Insulin-like Growth Factor-I Receptor and PTEN Protein Expression in Endometrial Carcinoma

Correlation With bax and bcl-2 Expression, Microsatellite Instability Status, and Outcome

Gloria Peiró, MD,1 Peter Lohse, MD,2 Doris Mayr, MD,1 and Joachim Diebold, MD1

Key Words: Insulin-like growth factor-I receptor; IGF-IR; PTEN; Protein expression; Microsatellite instability; MSI; Endometrial carcinoma

Abstract

We immunohistochemically analyzed 89 endometrial carcinomas for insulin-like growth factor-I receptor (IGF-IR) and PTEN (phosphatase and tensin homolog deleted on chromosome 10) expression. Results were compared with clinicopathologic factors, bcl-2 and bax expression, microsatellite instability (MSI) status, and prognosis. Increased expression of IGF-IR and bcl-2 (>50% cells) was seen in 60 cases (67%) and 15 cases (17%), respectively; loss of PTEN was seen in 13 cases (15%) and of bax in 11 cases (12%). No significant correlation was observed between the proteins or with clinicopathologic factors. Loss of PTEN was more frequent in MSI-positive tumors (4/10 [40%]) than in negative tumors (9/79 [11%]; P = .016). Longer survival was observed for patients with endometrioid tumors, International Federation of Gynecology and Obstetrics stage I or II tumors, grade 1 tumors, superficial myometrial infiltration (<50%), less than 5% necrosis, no vascular invasion, or low level IGF-IR (<10% of cells) (P ≤ .05). Cox analysis showed independent value only for stage, grade, type, and lymph-vascular invasion (P < .05). Our data demonstrate that IGF-IR overexpression occurs in a subset of endometrioid carcinomas, which has potential prognostic value, while loss of PTEN often is associated with the MSI phenotype.

Endometrial carcinomas, like other neoplasias, are believed to arise as a result of molecular alterations in tumor suppressor genes, proto-oncogenes, and DNA damage recognition and repair genes.1

The type 1 insulin-like growth factor receptor (IGF-IR) (15q26.1) is a member of the tyrosine-kinase receptor superfamily involved in cell growth control, malignant transformation, and inhibition of apoptosis. Activated by insulin-like growth ligands, the main signaling pathway rests on the activation of phosphatidylinositol-3-kinase (PI-3K), Akt (also known as protein kinase B [PKB]), and phosphorylation and inactivation of bad.2 IGF-IR has been found to be highly expressed in a number of different human tumors and cell lines such as breast carcinoma, melanoma, astrocytoma, synovial sarcoma, neuroblastoma, pancreatic carcinoma, and prostate carcinoma.3-10 IGF-IR gene alterations and/or expression levels in normal and neoplastic endometrium, however, have not been evaluated extensively.11-14

PTEN (phosphatase and tensin homolog deleted on chromosome 10) (also called MMAC1 or TEP1) is a tumor suppressor gene located on human chromosome 10q23.15 It is a bifunctional phosphatase that is able to dephosphorylate phosphoserines in proteins and phosphatidylinositol phosphatases, thereby directly counteracting PI-3K activity and preventing the activation of Akt/PKB. It is thought that the enzyme regulates cell cycle progression, migration, and survival from apoptosis.16 PTEN germline mutations have been reported in patients affected with familial cancer syndromes.17 Moreover, genetic defects and reduction or loss of expression are found frequently in a large number of sporadic human tumors, including glioblastoma, neuroblastoma, and breast and prostate carcinomas, and this finding has been
associated with adverse pathologic markers and a poor prognosis.\textsuperscript{8,10,15,18,19} Recent reports show that, in endometrial carcinomas, PTEN can be mutated,\textsuperscript{20-29} deleted,\textsuperscript{21} or hypermethylated,\textsuperscript{30,31} leading to loss of protein expression\textsuperscript{28,32,33} and representing one of the most frequent genetic alterations described in this neoplasia.\textsuperscript{17} Several authors have detected PTEN defects also in the adjacent hyperplastic endometrium (with and without atypia), and coexistence with the microsatellite instability (MSI) phenotype has been described.\textsuperscript{20-29} However, regarding the association with clinicopathologic features and prognostic relevance, discordant results have been published.\textsuperscript{20,23,27,30,33}

Finally, it is not clear whether the role of IGF-IR and PTEN on apoptosis involves the known apoptotic regulators of the mitochondrial pathway such as bcl-2 and bax.\textsuperscript{3,4} Therefore, we studied the expression of IGF-IR and PTEN in a series of endometrial carcinomas that had been analyzed previously for MSI status,\textsuperscript{34} as well as for bcl-2 and bax expression.\textsuperscript{35} We correlated these data with clinicopathologic features and survival. Our results show overexpression of IGF-IR and loss of PTEN in a subset of endometrial carcinomas, independent of bcl-2 and bax expression.\textsuperscript{35} A low-level of IGF-IR protein expression was associated with longer survival. Absence of PTEN protein was seen more frequently in tumors with the MSI phenotype.

Materials and Methods

Cases and Tumor Samples

This study analyzed the expression of IGF-IR and PTEN in a total of 89 cases of uterine endometrial carcinoma selected from the surgical pathology files of the Department of Pathology, Grosshadern Hospital, University of Munich, Munich, Germany, for the period January 1984 to December 1994. Patients’ ages ranged from 39 to 92 years (mean, 66 years). Follow-up was available for 84 patients (94%; median follow-up, 2,500 days).

Histologic classification of the tumors and of the adjacent endometrium was performed according to the World Health Organization criteria\textsuperscript{36} and histologic grading and staging according to the International Federation of Obstetrics and Gynecology (FIGO) criteria.\textsuperscript{37} Nonneoplastic endometrium was stratified in 3 categories: (1) atrophic or inactive, (2) simple hyperplasia with no atypia, and (3) complex hyperplasia with and without atypia. Additional histologic features recorded were lymph-vascular invasion (LVI) (nondefinitive and absent or present), depth of myometrial invasion (<50% or >50%), and tumor necrosis (<5% or ≥5%).

DNA Extraction, Microsatellite Markers, and MSI Analyses

Methods for genomic DNA isolation, amplification of microsatellite markers (D2S123, DSS346, Mfd15, BAT25, and BAT26), and analysis of the obtained fragments have been described.\textsuperscript{34} Tumors showing instability at 2 or more (≥40%) loci were defined as high-frequency MSI (MSI-H) or positive, as low-frequency MSI if only 1 of the 5 markers showed instability, and as microsatellite-stable if none of the markers showed instability.

Immunohistochemical Analysis

In each case, all H&E-stained slides were reviewed, and 1 or 2 representative tumor tissue blocks were selected for immunohistochemical analyses. Two- to four-micrometer-thick sections were mounted on poly-L-lysine–coated slides (Sigma Chemical, St Louis, MO), deparaffinized, and rehydrated through graded alcohols to water. In Table 1, the antibodies, clones, sources, pretreatments, working dilutions, incubation period, and localization of the immunostaining are given. The IGF-IR antibody (chicken polyclonal IgY) recognizes the IGF-IR \( \alpha \) subunit, while the PTEN antibody is a synthetic peptide derived from the C-terminal region of human PTEN conjugated to carrier protein.
Staining for IGF-IR and PTEN on formalin-fixed, paraffin-embedded sections was performed with the labeled avidin-biotin complex–peroxidase-AEC (3-amino-9-ethylcarbazol) system. After microwave pretreatment, the slides were rinsed in tris(hydroxymethyl)aminomethane (Tris)-buffered saline (0.05-mol/L concentration of Tris hydrochloride, pH 7.4-7.6) twice for 5 minutes. Endogenous peroxidase activity was blocked by incubation with 10% hydrogen peroxide for 10 minutes, followed by 10 minutes in water. Sections were immersed in Tris-buffered saline twice for 5 minutes. The Vectastain Elite Kit (Vector Laboratories, Burlingame, CA) was used as described by the manufacturer. The primary antibodies were applied and incubated. In negative control samples, the first antibodies were omitted. We also used sections of breast carcinoma with known PTEN status as positive and negative control samples to prove the antibody specificity in our material. After a brief rinsing in Tris with 0.05% of Brij 35 Solution (Merck, Darmstadt, Germany), sections were immersed in 3-amino-9-ethylcarbazole substrate (Sigma, Steinheim, Germany) for 15 minutes, and then were counterstained lightly with hematoxylin and mounted with Glycergel (Merck).

According to the number of positive tumor cells, the staining was scored semiquantitatively as follows: 0, 0%; 1, less than 10%; 2, 10% to 50%; 3, 51% to 80%; or 4, more than 80%. The intensity of the staining was evaluated as weak (1+), moderate (2+), or strong (3+). For each tumor case, the values of the 2 parameters were multiplied, resulting in scores ranging from 0 to 12 (Remmele score).35 Tissue sections contained different proportions of immunostained nontumor cells (residual endometrium, endometrial stroma, myometrium, endothelial cells, or lymphocytes), which served as an internal positive control. Specimens in which tumor cells and normal control cells were completely negative for antibody immunostaining were excluded from the analysis.

For the purposes of the study, expression of IGF-IR was determined by membranous or cytoplasmic staining (<10%, 10%-50%, and >50% positive cells). For the final scoring of the tumors, intensity was not taken into account because the majority stained strongly. Staining for PTEN (cytoplasm) was evaluated as absent (no protein) or present (any evidence), independent of the intensity.

Statistical Analyses

Associations between IGF-IR and PTEN protein levels and several clinicopathologic features, the expression of bax and bcl-2, and the presence of MSI34,35 were examined ($\chi^2$ test and Fisher exact test, as appropriate). Survival curves were drawn by the Kaplan-Meier method, and differences in survival were calculated by using the log-rank test. For multivariate analysis, the Cox proportional hazards model was applied. For all calculations, SPSS-10 statistical software (SPSS, Chicago, IL) was used. $P$ values less than .05 were considered statistically significant.

Results

Tumors were classified into 2 groups: (1) endometrioid type (pure endometrioid, mixed endometrioid and mucinous or squamous, and mixed malignant müllerian tumors owing to the presence of endometrioid characteristics; 76/89 [85%]) and (2) special variants (papillary serous carcinoma or mixed serous and clear cell carcinomas; 13/89 [15%]). Grade 1 was seen in 35 (39%), grade 2 in 29 (33%), and grade 3 in 25 (28%). Tumors in most cases were in a low FIGO stage (I, 61 [69%]; II, 15 [17%]); only 11 (12%) were in stage III, and 2 (2%) were in stage IV. Of the patients, 83 (93%) were older than 50 years (mean age, 66 years).

Adjacent nonneoplastic endometrium, analyzed in 58 cases (65%), showed atrophy in 24 (41%), simple hyperplasia with no atypia in 12 (21%), and complex hyperplasia (with and without atypia) in 22 (38%). No cases of simple hyperplasia with atypia were found. Cases of complex hyperplasia with and without atypia were analyzed together owing to the small number of cases.

Image 1 shows examples of IGF-IR and PTEN protein staining and Table 2 their distribution in relation to clinicopathologic features. IGF-IR staining with the polyclonal IGF-IR $\alpha$ subunit antibody was seen predominantly in the cytoplasm and in the membrane.

Increased expression of IGF-IR (>50% positive cells) was present in 60 (67%) of 89 tumors. Interestingly, the cells of cystic or dilated glands frequently were negative. Among 53 adjacent endometrium specimens evaluable for IGF-IR staining, 30 cases (57%) showed high expression in both the endometrium and the coexisting carcinoma ($P = .018$) but with lower intensity in the adjacent endometrium (strong, 8/53 [15%] vs weak, 22/53 [42%], respectively; $P = .024$). Combined analysis of positive cells and intensity showed lower scores for nonneoplastic endometrium (Remmele score, 2-6 in 29/53 [55%]) compared with carcinoma samples (Remmele score, 8-12 in 30/53 [57%]) ($P = .04$). However, only a trend was observed when the adjacent endometrium was stratified by histologic features (atrophic or inactive vs simple hyperplasia vs complex hyperplasia: $P = .091$).

IGF-IR protein expression in tumors showed no association with the age of the patients; histologic type, grade, or stage of the disease; myometrial wall infiltration; presence of LVI; or necrosis.

Both the adjacent endometrial stroma and the endothelial cells were moderately PTEN protein–positive. Among
58 specimens with adjacent endometrium evaluable for PTEN expression, a heterogeneous pattern of staining was noted in about one third. Absence of the protein was seen in 2 (8%) of 24 cases with atrophic or inactive endometrium and in 3 (14%) of 22 cases with complex hyperplasia and occurred exclusively in PTEN-negative tumors ($P < .0001$).

Regarding PTEN expression in the tumor tissue, absence of the protein was detected in 13 (15%) of 89. We

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Microsatellite Instability Status in Relation to Clinicopathologic Features and Insulin-like Growth Factor-I Receptor (IGF-IR) and PTEN Protein Expression*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IGF-IR</td>
</tr>
<tr>
<td></td>
<td>&lt;10%</td>
</tr>
<tr>
<td>Total</td>
<td>13 (15)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>&lt;50 (n = 6)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>≥50 (n = 83)</td>
<td>12 (14)</td>
</tr>
<tr>
<td>Histological type</td>
<td></td>
</tr>
<tr>
<td>Endometrioid (n = 76)</td>
<td>12 (16)</td>
</tr>
<tr>
<td>Nonendometrioid (n = 13)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>Histological grade</td>
<td></td>
</tr>
<tr>
<td>1 (n = 35)</td>
<td>7 (20)</td>
</tr>
<tr>
<td>2 (n = 29)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>3 (n = 25)</td>
<td>4 (16)</td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
</tr>
<tr>
<td>I (n = 61)</td>
<td>11 (18)</td>
</tr>
<tr>
<td>II (n = 15)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>III (n = 11)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>IV (n = 2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Myometrial invasion</td>
<td></td>
</tr>
<tr>
<td>≤50% (n = 52)</td>
<td>7 (13)</td>
</tr>
<tr>
<td>&gt;50% (n = 37)</td>
<td>6 (16)</td>
</tr>
<tr>
<td>Necrosis</td>
<td></td>
</tr>
<tr>
<td>&lt;5% (n = 72)</td>
<td>11 (15)</td>
</tr>
<tr>
<td>≥5% (n = 17)</td>
<td>2 (12)</td>
</tr>
<tr>
<td>Lymph-vascular invasion</td>
<td></td>
</tr>
<tr>
<td>Absent or nondefinitive (n = 72)</td>
<td>11 (15)</td>
</tr>
<tr>
<td>Present (n = 17)</td>
<td>2 (12)</td>
</tr>
</tbody>
</table>

FIGO, International Federation of Gynecology and Obstetrics; NS, not significant; PTEN, phosphatase and tensin homolog deleted on chromosome 10.

* Data are given as number (percentage). $P$ values were calculated by using the $\chi^2$ test or the Fisher exact test.
found trends between loss of expression and lower FIGO stage (12/13 in stage I or II vs 1/13 in stage III or IV; \( P = .15 \)) and between loss of expression and less than 50% myometrial invasion (10/13; \( P = .14 \)).

Increased expression of bcl-2 (>50% positive cells) was seen in 15 (17%) of 89 cases and loss of bax in 11 (12%) of 89. There was no significant correlation with IGF-IR or PTEN expression (\( P > .05 \)).

MSI was detectable at only 1 locus in 8 (9%), at 2 loci in 7 (8%), and at 3 loci in 3 (3%) of the 89 primary endometrial carcinomas. The last 2 groups (10/18) were classified as MSI-H (≥2 loci), representing 11% of the cases under study. Loss of PTEN was seen more frequently in tumors showing the MSI phenotype (4/10 [40%]) than in negative cases (9/79 [11%]) (\( P = .016 \)). The statistical results were even more significant when we analyzed our cases stratified according to the level of instability: no MSI, 6 (8%) of 71; at 1 locus, 3 (38%) of 8; and at 2 or more loci, 4 (40%) of 10 (\( P = .005 \)). In contrast, no correlation was found with bax expression; loss of bax was seen in 2 (20%) of 10 MSI-H and in 9 (11%) of 79 MSI-negative tumors (\( P = .43 \)).

Longer survival (Kaplan-Meier; log-rank test) was observed in patients with endometrioid tumors (\( P = .0036 \)), FIGO stage I or II tumors (\( P < .0001 \)), grade 1 tumors (\( P < .0001 \)), superficial myometrial infiltration (≤50%) (\( P = .046 \)), no LVI (\( P = .042 \)), and less than a 5% area of tumor necrosis (\( P = .032 \)). In addition, among patients with known follow-up, we observed that those whose tumors contained fewer than 10% cells positive for IGF-IR had a better prognosis than those with 10% or more positive cells (11/12 [92%] and 42/72 [58%], respectively; \( P = .05 \)).

Patients whose tumors were characterized by loss of PTEN also lived longer than those whose tumors contained 10% or more positive cells (median, 36 months; 95% confidence interval, 23 to 48 months vs 12 months; 95% confidence interval, 7 to 17 months; \( P = .001 \)).

### Table 3

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (y)</th>
<th>Type of Carcinoma</th>
<th>Stage</th>
<th>Grade</th>
<th>MSI†</th>
<th>PTEN†</th>
<th>bax†</th>
<th>Survival Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57</td>
<td>Endometrioid</td>
<td>IIb</td>
<td>2</td>
<td>3/5</td>
<td>Loss</td>
<td>Loss</td>
<td>Alive</td>
</tr>
<tr>
<td>2</td>
<td>73</td>
<td>Mixed papillary serous</td>
<td>IIIa</td>
<td>2</td>
<td>2/4</td>
<td>High</td>
<td>High</td>
<td>Dead</td>
</tr>
<tr>
<td>18</td>
<td>61</td>
<td>Mixed endometrioid-mucinous</td>
<td>la</td>
<td>2</td>
<td>3/5</td>
<td>Loss</td>
<td>Loss</td>
<td>Alive</td>
</tr>
<tr>
<td>29</td>
<td>65</td>
<td>Mixed endometrioid-mucinous</td>
<td>la</td>
<td>1</td>
<td>3/5</td>
<td>Loss</td>
<td>High</td>
<td>Alive</td>
</tr>
<tr>
<td>41</td>
<td>71</td>
<td>Endometrioid</td>
<td>IIIa</td>
<td>3</td>
<td>2/5</td>
<td>Loss</td>
<td>High</td>
<td>Alive</td>
</tr>
<tr>
<td>52</td>
<td>84</td>
<td>Mixed papillary serous</td>
<td>lc</td>
<td>3</td>
<td>2/5</td>
<td>High</td>
<td>High</td>
<td>Dead</td>
</tr>
<tr>
<td>62</td>
<td>71</td>
<td>Mixed endometrioid-mucinous</td>
<td>la</td>
<td>1</td>
<td>2/5</td>
<td>High</td>
<td>High</td>
<td>Alive</td>
</tr>
<tr>
<td>76</td>
<td>56</td>
<td>Papillary serous</td>
<td>IIIa</td>
<td>2</td>
<td>2/5</td>
<td>High</td>
<td>High</td>
<td>No follow-up</td>
</tr>
<tr>
<td>83</td>
<td>70</td>
<td>Mixed endometrioid-mucinous</td>
<td>llb</td>
<td>1</td>
<td>2/5</td>
<td>High</td>
<td>Low</td>
<td>Alive</td>
</tr>
<tr>
<td>88</td>
<td>57</td>
<td>Endometrioid</td>
<td>IIIa</td>
<td>3</td>
<td>2/5</td>
<td>High</td>
<td>Low</td>
<td>Dead</td>
</tr>
</tbody>
</table>

MSI, microsatellite instability; PTEN, phosphatase and tensin homolog deleted on chromosome 10.

† For MSI analysis, 5 markers were used; data are given as the number of markers revealing instability/number of markers tested.

‡ Low (≤50% positive cells) and high (≥50% positive cells) protein expression. Loss indicates no expression.

### Figure 1

Univariate survival analysis (Kaplan-Meier). **A**, Insulin-like growth factor-I receptor and percentages of positive cells (<10%, dotted line, 92% of cases; ≥10%, solid line, 58% of cases; \( P = .05 \); log-rank test). **B**, Absent (dotted line, 77% of cases) vs present (solid line, 59% of cases) PTEN (phosphatase and tensin homolog deleted on chromosome 10) protein expression (\( P = .38 \); log-rank test).
longer than those with the PTEN protein present, but the results did not reach statistical significance (10/13 [77%] and 42/71 [59%], respectively; P = .38)  

Cox analysis showed independent value for stage, grade, histologic type, and LVI (all P < .05). PTEN, bax, and bcl-2 protein levels, age, presence of necrosis, and MSI status did not influence the outcome for the patients.

**Discussion**

There is growing interest in identifying genetic factors involved in controlling cell growth and survival whose overexpression or loss of expression eventually may lead to malignant transformation. In the present study, we observed in a subset of endometrial carcinomas the overexpression of IGF-IR (60/89 [67%]) and loss of PTEN (13/89 [15%]), which both are involved in regulating proliferation and apoptosis of normal cells.1,2,16

In normal endometrium, IGF-IR levels are menstrual cycle–dependent (higher during the late proliferative and early to mid secretory phase), consistent with the protein’s role in cell proliferation and differentiation.11 We noticed a high percentage of IGF-IR expression in the endometrium adjacent to the tumor, which was, however, of lesser intensity than in the coexisting carcinoma, and this was independent of the histologic changes (atrophic or inactive vs simple hyperplasia vs complex hyperplasia) and of the tumor type. Our findings suggest that alterations of IGF-IR expression may precede detectable morphologic changes, since they also were present in the nonhyperplastic endometrium. Increased intensity in the tumor tissue, on the other hand, may reflect IGF-IR up-regulation in neoplastic cells. Nevertheless, we did not study IGF-IR expression in cases of normal endometrium not associated with carcinoma for comparison.

In a series of 42 samples of normal endometrium and of 11 endometrial carcinomas, Roy et al14 observed elevated IGF-IR messenger RNA levels in the tumor tissues. Moreover, demonstration of high messenger RNA levels of the ligands, IGF-I and IGF-II, in 2 cases of adenomatous hyperplasia suggested the possibility that the IGF-IR could be activated differently in 2 types of endometrial carcinoma, namely ligand-dependent in type I (endometrioid) endometrial carcinomas and ligand-independent in type II (nonendometrioid) endometrial carcinomas.14 Despite the notion that IGF-IR mediates steroid hormone actions,12 we found no correlation of expression levels with tumors occurring at younger age or with carcinomas of the endometrioid type, which are assumed to develop in the presence of an unopposed excess of estrogen.

In our series, protein expression did not correlate with grade, stage, LVI, myometrial invasion, or tumor necrosis. Nevertheless, patients whose tumors contained lower levels of positive cells survived longer, but the data were not significant when the classic clinicopathologic factors were considered. In vitro and in vivo studies have provided evidence that overexpression of IGF-IR is associated with a more aggressive phenotype. Increased expression of IGF-IR correlated with high Ki-67 indices,6,7 presence of metastases,7,10 progression from benign to malignant lesions,5 tumor recurrence,3 or higher invasiveness.9 In addition, high IGF-IR levels seemed to confer cellular radioresistance.3 Therefore, IGF-IR may be considered a potential new target for treatment.2 In fact, experimental strategies altering its expression and function, combined with the development of viral vectors to deliver the genetic information, could form the basis for the development of a specific therapy for tumors with known IGF-IR involvement.38

Loss of PTEN expression, considered to reflect the loss of gene function induced by mutations,20-29 deletions,21 or promoter hypermethylation,31,33 was observed in 13 (15%) of our 89 cases, which is low compared with previously reported data (20%-61%).20,25,28,32,33 Mutter et al, 25 for example, found a high rate of loss of PTEN (61%). This discrepancy may be explained by the selection of patients with endometrioid-type carcinomas and premalignant lesions. The wide variation also may be due partially to different antibodies and methods of detection or to nonuniform scoring systems used in the studies.

In the present series, we found no association between the absence of the PTEN protein and age, histologic type, grade, stage, LVI, myometrial invasion, or tumor necrosis, as observed by other investigators.21,27 Interestingly, the correlation with the endometrioid type20,23 and a tendency toward a greater proportion of nonendometrioid tumors33 have been reported. Lax et al28 recently observed loss of PTEN (<10% cells) in 52% of clear cell carcinomas, 60% of mixed clear cell or secretory endometrial carcinomas, and all secretory endometrial carcinomas. No relationship between PTEN mutations or loss of PTEN and estrogen or progesterone receptor expression, obesity, premenopause, or history of hormone replacement has been observed,20,27 suggesting that PTEN alteration may be contributing to carcinogenesis through an estrogen-independent pathway. Furthermore, no significant correlation was seen with DNA index, S phase, microvessel density, or expression of Ki-67, p53, p21, or p16 in a recent study by Salvesen et al.33

In the present series, absence of PTEN was associated highly with the MSI phenotype, as previously reported for tumors with a PTEN mutation20-29 or with hypermethylation of the PTEN promoter,31 supporting its role as a target gene in endometrial carcinomas with a deficiency in DNA repair.

In contrast, loss of bax (observed in 11 [12%] of our tumors) did not correlate with MSI status. Previous studies
also have shown a low incidence of \textit{bax} mutations, strongly suggesting that \textit{bax} is only rarely a target gene in endometrial carcinomas.\textsuperscript{21,26}

Our observation of a correlation, although only as a trend, in the absence of \textit{PTEN} protein expression, lower FIGO stages, and longer survival is in line with recent findings showing \textit{PTEN} mutations in endometrial cancers that are less likely to metastasize and that have a favorable prognosis.\textsuperscript{20,27} These results contrast with those reported by Salvesen et al, regarding \textit{PTEN} hypermethylation\textsuperscript{30} and loss of protein expression,\textsuperscript{33} who found an association with advanced stages, although a significant correlation with prognosis could not be reached. Conversely, \textit{PTEN} is lost or mutated at high frequency in glioblastoma\textsuperscript{18} and to a lesser extent in neoplasias of the prostate\textsuperscript{10} and breast\textsuperscript{15,19} and in neuroblastoma,\textsuperscript{8} and this loss seems to be associated with adverse pathologic markers and a poor prognosis.

In the adjacent endometrium evaluable for \textit{PTEN} expression, absence of the protein in 2 (8\%) of 24 atrophic or inactive endometrial samples and in 3 (14\%) of 22 cases of complex hyperplasia occurred exclusively in \textit{PTEN}-negative tumors. Mutational inactivation of \textit{PTEN} in about one fourth to one half of endometrial hyperplasia cases with and without atypia,\textsuperscript{21,22,25} as well as in nonhyperplastic endometrial carcinomas,\textsuperscript{29} indicates that \textit{PTEN} is involved in the earliest stages of tumor development. Interestingly, Levine et al\textsuperscript{22} analyzed cases of complex hyperplasia with atypia with or without synchronous carcinoma for \textit{PTEN} mutations and found that the frequency did not increase with the presence of a synchronous carcinoma. This suggests that \textit{PTEN} mutations may not facilitate the malignant transformation of all tumors. Recent in vitro analyses have pointed out that \textit{PTEN} may be a good candidate for gene therapy in the future.\textsuperscript{39}

The antiapoptotic effect of IGF-IR is mediated by the insulin receptor substrate–1 (IRS-1)/PI-3K-Akt/PKB pathway, and is counteracted by \textit{PTEN}.\textsuperscript{2,16} The process of apoptosis involves various discrete levels, ultimately leading to the activation of cysteine-aspartate specific proteases (caspases). Among the proteins involved upstream of caspase activation, the \textit{bcl}-2 protein family (ie, \textit{bcl}-2, \textit{bax}, \textit{bcl}-x\textit{L}) determines the susceptibility to cell death by their relative levels and interactions.\textsuperscript{40} Some results indicate that the activated IGF-IR may modulate the expression of \textit{bcl}-XL by increasing the protein levels, supporting a link between the IGF-IR and the \textit{bcl}-2 family proteins.\textsuperscript{41} We found, however, no association between IGF-IR and \textit{PTEN} on one side and \textit{bcl}-2 and \textit{bax} on the other, suggesting that IGF-IR and \textit{PTEN} do not interact with the expression levels of these apoptosis regulators. In a series of endometrial carcinomas, Kanamori et al\textsuperscript{52} demonstrated that activation of Akt caused by a loss of \textit{PTEN} may be involved in the mechanism of carcinogenesis by preventing apoptosis through inactivation of bad. A heterodimeric partner for both \textit{bcl}-\textit{XL} and \textit{bcl}-2, bad neutralizes their protective effect by precluding the binding of \textit{bax}, thereby promoting cell death.\textsuperscript{40}

Our results suggest a contributory role for IGF-IR overexpression and loss of \textit{PTEN} in the pathogenesis of a subset of endometrial carcinomas. Additional studies in preneoplastic and neoplastic endometrial cells are necessary to clarify the involvement of IGF-IR and \textit{PTEN} in the deregulation of the complex processes of proliferation and apoptosis. If this involvement is confirmed, as for other mitogenic tyrosine-kinase receptors (ie, epidermal growth factor receptor, HER-2/neu), novel therapeutic agents and strategies that target these pathways would be especially beneficial to patients with endometrial cancer who are at a high risk of death, such as those who have primary tumors that overexpress IGF-IR.

\textbf{From the \textsuperscript{1}Institute of Pathology, Ludwig-Maximilians University, and the \textsuperscript{2}Department of Clinical Chemistry-Grosshadern, Ludwig-Maximilians University, Munich, Germany.}

\textbf{Presented in part at the 91st United States and Canadian Academy of Pathology Annual Meeting, February 23-March 1, 2002, Chicago, IL.}

\textbf{Address reprint requests to Dr Peiró : Pathology Dept, Hospital General Universitari d’Alacant, C/ Pintor Baeza s/n, 03010-Alacant, Spain.}

\textbf{Acknowledgments: We thank Pia Lohse, Heike Ruebsamen, Andrea Sendelhofert, and Sina Heidrich for technical assistance.}

\textbf{References}


