Modern Pathology (2008) 21, 131–139

www.modernpathology.org

PIK3CA mutations in the kinase domain (exon 20) of uterine endometrial adenocarcinomas are associated with adverse prognostic parameters

Lluis Catasus, Alberto Gallardo, Miriam Cuatrecasas and Jaime Prat

Department of Pathology, Hospital de la Santa Creu i Sant Pau, Autonomous University of Barcelona, Barcelona, Spain

Mutations of the oncogene *PIK3CA* occur frequently in endometrial carcinomas, but their prognostic significance is unclear. To determine the clinicopathological and molecular implications of these mutations, *PIK3CA* status was investigated in 109 endometrial (102 endometrioid and 7 mixed) carcinomas and the results were compared with clinicopathological parameters associated with prognosis. Tumors were also investigated for microsatellite instability and *PTEN*, β-catenin gene (*CTNNB1*), *K-RAS*, and *B-RAF* mutations. We found 35 *PIK3CA* somatic missense mutations in 32 (29%) endometrial carcinomas. Eighteen mutations occurred in exon 20 (kinase domain), and 17 in exon 9 (helical domain). Almost all mutated tumors were pure endometrioid adenocarcinomas. All tumors with *PIK3CA* mutations exhibited myometrial invasion ($P=0.032$). Lymphovascular invasion was found more frequently in mutated (28%) than nonmutated carcinomas (18%). Histological grade varied significantly according to the location of the *PIK3CA* mutations whether in exon 9 or exon 20 ($P=0.033$). The frequency of exon 9 mutations was higher in grade 1 carcinomas (57%) than in grade 2 (29%) or grade 3 (14%) tumors. Conversely, mutations in exon 20 were more common in grade 3 (60%) than in grade 2 (20%) or grade 1 (20%) carcinomas. None of the tumors confined to the endometrium (stage IA) had *PIK3CA* mutations. Furthermore, whereas 64% of adenocarcinomas with exon 9 mutations had invaded $\leq$1/2 of the myometrial thickness (stage IB), 73% of tumors with exon 20 mutations had either deeper myometrial invasion (stage IC) or cervical involvement (stage II) ($P=0.045$). *PIK3CA* mutations coexisted with microsatellite instability and mutations in *PTEN*, *CTNNB1*, *K-RAS*, and *B-RAF* genes. These results favor that *PIK3CA* mutations are associated with myometrial invasion and, moreover, that tumors harboring *PIK3CA* mutations in exon 20 are frequently high-grade, deeply invasive endometrial carcinomas that tend to exhibit lymphovascular invasion.

Modern Pathology (2008) **21,** 131–139; doi:10.1038/modpathol.3800992; published online 14 December 2007

Keywords: endometrial carcinoma; histological grade; myometrial invasion; *PIK3CA*

Endometrial carcinoma is the most common malignant tumor of the female genital tract in the Western world.[1] Over 80% of cases are estrogen-related well-differentiated to moderately well-differentiated endometrioid adenocarcinomas, which are confined to the uterine corpus at diagnosis (stage I), and thus most of them can be cured. While favorable in most cases, the prognosis of stage I endometrioid adenocarcinomas varies significantly depending on histological grade, depth of myometrial invasion, and presence or absence of lymphovascular invasion. Patients with high-grade tumors that invade >1/2 of the myometrial thickness or exhibit lymphovascular invasion have a high risk of regional lymph node metastases and require adjuvant therapy.[2]

Within the last decade, several genetic alterations, including microsatellite instability,[3–5] *PTEN* alterations,[6–8] and mutations of *K-RAS*[9–11] and *CTNNB1* (β-catenin),[12,13] have been discovered in endometrioid adenocarcinomas and their prognostic significance widely investigated. Although microsatellite instability was initially thought to be associated with a favorable outcome,[14] recent data, obtained after excluding nonendometrioid carcinomas, have

Correspondence: Professor J Prat, MD, FRCPath, Department of Pathology, Hospital de la Santa Creu i Sant Pau, Avda. Sant Antoni M Claret 167, Barcelona 08025, Spain.
E-mail: jprat@santpau.es
Received 10 September 2007; revised 09 November 2007; accepted 15 November 2007; published online 14 December 2007

shown that the frequency of microsatellite instability is higher in high-grade and advanced stage tumors with deep myometrial and lymphovascular invasion.[15,16] Similarly, earlier investigations suggested that *PTEN* mutations were associated with low-stage, nonmetastatic disease, and prolonged survival;[17] however, subsequent studies reported that mutations only outside exons 5–7 of *PTEN* might represent a molecular predictor of favorable survival, independent of the clinical and pathological characteristics of the tumors.[18] Also, promoter methylation of *PTEN* has been associated with advanced stage in endometrial carcinoma.[19] In contrast, *CTNNB1* (β-catenin) mutations are thought to represent a separate pathway in estrogen-driven endometrial carcinogenesis, resulting in low-grade, low-stage endometrioid adenocarcinomas with squamous differentiation and excellent prognosis.[20,21]

The phosphatidylinositol 3′-kinase (PI3K)/AKT signaling pathway is frequently activated in multiple human epithelial cancers, including endometrioid adenocarcinomas.[22–25] In response to cell-surface receptor signals, PI3K phosphorylates phosphatidylinositol 4,5-biphosphate to generate the second messenger phosphatidylinositol 3,4,5-triphosphate. This, in turn, activates the AKT oncogenic pathway, directly counteracting the actions of the lipid phosphatase PTEN.[26] Activated AKT regulates the expression of various downstream target genes that inhibit apoptosis and promote cell proliferation. A high frequency of mutations of the oncogene *PIK3CA*, which encodes the p110α catalytic subunit of PI3K, has been recently identified in endometrioid adenocarcinomas.[25,27,28] These mutations are usually of the missense type and clustered in exon 9 (residues E542 and E545 of the helical domain) and exon 20 (residue H1047 of the kinase domain). However, in contrast to other epithelial cancers, no association has been found between the distribution of *PIK3CA* mutations and the traditional prognostic parameters of endometrioid adenocarcinomas.[25,28] Given the reported role of *PIK3CA* mutations in tumor invasion and metastases,[29] we were interested in determining the clinicopathological impact of *PIK3CA* mutations in a large series of endometrial adenocarcinomas and their relationship with other genetic alterations also common in these tumors. We found that all carcinomas with *PIK3CA* mutations exhibited myometrial invasion and, furthermore, histological grade and depth of myometrial invasion varied significantly according to the distribution of mutations between exons 9 and 20.

Materials and methods

Tumor Samples and Genomic DNA

One hundred and nine unselected endometrial adenocarcinomas were retrieved from the Tumor Bank of the Department of Pathology, Hospital de la Santa Creu i Sant Pau, Autonomous University, Barcelona, Spain. All available hysterectomy slides were reviewed using the World Health Organization classification,[30] and clinical data were obtained from the medical record. Tumors were staged and graded according to the International Federation of Gynaecology and Obstetrics (FIGO) guidelines. Genomic DNA was extracted by a standard procedure from frozen tumor and corresponding normal tissues. All cases were anonymized, and the study was approved by the institutional ethics committee.

PIK3CA, *PTEN*, β-Catenin Gene, *K-RAS*, and *B-RAF* Mutational Analysis

PIK3CA, *PTEN*, β-catenin gene (*CTNNB1*), *K-RAS*, and *B-RAF* mutations were assessed on tumor DNA by polymerase chain reaction (PCR) amplification and subsequent sequencing analysis. Mutational analysis was performed using previously reported PCR conditions and primers for exons 9 and 20 of *PIK3CA*,[31] exons 1–9 of *PTEN*,[8] exon 3 of *CTNNB1*,[13] exon 1 of *K-RAS*,[11] and exons 11 and 15 of *B-RAF* genes.[32] 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.[33] The PCR products were purified using the exoSAP-IT (USB, Cleveland, OH, USA) and subjected to direct sequencing using 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).

Microsatellite Analysis

Microsatellite instability status was determined by PCR using fluorochrome-labeled primers for the five recommended microsatellite markers (BAT 25, BAT 26, D2S123, D5S346, and D17S250).[34,35] Primers and PCR amplification conditions were made as previously described.[5] Tumors were considered microsatellite instability positive when two or more altered markers were found. Individual PCRs were performed for each marker, and then each sample was capillary electrophoresed on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

Statistical Analysis

Statistical analysis was performed with the statistical package SPSS/win 14.0 (SPSS, Chicago, IL, USA). The following parameters were evaluated:

age, tumor size, histological type and grade, depth of myometrial invasion, lymphovascular invasion, clinicopathological stage, hormone receptor status, p53 protein expression, microsatellite instability status, presence of *PIK3CA*, *PTEN*, *CTNNB1*, *K-RAS*, and *B-RAF* mutations, and patients' outcome. A value of $P = 0.05$ was considered statistically significant.

Results

Clinical and Pathologic Findings

The age of the patients ranged from 35 to 88 years (mean: 66 years). Tumor size varied from 0.4 to 8.8 cm (mean: 4 cm). Of the 109 cases, 102 (94%) were endometrioid adenocarcinomas and 7 (6%) were mixed carcinomas (5 endometrioid carcinomas (clear cell), and 2 endometrioid carcinomas (serous)). Thirty-four tumors were grade 1, 40 grade 2, and 35 grade 3. Most tumors were FIGO stage I (80; 73%), 14 stage II, 14 stage III, and 1 stage IV. Myometrial invasion was $\leq 1/2$ of the myometrial thickness in 63 cases and $> 1/2$ in 46 cases. Twenty-two tumors (18 endometrioid adenocarcinomas and 4 mixed carcinomas; 20%) had lymphovascular invasion. Follow-up information was obtained in 105 of 109 cases (96%). Ninety-four (90%) patients were alive without clinical evidence of tumor at a mean follow-up interval of 3.9 years (range: 2 months to 10 years). Tumor persisted or recurred in 3 (3%) patients at a mean follow-up interval of 2.9 years. Two had grade 3 carcinomas (stages IIA and IIIA) and the other had a grade 2 carcinoma (stage IVB). One of the former tumors had lymphovascular invasion. Six (6%) patients died of tumor between 10 months and 8.4 years (mean: 3 years) postoperatively. Three had grade 3 carcinomas (stages IB, IC, and IIIA) and the other three patients had grade 2 tumors with lymphovascular invasion (one stage IC and two stage IIIC). Two (2%) patients died of unrelated causes.

PIK3CA Mutations

Thirty-five *PIK3CA* somatic missense mutations were identified in 32 of the 109 (29%) endometrial carcinomas. Eighteen occurred in the kinase domain (exon 20) and 17 in the helical domain (exon 9). Three carcinomas carried more than one mutation in

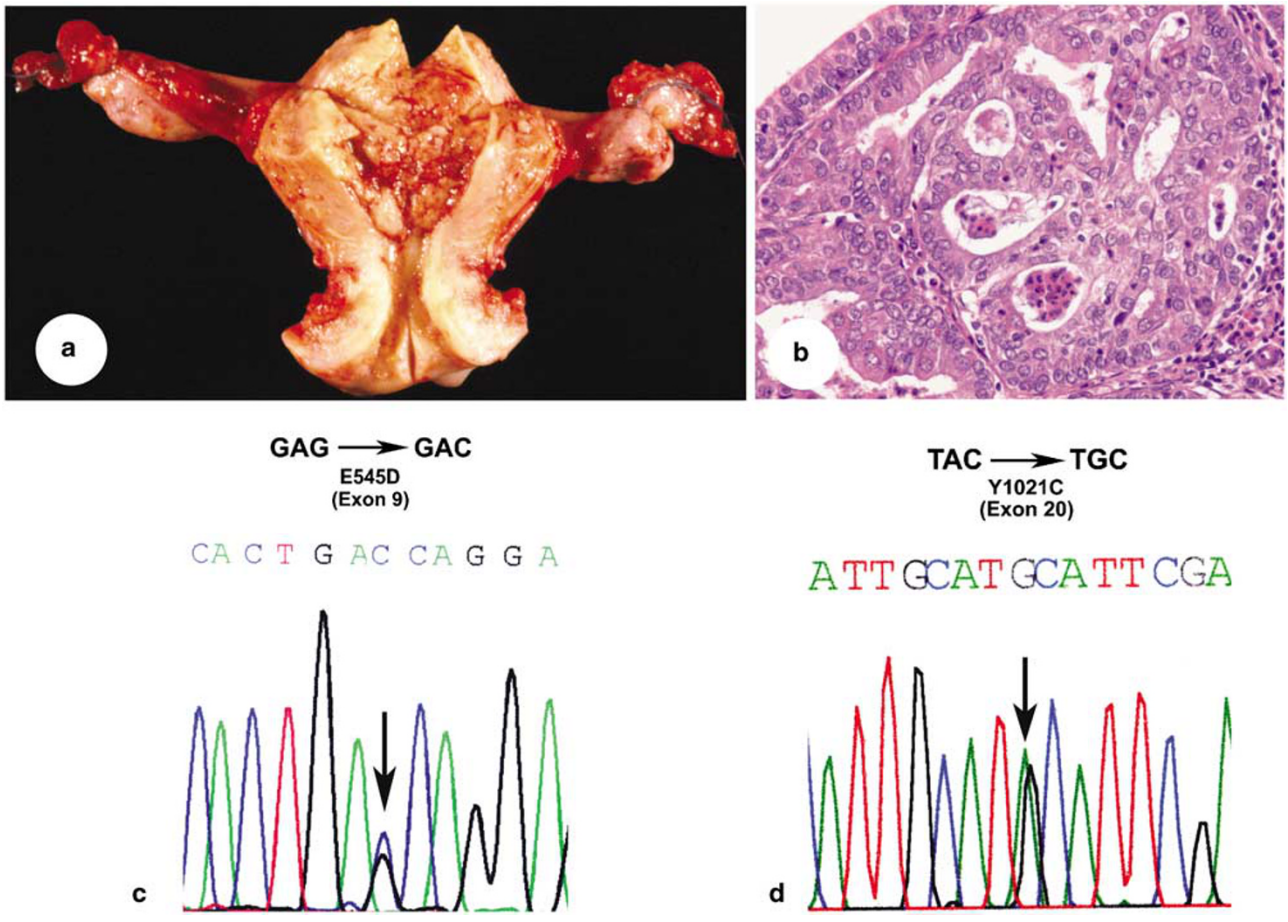

Figure 1 Gross and histological features of an endometrioid carcinoma (**a** and **b**) with double mutation in *PIK3CA*. Sequencing histogram of missense mutations (arrows in **c** and **d**) in exon 9 (E545D) and exon 20 (Y1021C).

Table 1 *PIK3CA* mutations in endometrial carcinomas

Exon	*Nucleotide*	*Amino acid*	*Frequency*
9	A1625T	E542V	1
9	G1624A	E542K	3
9	G1635C	E545D	4
9	A1634G	E545G	2
9	G1633A	E545K	4
9	A1634C	E545A	2
9	A1637G	Q546R	1
20	G3052C	D1017H[a]	1
20	T3061C	Y1021H	1
20	A3062C	Y1021C	2
20	A3073G	T1025A	1
20	G3103A	A1035T	1
20	A3127G	M1043V	1
20	A3140G	H1047R	8
20	C3155A	T1052K	1
20	A3194T	H1065L	2

[a]Novel *PIK3CA* mutation.

exons 9 and 20 (Figure 1). The most commonly mutated codons were 542 (4 cases; 11%) and 545 (12 cases; 33%) in exon 9 and 1047 (8 cases; 22%) in exon 20. In addition, a novel mutation was found and confirmed in codon 1017 of exon 20. This mutation, which was a G to C transversion converting aspartic to histidine (D1017H), has not been reported previously either in the literature or in the Catalog of Somatic Mutations in Cancer (http://www.Sanger.ac.uk/genetics/CGP/cosmic). All mutations are listed in Table 1.

Correlations with Clinicopathological Variables

Almost all (91%) mutated tumors were pure endometrioid adenocarcinomas, whereas only three of the seven mixed carcinomas (two endometrioid carcinomas (clear cell) and one endometrioid carcinomas (serous)) had *PIK3CA* mutations. Microdissection was performed in the mixed carcinomas to sample the different components. *PIK3CA* mutations were found in the endometrioid component in all three cases. All tumors with *PIK3CA* mutations exhibited myometrial invasion ($P=0.032$). Although mutated tumors had a higher frequency of lymphovascular invasion (9 of 32; 28%) than nonmutated carcinomas (13 of 73; 18%), this association was not statistically significant ($P=0.198$).

Histological grade varied significantly according to the distribution of *PIK3CA* mutations between exons 9 and 20 ($P=0.033$). Mutations in exon 9 were found more frequently in grade 1 (8 of 14; 57%) than grade 2 (4 of 14; 29%) or grade 3 (2 of 14; 14%) tumors. In contrast, mutations in exon 20 were more common in grade 3 (9 of 15; 60%) than grade 2 (3 of 15; 20%) or grade 1 (3 of 15; 20%) carcinomas. Such association was lost when all mutations (exons 9 and 20) were taken into account (Table 2). In addition, both the presence and distribution of

Table 2 Histological grade of endometrial carcinomas according to the presence and distribution of *PIK3CA* mutations

	Grade 1	*Grade 2*	*Grade 3*
Endometrial carcinomas Total ($n=109$)	34 (31%)	40 (37%)	35 (32%)
Endometrial carcinomas with PIK3CA mutations Total ($n=32$)	12 (37%)	7 (22%)	13 (41%)
Exon 9 ($n=14$)	8 (57%)	4 (29%)	2 (14%)
Exon 20 ($n=15$)	3 (20%)	3 (20%)	9 (60%)
Exons 9 and 20 ($n=3$)	1 (33%)	0 (0%)	2 (67%)
Endometrial carcinomas without PIK3CA mutations Total ($n=77$)	22 (28.5%)	33 (43%)	22 (28.5%)

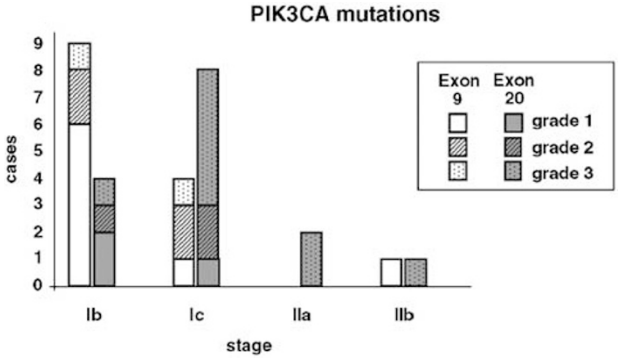

Figure 2 Histological grade and stage of 29 endometrial carcinomas according to the distribution of *PIK3CA* mutations whether in exon 9 or exon 20.

PIK3CA mutations correlated with stage. None of the tumors confined to the endometrium (stage IA) had *PIK3CA* mutations. Furthermore, whereas 9 of 14 adenocarcinomas (64%) with exon 9 mutations had invaded $\leq 1/2$ of the myometrial thickness (stage IB), 11 of 15 (73%) tumors with exon 20 mutations either had deeper myometrial invasion (stage IC) or cervical involvement (stage II) ($P=0.045$). The three tumors carrying more than one (both exons 9 and 20) mutation exhibited clinicopathological features similar to those of carcinomas with exon 20 mutations only. The distribution of *PIK3CA* mutations between exons 9 and 20, according to the histological grade and stage of the tumors, is shown in Figure 2. No correlations were found between *PIK3CA* mutations and other clinicopathological parameters.

Correlations of PIK3CA Mutations with Other Molecular Alterations

A summary of the molecular genetic findings is shown in Tables 3 and 4. *PTEN* mutations were

Table 3 Association of *PIK3CA* mutations with MI, and *PTEN*, *CTNNB1*, *K-RAS*, and *B-RAF* mutations

	Microsatellite		*PTEN*		*CTNNB1*		*K-RAS*		*BRAF*	
	MI	*MS*	*WT*	*Mut.*	*Mut.*	*WT*	*Mut.*	*WT*	*Mut.*	*WT*
PIK3CA mutation	12/37 (32%)	20/72 (28%)	13/50 (26%)	19/59 (32%)	5/24 (21%)	27/85 (32%)	4/19 (21%)	28/90 (31%)	2/2 (100%)	30/107 (28%)

MI, microsatellite instability.

Table 4 Molecular genetic findings in endometrial carcinomas with *PIK3CA* mutations

Histology	*Grade*	*Stage*	*Vascular invasion*	*Outcome*	*Age (years)*	*Tumor size (cm)*	*PIK3CA mut. (exon)*	*MI*	*PTEN mut.*	*CTNNB1 mut.*	*K-RAS mut.*	*B-RAF mut.*
EEC	1	IB	Yes	DOC	58	2.5	E545A (9)	No				
EEC	1	IIB	No	NED	69		E545K (9)	Yes		T41A	G12D	
EEC	2	IC	No	NED	77	4	E545D (9)	Yes	963–968insA			
EEC	3	IC	Yes	NED	88	4.5	E545G (9)	Yes	G132D			
EEC	2	IB	No	NED	63	6	Q546R (9)	Yes	963–968insA			
EEC	3	IB	No	NED	61	4.5	E542K (9)	No				
EEC	2	IC	Yes	NED	82	7	E545K (9)	Yes	643–645delT			
EEC	2	IB	Yes	NED	68	2	E545A (9)	No				
EEC	1	IC	No	NED	71	5	E545D (9)	No	R130G			
EEC	1	IB	No	NED	62	2	E545G (9)	Yes	721–723insT			
EEC	1	IB	No	NED	64	5	E545D (9)	No			G12D	
EEC	1	IB	No	NED	73	5	E545K (9)	No	519–520delCT	S33F		
EEC	1	IB	No	NED	55	3	E542K (9)	Yes	795–800insA			
EEC	1	IB	No	NED	69	4	E545K (9)	No	R130Q		G12A	
EEC	3	IC	No	NED	72	8.8	E542K (9), D1017H (20)	No	433–437insT	S45P		R442T
EEC	3	IIIA	No	NED	59	3.8	E545D (9), Y1021C (20)	No	F81L			
EEC	1	IIA	No	NED	62	3.5	E542V (9), T1052K (20)	No		S45P		
EEC	2	IC	Yes	NED	61	5	A1035T (20)	No	E7X, R15I			
EEC	1	IC	No	NED	65	5	H1047R (20)	No		G34V		
EEC	3	IC	No	NED	58	1.5	H1047R (20)	Yes	K128N		G12D	
EEC	1	IB	No	NED	54	1.5	H1047R (20)	No	S59X, L70F			R443W
EEC	3	IC	Yes	NED	77	4	H1047R (20)	Yes	433delTTTTTA			
EEC	3	IIB	No	NED	69	5	Y1021H (20)	Yes	L146X, 466–469insG			
Mixed	3	IIA	Yes	NED	64	4.2	H1047R (20)	No				
Mixed	3	IIA	Yes	AWD	71	7.5	H1047R (20)	No				
EEC	2	IB	No	NED	58	4.5	H1065L (20)	Yes				
EEC	2	IC	No	NED	77	4.5	H1065L (20)	No	993delC			
EEC	3	IC	No	NED	71	8	M1043V (20)	Yes	390-391delA			
EEC	1	IB	No	NED	66	5	H1047R (20)	No	R173C			
EEC	3	IB	No	NED	72	3.3	Y1021C (20)	No				
EEC	3	IC	Yes	NED	79	3.5	T1025A (20)	No				
Mixed	3	IC	No	NED	74	6	H1047R (20)	No				

MI, microsatellite instability.

detected in 54% (59/109), microsatellite instability in 34% (37/109), β-catenin gene (*CTNNB1*) mutations in 22% (24/109), *K-RAS* mutations in 17% (19/109), and *B-RAF* mutations in 2% (2/109) of cases. *PIK3CA* mutations coexisted with microsatellite instability, or *PTEN*, *CTNNB1*, *K-RAS*, and *B-RAF* mutations. Association of *PIK3CA* and *PTEN* mutations was found in 19 of 109 (17%) endometrial carcinomas, and *PIK3CA* mutations were identified more frequently in tumors with *PTEN* mutations (19 of 59; 32%) than in those without *PTEN* mutations (13 of 50; 26%). Also, *PIK3CA* mutations coexisted with microsatellite instability in 12 of 109 (11%) endometrial carcinomas and were more common in tumors with microsatellite instability (12 of 37; 32%) than in microsatellite stable endometrial carcinomas (20 of 72; 28%). In contrast, coexistence of *PIK3CA* and *CTNNB1* mutations was found in only 5 of 109 (5%) cases, and the former were less common in endometrial carcinomas with *CTNNB*

mutations (5 of 24; 21%) than in those without *CTNNB* mutations (27 of 85; 32%). Similarly, a combination of *PIK3CA/K-RAS* mutations occurred in only 4 of 109 (4%) endometrial carcinomas, and the frequency of *PIK3CA* mutations was lower in endometrial carcinomas with *K-RAS* mutations (4 of 19; 21%) than in those without *K-RAS* mutations (28 of 90; 31%). Nevertheless, the coexistence of *PIK3CA* mutations with microsatellite instability, *PTEN*, *CTNNB1*, or *K-RAS* mutations did not reach statistical significance. The only two *B-RAF* mutations detected (2 of 109; 2%) were associated with *PIK3CA* mutations.

Discussion

The PI3K/AKT pathway is frequently activated in endometrial adenocarcinomas, and a high percentage of mutations of the oncogene *PIK3CA* have recently been reported.[25,27,28] However, in contrast to other epithelial cancers, such as colorectal[31,36] and breast carcinomas,[37,38] no correlation has been found between the distribution of *PIK3CA* mutations and grade and stage of disease. Given the recently reported role of *PIK3CA* mutations in tumor invasion and metastases,[29] we analyzed the *PIK3CA* status in a large series of endometrial adenocarcinomas and compared the results, between *PIK3CA*-mutated and *PIK3CA*-nonmutated groups, with the clinicopathological parameters known to be related to prognosis.

The reported frequency of *PIK3CA* mutations in endometrial carcinoma ranges from 24 to 39%.[25,27,28] Mutations were found predominantly in exon 20 in two studies,[25,27] and evenly distributed between exons 9 and 20 in a third.[28] In our series, we encountered 35 mutations in 32 (29%) tumors, and almost all (91%) were pure endometrioid adenocarcinomas. Microdissection of the different histologic components was performed in the three mixed carcinomas with *PIK3CA* mutations, which were found in the endometrioid component in all three cases. Eighteen mutations were identified in the kinase domain (exon 20) and 17 in the helical domain (exon 9). Three tumors had more than one mutation in exons 9 and 20. Although, in agreement with previous studies, most mutations (67%) were clustered in hot spots E542 and E545 of exon 9 and H1047 of exon 20, rare mutations were also found. It was initially thought that rare mutations would not confer as much growth advantage as hot spot mutations; however, recent data indicate that rare *PIK3CA* mutations in those domains may also result in gain of lipid kinase activity.[39] Only the helical (exon 9) and kinase (exon 20) domains of *PIK3CA* have been analyzed in this study. Recently, the frequency and distribution of *PIK3CA* mutations were reviewed in a total of 2.334 tumor samples corresponding to colon, breast, liver, brain, stomach, lung, and ovary.[40] It was found that 91% of the mutations occurred in exons 9 and 20. Moreover, the role of mutations outside of those domains is unclear.

Previous studies in various epithelial cancers have found that *PIK3CA* mutations are mainly associated with high-grade and invasive tumors.[31,36–38] For instance, in colorectal carcinomas, their frequency was significantly greater in high-grade and advanced carcinomas (32%) than in low-grade tumors (3%).[31] Similarly, a higher rate of *PIK3CA* mutations was found in invasive lobular and ductal breast carcinomas than in other histologic types associated with more favorable prognosis.[37] Also, it has been shown that *PIK3CA* mutations occur in colorectal adenomas when the neoplastic cells acquire the ability to invade.[31] In a comparative study of 44 endometrioid adenocarcinomas and 29 precursor lesions (complex atypical hyperplasias), *PIK3CA* mutations were identified in 39% of the former but only 7% of the latter, suggesting that such mutations represent a late event in endometrial carcinogenesis and are associated with invasion.[28] However, in contrast to colorectal and breast carcinomas, a correlation between *PIK3CA* mutations and tumor grade or clinicopathological stage was neither encountered in this[28] nor in a previous mutational study of 66 endometrial carcinomas.[25] Conversely, we found that tumor grade varied significantly according to the distribution of *PIK3CA* mutations between exons 9 and 20 ($P=0.033$). Whereas in most grade 1 carcinomas the mutations were identified in exon 9 (57.1%), the majority of grade 3 tumors (60%) had mutations in exon 20. In addition, all tumors with *PIK3CA* mutations exhibited myometrial invasion ($P=0.032$) and, moreover, the depth of myometrial invasion correlated also with the occurrence of the mutations either in exon 9 or exon 20. While most adenocarcinomas with exon 9 mutations (64%) had invaded only $\leq 1/2$ of the myometrial thickness (stage IB), the majority of tumors with exon 20 mutations (73%) had either deeper myometrial invasion (stage IC) or cervical involvement (stage II) ($P=0.045$). Also, mutated tumors had a higher frequency of lymphovascular invasion (28%) than nonmutated carcinomas (18%).

Our findings provide evidence that not only *PIK3CA* mutations are associated with adverse prognostic parameters in endometrial adenocarcinomas but also their location either in exon 9 or exon 20 carries a different prognosis. The distinct association of different *PIK3CA* mutations with histologic grade and depth of myometrial invasion is consistent with the recent suggestion that different categories of mutants, as defined by their structural and functional domains, would increase PI3K function by different mechanisms.[39] Mutations in the kinase domain of exon 20 are close to the hinge region of the catalytic loop and would lead to its activation; in contrast, mutations in the helical domain of exon 9 are clustered on an exposed

surface patch of the protein and would change its ability to interact with other regulatory proteins, which may vary for each tissue.[39] Thus, the different mechanisms by which the two categories of *PIK3CA* mutations induce a gain of function may explain their different impact on tumor grade and myometrial invasion, as found in our study.

Three tumors in our series carried more than one mutation, each in different functional domains of exons 9 and 20. Rare cases of double mutations have also been reported in gastric and breast cancers,[23,38] but their significance is unknown. Recent investigations suggest that mutations located in the same exon do not produce an additive gain of function, whereas mutations in different exons increase the kinase activity.[39] Accordingly, the three cases in our study with more than one mutation were advanced tumors and exhibited pathologic features similar to those of carcinomas with mutations in exon 20 only.

We also investigated the occurrence of microsatellite instability and mutations of *PTEN*, *CTNNB1*, *K-RAS*, and *B-RAF* in endometrial carcinomas with *PIK3CA* mutations. Recent studies in colorectal and breast cancer[38,41] have claimed that *PIK3CA* and *PTEN* mutations are mutually exclusive, suggesting that carcinogenic signaling through this pathway can occur either through activation of *PIK3CA* or inactivation of *PTEN*. However, in endometrial carcinoma, three recent studies[25,27,28] have found the coexistence of *PIK3CA* and *PTEN* mutations ranging from 15 to 27%. In one of these investigations,[28] *PTEN* mutations occurred in 48% of complex atypical hyperplasias and 57% of endometrioid adenocarcinomas; however, the frequency of PIK3CA mutations was significantly lower in hyperplasias than in endometrioid adenocarcinomas (7 *vs* 39%). In our series, *PTEN* mutations were detected in 54% of endometrioid adenocarcinomas and coexisted with *PIK3CA* mutations in 17% of cases. *PIK3CA* mutations were more common in tumors with *PTEN* mutations (32%) than in those without *PTEN* mutations (26%); nevertheless, they were not found to be significantly clustered with *PTEN* mutations. Microsatellite instability was found in approximately one-third of the tumors in our series; however, association of microsatellite instability with *PIK3CA* mutations occurred in only 11% of cases. Although *PIK3CA* mutations were slightly more frequent in microsatellite instability (32%) than in microsatellite stable carcinomas (28%), the difference was not statistically significant.

We detected *CTNNB1* mutations in 22% of carcinomas and coexistence with *PIK3CA* mutations in 5%. We found a lower rate of *PIK3CA* mutations in tumors with *CTNNB1* mutations than in carcinomas without *CTNNB1* mutations (21 *vs* 32%). Our results are consistent with the finding of *CTNNB1* (*β*-catenin gene) mutations predominantly in early-stage tumors associated with favorable prognosis.[13,21,42]

PI3K/AKT and RAS are closely related signaling pathways. RAS can activate PI3K both directly and indirectly.[31] Mutations in the *K-RAS* proto-oncogene are identified in approximately 15–30% of endometrioid adenocarcinomas.[9–11] In contrast, the frequency of *B-RAF* mutations is significantly lower.[43–45] It has been suggested that *PIK3CA* and *K-RAS* mutations are mutually exclusive alterations.[27] However, in our study, *K-RAS* mutations were detected in 17% of cases and coexisted with *PIK3CA* mutations in 4%. This discrepancy might be caused by the difference in the number of cases studied. We found *B-RAF* mutations in only two cases and, interestingly, they coexisted with *PIK3CA* mutations, suggesting a possible synergistic effect.

In summary, our study confirms that *PIK3CA* mutations are frequent in endometrial adenocarcinomas and coexist with microsatellite instability and mutations in *PTEN*, *CTNNB1*, *K-RAS*, and *B-RAF*. It provides evidence that tumors carrying *PIK3CA* mutations are often high-grade carcinomas associated with myometrial invasion. Moreover, it shows evidence that carcinomas with *PIK3CA* mutations in exon 20 exhibit higher histological grade and deeper myometrial invasion than those with exon 9 mutations.

Acknowledgement

This work was supported by Grants FIS PI04-1891, PI06-0950, and RTICCCFIS RD06/0020/0015, Department of Health, Spain, and Marató TV3 2005-47.

Conflict of interest

None.

References

1 American Cancer Society. Cancer Facts and Figures 2007. American Cancer Society: Atlanta, GA, 2007.
2 Prat J. Prognostic parameters of endometrial carcinoma. Hum Pathol 2004;35:649–662.
3 Risinger JI, Berchuck A, Kohler MF, *et al.* Genetic instability of microsatellites in endometrial carcinoma. Cancer Res 1993;53:5100–5153.
4 Caduff RF, Johnston CM, Svoboda-Newman SM, *et al.* Clinical and pathological significance of microsatellite instability in sporadic endometrial carcinoma. Am J Pathol 1996;148:1671–1678.
5 Catasus L, Machin P, Matias-Guiu X, *et al.* Microsatellite instability in endometrial carcinomas clinicopathologic correlations in a series of 42 cases. Hum Pathol 1998;29:1160–1164.
6 Tashiro H, Blazes MS, Wu R, *et al.* Mutations in PTEN are frequent in endometrial carcinoma but rare in other common gynecological malignancies. Cancer Res 1997;57:3935–3940.

7 Kong D, Suzuki A, Zou TT, *et al.* PTEN is frequently mutated in primary endometrial carcinomas. Nat Genet 1997;17:143–144.
8 Bussaglia E, del Rio E, Matias-Guiu X, *et al.* PTEN mutations in endometrial carcinomas: a molecular and clinicopathologic analysis of 38 cases. Hum Pathol 2000;31:312–317.
9 Enomoto T, Inoue M, Perantoni AO, *et al.* K-Ras activation in premalignant and malignant epithelial lesions of the human uterus. Cancer Res 1991;51: 5308–5314.
10 Caduff RF, Johnston CM, Frank TS. Mutations of the c-Ki-Ras oncogene in carcinoma of the endometrium. Am J Pathol 1995;146:182–188.
11 Lagarda H, Catasus L, Arguelles R, *et al.* K-ras mutations in endometrial carcinomas with microsatellite instability. J Pathol 2001;193:193–199.
12 Fukuchi T, Sakamoto M, Tsuda H, *et al.* Beta-catenin mutations in carcinoma of the uterine endometrium. Cancer Res 1998;58:3526–3528.
13 Machin P, Catasus L, Pons C, *et al.* CTNNB1 mutations and beta-catenin expression in endometrial carcinomas. Hum Pathol 2002;33:206–212.
14 Maxwell GL, Risinger JI, Alvarez AA, *et al.* Favorable survival associated with microsatellite instability in endometrioid endometrial cancers. Obstet Gynecol 2001;97:417–422.
15 Hirasawa A, Aoki D, Inoue J, *et al.* Unfavorable prognostic factors associated with high frequency of microsatellite instability and comparative genomic hybridization analysis in endometrial cancer. Clin Cancer Res 2003;9:5675–5682.
16 An HJ, Kim KI, Kim JY, *et al.* Microsatellite instability in endometrioid type endometrial adenocarcinoma is associated with poor prognostic indicators. Am J Surg Pathol 2007;31:846–853.
17 Risinger JI, Hayes K, Maxwell GL, *et al.* PTEN mutation in endometrial cancers is associated with favorable clinical and pathologic characteristics. Clin Cancer Res 1998;4:3005–3010.
18 Minaguchi T, Yoshikawa H, Oda K, *et al.* PTEN mutation located only outside exons 5, 6, and 7 is an independent predictor of favorable survival in endometrial carcinomas. Clin Cancer Res 2001;7: 2636–2642.
19 Salvesen HB, MacDonald N, Ryan A, *et al.* PTEN methylation is associated with advanced stage and microsatellite instability in endometrial carcinoma. Int J Cancer 2001;91:22–26.
20 Saegusa M, Hashimura M, Yoshida T, *et al.* Beta-catenin mutations and aberrant nuclear expression during endometrial tumorigenesis. Br J Cancer 2001;84:209–217.
21 Brachtel E, Sanchez C, Moreno G, *et al.* Distinct molecular alterations in complex endometrial hyperplasia (CEH) with and without immature squamous metaplasia (squamous morules). Am J Surg Pathol 2005;29:1322–1329.
22 Campbell IG, Russell SE, Choong DY, *et al.* Mutation of the PIK3CA gene in ovarian and breast cancer. Cancer Res 2004;64:7678–7681.
23 Lee JW, Soung YH, Kim SY, *et al.* PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. Oncogene 2005;24: 1477–1480.
24 Samuels Y, Velculescu VE. Oncogenic of PIK3CA in human cancers. Cell Cycle 2004;3:1221–1224.
25 Oda K, Stokoe D, Taketani Y, *et al.* High frequency of coexistent mutations of PIK3CA and PTEN genes in endometrial carcinoma. Cancer Res 2005;65: 10669–10673.
26 Stambolic V, Suzuki A, de la Pompa JL, *et al.* Negative regulation of PKB/AKT dependent cell survival by the tumor suppressor PTEN. Cell 1998;95:29–39.
27 Velasco A, Bussaglia E, Pallares J, *et al.* PIK3CA gene mutations in endometrial carcinoma. Correlation with PTEN and K-Ras alterations. Hum Pathol 2006;37: 1465–1472.
28 Hayes MP, Wang H, Espinal-Witter R, *et al.* PIK3CA and PTEN mutations in uterine endometrioid carcinoma and complex atypical hyperplasia. Clin Cancer Res 2006;12:5932–5935.
29 Samuels Y, Diaz Jr LA, Schmidt-Kittler O, *et al.* Mutant PIK3CA promotes cell growth and invasion of human cancer cells. Cancer Cell 2005;7:561–573.
30 Silverberg SG, Kurman RJ, Nogales F, *et al.* Tumours of the uterine corpus. In: Tavassoli FA, Devilee P (eds). World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Breast and Female Genital Organs. IARC Press: Lyon, 2003, pp 217–257.
31 Samuels Y, Wang Z, Bardelli A, *et al.* High frequency of mutations of the PIK3CA gene in human cancers. Science 2004;304:554.
32 Feng YZ, Shiozawa T, Miyamoto T, *et al.* BRAF mutation in endometrial carcinoma and hyperplasia: correlation with KRAS and p53 mutations and mismatch repair protein expression. Clin Cancer Res 2005;11:6133–6138.
33 Müller C, Miller CW, Hofmannn W-K, *et al.* Rare mutations of the PIK3CA gene in malignancies of the hematopoietic system as well as endometrium, ovary, prostate and osteosarcomas, and discovery of a PIK3CA pseudogene. Leuk Res 2007;31:27–32.
34 Bocker T, Diermann J, Friedl W, *et al.* Microsatellite instability analysis: a multicenter study for reliability and quality control. Cancer Res 1997;57:4739–4743.
35 Boland CR, Thibodeau SN, Hamilton SR, *et al.* A National Cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58:5248–5257.
36 Samuels Y, Ericson K. Oncogenic PI3K and its role in cancer. Curr Opin Oncol 2006;18:77–82.
37 Buttitta F, Felicioni L, Barassi F, *et al.* PIK3CA mutation and histological type in breast carcinoma: high frequency of mutations in lobular carcinoma. J Pathol 2006;208:350–355.
38 Saal LH, Holm K, Maurer M, *et al.* PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. Cancer Res 2005;65:2554–2559.
39 Gymnopoulos M, Elsliger M-A, Vogt PK. Rare cancer-specific mutations in PIK3CA show gain of function. Proc Natl Acad Sci 2007;104:5569–5574.
40 Bader AG, Kang S, Zhao L, *et al.* Oncogenic PI3K deregulates transcription and translation. Nat Rev 2005;5:921–929.
41 Frattini M, Signoroni S, Pilotti S, *et al.* Phosphatase protein homologue to tensin expression and phosphatidylinositol-3 phosphate kinase mutations in colorectal cancer. Cancer Res 2005;65:11227.

42 Palacios J, Catasus L, Moreno-Bueno G, *et al.* Beta- and gamma-catenin expression in endometrial carcinoma. Relationship with clinicopathological features and microsatellite instability. Virchows Arch 2001;438: 464–469.
43 Mutch DG, Powell MA, Mallon MA, *et al.* RAS/RAF mutation and defective DNA mismatch repair in endometrial cancers. Am J Obstet Gynecol 2004;190:935–942.
44 Salvesen HB, Kumar R, Stefansson I, *et al.* Low frequency of BRAF and CDKN2A mutations in endometrial cancer. Int J Cancer 2005;115: 930–934.
45 Pappa KI, Choleza M, Markaki S, *et al.* Consistent absence of BRAF mutations in cervical and endometrial cancer despite K-Ras mutation status. Gynecol Oncol 2006;100:596–600.