-grade endometrioid adenocarcinomas (5/29; 17%) ($P=0.000$) (Figure 3). *p53* alterations were found in 7/12 (58%) serous carcinomas and in 5/12 (42%) clear cell carcinomas, regardless of whether they were pure or mixed with an endometrioid adenocarcinomas component. In mixed serous-clear cell carcinomas, the *p53* alterations were found in 3/4 (75%) of cases. Tumors with *p53* alterations had lymphovascular invasion more frequently (8/21; 38%) than tumors with wild-type *p53* (18/97; 18.5%). However, the difference did not reach statistical significance ($P=0.078$). The *P53* mutations in exons 5–8 were associated with strong p53 immunostaining in 65% of cases. There was a coexistence of the *PIK3CA* mutations and *p53* alterations in 7% of cases (8/118), almost all of them were pure endometrioid adenocarcinomas or mixed carcinomas with an endometrioid component.

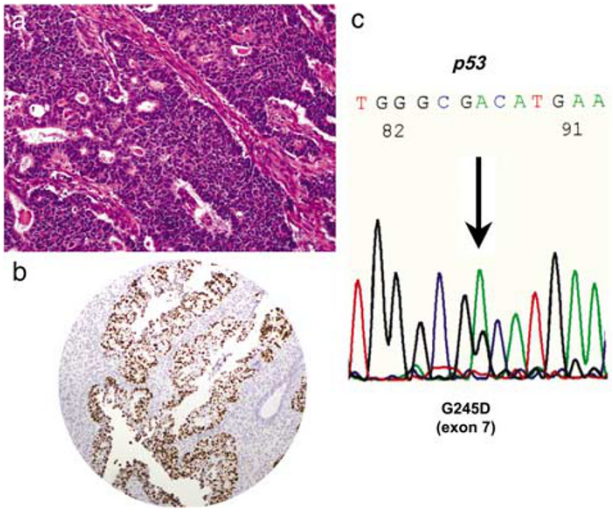


Figure 3 (**a**) Grade 3 endometrioid carcinoma. (**b**) Strong p53 immunoreaction. (**c**) Sequence analysis of *p53* missense mutation in exon 7 (G245D).

PTEN Mutations

Mutations of *PTEN* had been analyzed earlier in 102 endometrioid adenocarcinomas.[16] The molecular genetic analysis was expanded with 30 additional high-grade tumors (non-endometrioid adenocarcinomas and mixed endometrioid adenocarcinomas-non-endometrioid adenocarcinomas). The *PTEN* mutations were more common in pure endometrioid adenocarcinomas compared with that in non-endometrioid adenocarcinomas and mixed endometrioid adenocarcinomas-non-endometrioid adenocarcinomas. Results of the *PIK3CA* and *PTEN* mutations and of the p53 alterations are summarized in Table 1.

Correlations of *PIK3CA*, *PTEN*, and *P53* Alterations with Survival

The prognostic significance of *PIK3CA*, *PTEN*, and *p53* alterations was analyzed using the Kaplan–Meier method. Patients with *p53* alterations (strong immunoexpression and/or mutations) had a shorter survival than those without them ($P=0.000$) (Figure 4a). In contrast, no correlation with survival was encountered in carcinomas with or without mutations in the *PIK3CA* or *PTEN* genes only (Figure 4b). Moreover, the survival of patients with or without the PIK3–AKT pathway alterations (*PIK3CA* and/or *PTEN* mutations) was compared with p53 status (Figure 4c and d). Patients with concomitant p53 and PI3K–AKT alterations had shorter survival than patients with p53 alterations alone ($P=0.000$). For this analysis, exon 9 *PIK3CA* mutations were excluded. In a multivariate analysis, PI3K–AKT and *p53* concomitant alterations and vascular

invasion were independent predictors of survival ($P = 0.000$ and $P = 0.007$, respectively).

Discussion

We have carried out a comparative clinicopathologic and molecular genetic study of 132 endometrial carcinomas, including 102 endometrioid adenocarcinomas reported earlier,[16] and 30 additional high-grade non-endometrioid adenocarcinomas and mixed carcinomas. Our study focused on the status of the PI3K—AKT signaling pathway and p53.

Table 1 Molecular genetic alterations found in 132 endometrial carcinomas

	EEC low-grade	*EEC high-grade*	*NEEC*	*Mix EEC–NEEC*	*Total*
PTEN	55%	59%	0%	38%	48%
PIK3CA	26%	34%	21%	44%	29.5%
P53	2%	17%	54%	50%	16%

EEC, endometrioid endometrial carcinoma; NEEC, non-endometrioid endometrial carcinoma; Mix, mixed.

The reported frequency of the *PIK3CA* mutations in endometrial carcinomas ranges from 24 to 39%.[6,15,16,24] However, none of the series investigated included non-endometrioid adenocarcinomas. To the best of our knowledge, our study is the first to describe *PIK3CA* mutations in non-endometrioid adenocarcinomas. We found 29.5% (39/132) *PIK3CA* mutations in endometrial carcinomas including all histologic types. Mutations were more frequent in mixed endometrioid adenocarcinomas-non-endometrioid adenocarcinomas (44%) than in pure endometrioid adenocarcinomas (28%) or pure non-endometrioid adenocarcinomas (21%). It should be noted that, type and histologic grade of the tumors varied significantly according to location of the *PIK3CA* mutations, either in exon 9 (helical domain) or in exon 20 (kinase domain). As reported earlier, the frequency of exon 9 mutations was higher in low-grade endometrioid adenocarcinomas than in high-grade endometrioid adenocarcinomas. Non-endometrioid adenocarcinomas and mixed

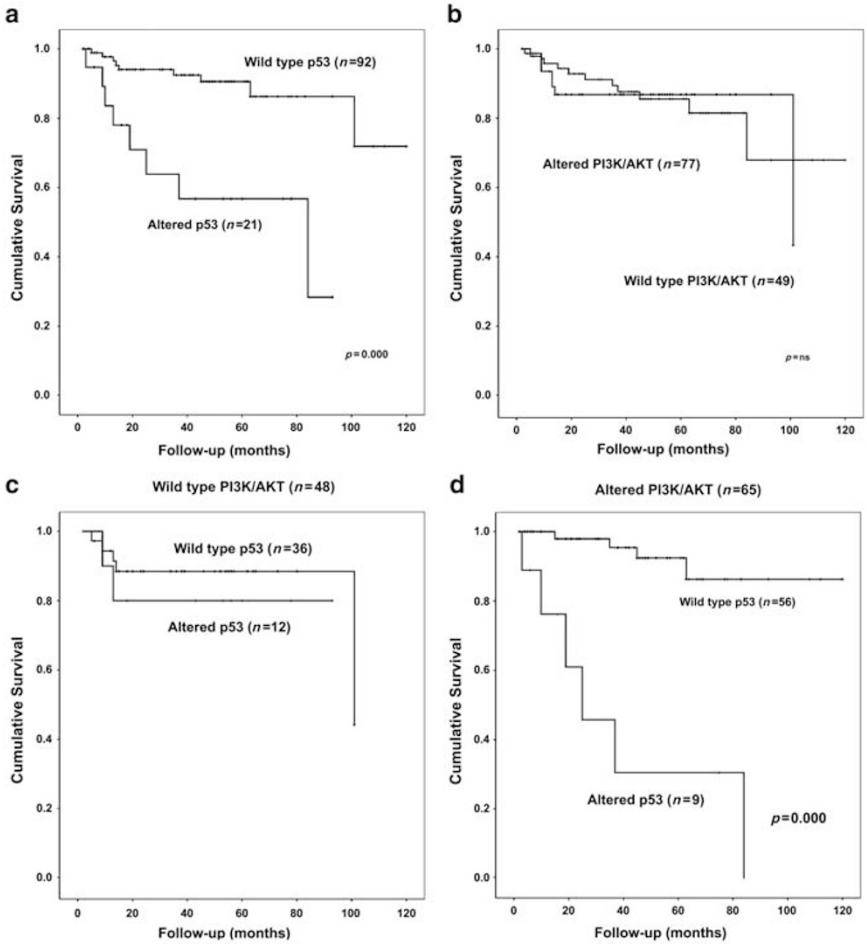


Figure 4 The Kaplan–Meier survival curves in endometrial carcinomas. (**a**) Patients with and without p53 alterations ($P = 0.000$). (**b**) Patients with and without PI3K–AKT pathway alterations ($P =$ ns). (**c** and **d**) Patients with and without PI3K–AKT and p53 alterations ($P = 0.000$).

carcinomas exhibited exon 20 mutations exclusively. We also found that the *PIK3CA* mRNA expression was increased in non-endometrioid adenocarcinomas compared with that in endometrioid adenocarcinomas, mixed carcinomas, and normal tissues. Almost all tumors with exon 20 *PIK3CA* mutations and/or *PIK3CA* mRNA overexpression had myometrial invasion and tended to show lymphovascular invasion. Our findings provide further evidence that endometrial adenocarcinomas with mutations in the kinase domain (exon 20) or the *PIK3CA* mRNA overexpression are associated with adverse prognostic parameters. Although similar findings have been reported in high-grade ovarian carcinomas,[25] reports have been contradictory regarding the prognostic significance of the *PIK3CA* helical and kinase domain mutations in breast carcinomas.[26–29] However, it has been reported that chickens injected with exon 20 *PIK3CA* mutants developed larger tumors than those injected with exon 9 *PIK3CA* mutants.[30]

The distinct associations of the different *PIK3CA* mutations with histologic type, grade, and depth of myometrial invasion in endometrial carcinomas is consistent with the idea that different categories of mutants, defined by their structural and functional domains, would increase PI3K function by diverse mechanisms. Mutations in the kinase domain of exon 20 alter the catalytic loop and increase the specific activity of the enzyme, thus enhancing pathway activation. In contrast, mutations in the helical domain (exon 9) cluster on an exposed surface patch of the protein and may change its ability to interact with other regulatory proteins, which may be different for each tissue.[31–33]

As expected, analyses of the *PTEN* mutations revealed that these alterations are more common in pure endometrioid adenocarcinomas compared with that in non-endometrioid adenocarcinomas and mixed tumors. Different studies of colorectal and breast carcinomas have claimed that the *PIK3CA* and *PTEN* mutations are mutually exclusive and suggest that carcinogenic signaling through this pathway occurs either through the activation of *PIK3CA* or inactivation of *PTEN*.[34,35] However, in endometrial carcinoma, coexistence of the *PIK3CA* and *PTEN* mutations ranges from 15 to 27% and the association is not statistically significant.[6,15,16,24] In our series, the *PTEN* mutations were detected in low- and high-grade endometrioid adenocarcinomas and in mixed carcinomas with an endometrioid component, but were not found in non-endometrioid adenocarcinomas. The *PTEN* and *PIK3CA* mutations coexisted in 15% (20/132) of low- and high-grade endometrioid adenocarcinomas, and in one case of mixed endometrioid adenocarcinomas-non-endometrioid adenocarcinomas. Our results confirm that in endometrial carcinoma, the *PIK3CA* mutations coexist frequently with other alterations of genes upstream of the PI3K–AKT pathway, such as *PTEN* or *K-Ras*. A recent study[36] has reconfirmed that the *PIK3CA* mutations occur almost exclusively in invasive tumors, whereas upstream mutations of the PI3K–AKT pathway (*PTEN* and *K-Ras* mutations) occur with equal frequency in early-and late-stage tumors. This finding suggests that the *PIK3CA* mutations cooperate with these alterations in the malignant transformation.

In our study, *p53* alterations (strong immunoexpression and/or mutations) were more frequent in non-endometrioid adenocarcinomas (54%) and mixed endometrioid adenocarcinomas-non-endometrioid adenocarcinomas (50%) than in high-grade endometrioid adenocarcinomas (17%) or low-grade endometrioid adenocarcinomas (2%). The *PIK3CA* mutations, almost all located in exon 20, coexisted with *p53* alterations in high-grade endometrioid adenocarcinomas and mixed carcinomas more frequently than in non-endometrioid adenocarcinomas. However, the *PIK3CA* mRNA overexpression occurred concomitantly with *p53* alterations exclusively in non-endometrioid adenocarcinomas. Moreover, the *p53* alterations coexisted with *PTEN* mutations in only three cases (4%). In a recent study of ovarian carcinomas,[25] *p53* mutations coexisted with *PIK3CA* amplification in 7% of serous carcinomas. However, in ovarian endometrioid carcinomas, no association between the *PIK3CA* alterations and *p53* mutations was found.

Recent studies have shown important interactions between the PI3K–AKT and p53 signaling pathways.[19,20] A functional p53 binding site has been identified within the *PTEN* promoter region, suggesting that *p53* is able to regulate *PTEN* expression. Other interaction sites at HDM2 may enable *PTEN* to regulate p53 protein stability. Moreover, a physical association between p53 and PTEN proteins, which would protect p53 from degradation, has been described. These observations suggest that *p53* and *PTEN* form a positive feedback loop, in which *p53* induces PTEN expression and PTEN reduces p53 degradation. It has also been shown that p53 binds directly to the *PIK3CA* promoter region and inhibits its activity.[21] Moreover, activation of the PI3K–AKT signaling pathway by inactivating the *PTEN* mutations or activating the *PIK3CA* mutations would cause the activation of p53. Thus, activation of the PI3K–AKT pathway together with p53 inactivation would promote malignant transformation.[22,23]

Our results suggest that activation of PI3K–AKT and p53 inactivation may have a concomitant negative effect on prognosis in high-grade endometrial carcinomas. In fact, the Kaplan–Meier analysis indicates that, by itself, *p53* alterations (strong immunoexpression and/or mutations) are associated with poor prognosis ($P = 0.000$). In contrast, no correlation with survival was encountered in carcinomas with alterations in the *PIK3CA* or *PTEN* genes exclusively. Thus, as suggested earlier,[15] activated PI3K–AKT pathway alone is not a prognostic marker in endometrial carcinomas. In breast carcinoma, however, aberrant PTEN tumor-suppres-

sor activity has been associated with poor prognosis.[37] In our cases with *p53* alterations, deregulation of the PI3K–AKT signaling pathway (*PIK3CA* and/or *PTEN* alterations) was associated with worse prognosis than that of cases with *p53* alterations alone. We found lower survival rates in carcinomas with both *p53* and *PIK3CA* alterations (exon 20 mutations and/or mRNA overexpression) and/or *PTEN* mutations, than in cases with *p53* alterations exclusively using the Kaplan–Meier and multivariate analysis ($P=0.000$). This association was lost when exon 9 *PIK3CA* mutations were considered. All patients who died of tumors with alterations in both *p53* and PIK3–AKT had non-endometrioid adenocarcinomas or mixed endometrioid adenocarcinomas-non-endometrioid adenocarcinomas. Our data suggest that PIK3–AKT and *p53* alterations have a negative synergistic influence on prognosis in endometrial carcinomas.

In summary, our study confirms that activation of the PI3K–AKT signaling pathway alone is not an adverse prognostic factor in endometrial carcinomas. However, simultaneous alterations both in the PI3K–AKT and the *p53* pathways have a negative effect on prognosis and are associated with lower survival. Such an association is lost when mutations in exon 9 of *PIK3CA* are included. As exons 9 and 20 encode different domains, it is reasonable to speculate that they may have different oncogenic potential. Thus, the different mechanisms by which the two categories of *PIK3CA* mutations induce a gain of function may explain their different impact on prognosis. The *PIK3CA* mutations are frequent events in all histologic types of endometrial adenocarcinomas. We found that tumors with exon 20 *PIK3CA* mutations or *PIK3CA* mRNA overexpression are often high-grade carcinomas associated with myometrial invasion compared with those with exon 9 mutations.

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Conflict of interest

The authors declare no conflict of interest.

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