Combined PTEN and p27kip1 Protein Expression Patterns Are Associated with Obesity and Prognosis in Endometrial Carcinomas

Athanassios Dellas,1 Gernot Jundt,1,2 Gideon Sartorius,3 Mischa Schneider,4 and Holger Moch5,6

Abstract

Purpose: Phosphatase and tensin homologue deleted from chromosome 10 (PTEN) and p27kip1 proteins are key players of the Akt pathway, which is nutritionally regulated by insulin receptor signaling and influenced by estrogens. In this study, the prognostic relevance of the PTEN/p27kip1 protein expression in endometrial carcinoma in relationship to the body mass index (BMI) was determined.

Experimental Design: BMI and prognosis of 452 surgically treated patients with endometrial carcinoma were correlated with histologic subtype, International Federation of Gynecology and Obstetrics (FIGO) stage, and differentiation grade. The expression of PTEN and p27kip1 was examined in 257 tumors by immunohistochemistry using a tissue microarray approach.

Results: Lack of PTEN was observed in 136 of 257 (53%) tumors and absence of p27kip1 expression was observed in 106 of 225 (47%) tumors. Absence of both proteins was significantly associated with well-differentiated tumors [PTEN (P < 0.02) and p27kip1 (P < 0.009)]. Differentiation grade, tumor stage, and histologic type were independent of an increased BMI. Importantly, tumors of obese women expressed significantly less PTEN (P < 0.008) and less p27kip1 (P < 0.01) than tumors from nonobese patients. Combined absence of both PTEN and p27kip1 expression characterized a group of 75 (32%) tumors with favorable clinical outcome, particularly in the FIGO stages I and II (P = 0.003) of obese patients. Cox regression analysis revealed that PTEN/p27kip1 phenotype, FIGO stage, and histologic grade were independent predictors of prognosis in endometrioid endometrial carcinoma.

Conclusions: Inactivation of PTEN/p27kip1 proteins is a specific feature in the progression of endometrial carcinoma in obese patients. The phenotype of the combined loss of PTEN/p27kip1 protein expression in obese patients is associated with a significantly better prognosis in endometrioid endometrial carcinoma.

Endometrial cancer represents a major health problem. During the first half of the 20th century, the incidence of cervical cancer was greater than the cancer of the endometrium by a ratio of >3:1, but this trend reversed during the last 50 years (1). Today, endometrial carcinoma is the most common malignant tumor of the female genital tract and the fourth most common cancer in women after carcinomas of the breast, colon, and lung in the western world (2). Epidemiologic data provide a strong link between excess body weight and the risk of developing endometrial carcinoma (3–6).

Endometrial carcinomas are subdivided into two major types based on epidemiology, histopathology, and clinical behavior. Type I tumors are endometrioid endometrial carcinomas (EEC) and type II tumors are non-EEC (NEEC). Type I tumors occur predominantly in perimenopausal women under unopposed estrogen stimulation. These tumors are frequently preceded by atypical endometrial hyperplasia. They are usually low-grade and confined to the uterus, and most women are cured due to an early detection following the initial symptom of irregular uterine bleeding. In contrast, type II tumors develop mainly in elder women in whom the endometrium is atrophic due to the absence of an estrogenic effect. These tumors are predominantly high-grade serous or clear-cell carcinomas. NEEC invade early into the myometrium. They are aggressive and associated with a less favorable clinical outcome. Whereas EEC are due to increased estrogen hormone levels, mainly in obese, younger patients, NEEC are observed in older patients without increased estrogen levels and without an association with obesity. EEC account for ~80% to 85% of endometrial cancers (7, 8).

Phosphatase and tensin homologue deleted from chromosome 10 (PTEN) is a tumor suppressor gene located on chromosome 10q23.3, a genomic region undergoing loss of heterozygosity in many human cancers (9). Somatic deletions or mutations of the PTEN gene have been identified in a large
The cyclin-dependent kinase inhibitor p27kip1 has been suggested as a downstream target of cell cycle arrest of PTEN (15). The encoding gene is located on chromosome 12p13. This location is commonly affected by heterozygous deletions (15). The encoding gene is located on chromosome 12p13. 

PTEN inactivation may occur in endometrial carcinoma by mutation, loss of heterozygosity at 10q23, and promoter methylation. The identification of loss of heterozygosity of 10q23 was observed in ~40% of endometrial carcinoma, and PTEN mutations were subsequently detected in up to 83% of endometrial carcinoma (11, 13). This is the highest known frequency of PTEN mutations in any primary malignancy analyzed (14). In this cancer, PTEN mutations are mainly confined to the endometrioid subtype (EEC). In contrast to tumors of various other organs, PTEN mutations have been found in up to 55% of endometrial hyperplasias, suggesting that the loss of the tumor suppressor function represents an early event in the multistep process leading to endometrial carcinoma (8).

The cyclin-dependent kinase inhibitor p27kip1 has been suggested as a downstream target of cell cycle arrest of PTEN (15). The encoding gene is located on chromosome 12p13. This location is commonly affected by heterozygous deletions and may therefore influence the concentration of the p27kip1 protein. p27kip1 was found to play a role in regulating the progression from the G1 to the S phase (16, 17). An up-regulation of p27kip1 induced by the exogenous PTEN gene transfer was shown in different cell lines, and recent experimental studies have suggested p27kip1 to be a downstream mediator through which PTEN negatively regulates cell cycle progression (15).

Increased body mass index (BMI) is associated with an increased risk of several cancers in adults in western countries (18). Endometrial cancer was the first cancer to be recognized as being strongly associated to obesity (19–21). Recently, a strong positive and linear association of BMI with endometrial cancer risk was described (4). The fact that increased BMI increases risk has been attributed to changes in concentrations of endogenous hormones in obese women. Among women who do not use hormone therapy, adipose tissue provides the major source of circulating estrogens through increased conversion of androstenedione to estrone and decreased production of sex-binding globulin by the liver. Both effects increase the concentration and bioavailability of both circulating estrone and estradiol (3, 7). On the other hand, estrogens produced in adipose tissue have a direct mitogenic effect on endometrial cells, and in postmenopausal obese women, this effect is not counterbalanced by progesterone due to chronic anovulation and thereby much reduced progesterone synthesis (22, 23).

The Akt pathway with PTEN and p27 as key players is involved in the nutritional pathway of tumor cells. Via regulation of insulin growth factor receptors and insulin-binding proteins, the Akt pathway may have a different relevance in EEC and NEEC, but the exact distribution of PTEN and p27 in different tumor subtypes and their potential relation to excessive body weight is unknown.

In an attempt to elucidate these potential relationships, we examined the p27kip1 and PTEN expression in endometrioid (type I) and nonendometrioid (type II) endometrial carcinomas and correlated their expression with the BMI. In patients with an increased BMI, we identified a p27kip1 and PTEN-negative group of EEC characterized by a favorable prognosis.

Translational Relevance

This study of combined phosphatase and tensin homologue deleted from chromosome 10 and p27kip1 expression patterns shows different phenotypes associated with body weight. Based on our analysis of these patterns, we identify risk groups for disease progression among women with endometrial carcinoma. There is clear evidence that increased body mass index is associated with a significantly higher risk for endometrial carcinoma. Excessive body weight is a major modifiable cancer risk factor. Avoidance of overweight and obesity remains the main priority for endometrial carcinoma prevention.

Materials and Methods

**Patients.** Retrospectively, 452 surgically resected adenocarcinomas of the uterus were identified in the Archives of the Institute for Pathology, University of Basel. The endometrial carcinomas were treated between 1985 and 1998 in academic centers and in the community setting. Patients with endometrial cancer who had localized disease were treated by hysterectomy and bilateral salpingo-oophorectomy. Adjuvant radiation therapy was postoperatively given when invasion of the myometrium or histologic grade 3 tumor with myometrial invasion were found. All carcinomas were subsequently staged according to the 6th edition of the International Union Against Cancer and American Joint Committee on Cancer tumor-node-metastasis system (24, 25). Only carcinomas limited to the uterine corpus and the true pelvis were included in the analysis, whereas patients with metastases in the pelvic or paraaortic lymph nodes were excluded from the study [International Federation of Gynecology and Obstetrics (FIGO) stages IIIC (n = 6) and IVB (n = 13)]. The surgical assessment of the nodal spread was not consistently undertaken during the period of study; thus, data on the nodal status are not used. In the Basel region, the assessment of the nodal status in endometrial carcinomas was introduced as a recommended procedure ~10 years ago, to be done in cases with deep myometrial invasion (>50%) and in a surgically low-risk environment (26, 27). The histopathologic analysis was done on H&E-stained sections by one author (H.M.). Immunohistochemistry was done in questionable cases to evaluate the histologic subtype. Histologic grading was according to the FIGO grading system based on the ratio of glandular or papillary structures versus solid tumor growth (grade 1, <5% solid tumor; grade 2, 6-50% solid; and grade 3, >50% solid; refs. 28, 29).

Follow-up information, information about metastasis, and causes of death were obtained from the participating departments of gynecology at the University Hospital Basel, Cantonal Hospital in Liestal/Basel-County, and Women’s Hospitals in Loerrach and Rheinfelden and from the Basel Cancer Registry. Survival time was calculated from the time of biopsy diagnosis to the death of patients due to endometrial carcinoma.

**Assessment of body mass categories.** Information on height and weight at the time of the primary cancer treatment was obtained from the participating departments of gynecology. The height and weight were used to calculate the BMI, which was divided into categories that
incorporated the definitions of normal weight (18.5-24.9), overweight (25.0-29.9), and obesity (≥30.0) as proposed by the WHO classification (30). The underweight category (<18.5) was not used because no BMI < 18.5 occurred. In a subanalysis, we used the BMI to create two categories (nonobese with BMI < 30 and obese women with BMI ≥ 30) to improve the focus on the relationship with different PTEN/p27kip1 phenotypes.

Construction and sectioning of tissue microarrays. For construction of a tissue microarray, 257 endometrial carcinomas were selected. Selection criteria were based on availability, presence, and size of representative tumor areas in paraffin blocks. Tissue microarrays were constructed as described previously (31, 32). Briefly, a tissue arraying instrument (Beecher Instruments) was used to create holes in a recipient paraffin block and to acquire tissue cores from the donor block by a thin-walled needle with an inner diameter of 0.6 mm, held in a X-Y precision guide. One cylindrical sample per case was retrieved from the selected region in the donor and extruded directly into the recipient block with defined array coordinates. A solid steel wire, closely fit in the tube, was used to transfer the tissue cores into the recipient block. After the construction of the array block, multiple 5 mm sections were cut with a microtome using an adhesive-coated tape sectioning system (Instrumedics). H&E-stained sections were used for histologic verification of tumor tissue on the arrayed samples.

Immunohistochemistry. The incubation was done according to the standard procedure of the ABC method (ABC-Elitekit; Vector). Tissue microarray slides were used for antibodies against PTEN and p27kip1 proteins.

Tissue microarray slides were incubated with a mouse PTEN antibody from Cascade Bioscience (clone 6H2.1; working dilution 1:75) overnight at room temperature and with mouse p27kip1 antibody obtained from Transduction Laboratories (clone 57; working dilution 1:500) for 60 min at room temperature. Antigen localization was achieved by an indirect streptavidin-biotin-peroxidase method using the 3-aminopropylcarbazole peroxidase reaction for PTEN and the diaminobenzidine peroxidase reaction for p27kip1.

Sections were counterstained with hematoxylin, dehydrated, and finally, mounted with Crystal/Mount (Biomeda).

Evaluation of staining results. PTEN expression was semiquantitatively evaluated by one observer (H.M.) who estimated the staining intensity and percentage positivity. PTEN expression in normal glands and endometrial stroma was used as internal positive control in each case. PTEN expression was absent in an endometrioid carcinoma with known genetic PTEN alteration. PTEN scoring was done on tissue microarray cores. Therefore, a staining index, obtained as a product of cytoplasmic and nuclear staining intensity and the proportion of immunopositive tumor cells (≤10%, 10-50%, and >50%), was calculated. Cases with a PTEN staining index of ≤10% were defined as negative to exclude false positivity in cores of the tissue microarrays.

For p27kip1 labeling analysis, only nuclear staining was defined as positive. The area with the most intense p27kip1 staining, in accordance with others, was selected from each case and subjected to quantitation of p27kip1 expression by visually counting up to 500 nuclei. The nuclei were evaluated systematically by one observer (H.M.) using high power (×40 at 8-10 fields) and an eyepiece grid. The findings were recorded as the percentage of immunopositive nuclei. A dichotomous variable was used by calculating the median values as cutoff points.

Negative controls included sections stained while omitting the primary antibodies but following the same procedure.

Statistics. Results are given as mean and SD. Relationships between categorical features and counts were evaluated by the nonparametric methods of the Mann-Whitney U test or the Kruskal-Wallis test. Multiple comparisons were done by Fisher’s test. Contingency table analysis was used to analyze the correlations among endometrial carcinomas with and without combined PTEN/p27kip1 expression, histologic grade, and tumor-node-metastasis stage. Survival was defined as the time between primary treatment and death. Patients that survived were censored at the time of their last follow-up. Survival analysis was completed using the Kaplan-Meier method with a log-rank test. A Cox proportional hazards analysis was used to test for independent prognostic information. Statistical analyses were done by use of the JMP 7.0.2 software program (SAS Institute).

Results

Pathology. Based on all available information, correct staging was accomplished in all tumors. Two hundred ninety-five (65%) tumors were FIGO stage I, 75 (17%) tumors were FIGO stage II, 67 (15%) tumors were FIGO stage III, and 15 (3%) tumors were FIGO stage IV.

There were 381 (84%) endometrioid carcinomas and 71 (16%) nonendometrioid carcinomas. Nonendometrioid carcinomas included 30 serous, 9 undifferentiated, 12 clear cell, 9 mucinous, and 11 malignant mixed müllerian tumors. Survival data were available for all 452 patients. There was a mean follow-up of 67.3 ± 62.2 months. All patients were surgically staged during their first surgery and selected for not having metastases in the regional lymph nodes (tumor-node-metastasis: N0). Thus, the overall 5-year survival for all stages and all histologic types was 70%. The respective data for the individual FIGO stages, histologic subtypes (types I and II), histologic grade, and BMI are presented in Table 1. Of all endometrial carcinomas, 43% were well differentiated, 32% were moderately differentiated, and 25% were poorly differentiated.

Women with a grade 1 EEC in FIGO stage I had a significantly better survival than women with grade 2/3 tumors. However, 10% of patients ages ≤70 years and with FIGO stage I died due to tumor progression despite having a favorable histologic type and a low-grade carcinoma.

PTEN and p27kip1 expression. PTEN was negative in 53% of 257 endometrial carcinomas. There were 52% PTEN-negative type I endometrial carcinoma and 50% PTEN-negative type II endometrial carcinoma (Table 1). Grade 1 adenocarcinomas were significantly more frequent PTEN-negative compared with grades 2 and 3 tumors (P < 0.017). There was no association among PTEN protein expression, histologic type, tumor stage, or prognosis.

The p27kip1 expression was low or negative in 47% of all carcinomas. Type II endometrial carcinomas tend to express p27kip1 more frequently than type I tumors, but this difference did not reach significance (P < 0.07). Grade 1 adenocarcinomas showed significantly more frequent negative or low p27kip1 expression compared with grades 2 and 3 tumors (P < 0.013). There were no correlations between p27kip1 expression and FIGO stage or prognosis.

Combined PTEN and p27kip1 expression patterns. There was no correlation between PTEN and p27kip1 expression. The analysis of the combined protein expression revealed significant correlations for different expression phenotypes (Table 2). The combined loss of PTEN/p27kip1 was the most frequent phenotype.

Patients with a combined overexpression of PTEN/p27kip1 had the worst survival (Fig. 1A). A tumor stage-wise analysis revealed that tumors with a combined loss of PTEN/p27kip1 had a significantly better survival only in the FIGO stages I and II (P = 0.0259; Fig. 1B). The difference between both phenotypes disappeared in advanced stages III and IV.

The association between the PTEN/p27kip1 expression phenotype and the prognosis exists only in hormone-dependent
carcinomas. Tumors with a combined loss of PTEN/p27<sup>kip1</sup> expression had a significantly better survival compared with all other phenotypes only in EEC (Fig. 1C). No significant relationship between expression phenotype and prognosis was found in NEEC.

A combined loss of PTEN/p27<sup>kip1</sup> was significantly associated with grade 1 endometrial carcinomas, whereas all other phenotypes occurred more frequently in grade 2/3 tumors. There was no difference in survival between all PTEN/p27<sup>kip1</sup> phenotypes in grade 1 endometrioid carcinomas of FIGO stages I and II. However, the combined loss of PTEN/p27<sup>kip1</sup> was significantly associated with a better survival in grades 2 and 3 endometrioid carcinomas of stages I and II (Fig. 1D).

### BMI categories and pathologic parameters

Information on height and weight at the time of the primary cancer treatment was available for 230 women. Seventy-seven (34%) of 230 patients had a normal BMI, 86 (37%) were overweight, and 67 (29%) were obese. Overweight and obese patients had a higher frequency of endometrioid carcinomas and FIGO stages I and II compared with normal weight women ($P < 0.0001$). Patients with BMI $\geq 30.0$ presented more frequently well-differentiated endometrial carcinomas than normal weight or overweight patients ($P < 0.0001$). The p27 and PTEN expression was significantly associated with BMI categories (Table 2). Importantly, tumors of obese women expressed significantly less PTEN ($P < 0.008$) and less p27<sup>kip1</sup> ($P < 0.01$) than samples from nonobese patients (Table 1).

In addition, the combined loss of PTEN/p27<sup>kip1</sup> was significantly more present in tissue samples of obese carcinoma patients ($P < 0.005$).

By correlating the BMI with both phenotypes, the phenotype of the combined loss of PTEN/p27<sup>kip1</sup> was found to be related to a better survival in obese patients ($P = 0.01$). This phenotype was not associated with prognosis in patients with normal BMI and overweight patients.

### Multivariate survival analysis

A multivariate analysis was done for type I endometrial carcinoma with the variables FIGO stage, differentiation grade, and PTEN/p27<sup>kip1</sup> phenotype. FIGO stage ($P < 0.0001$) and histologic grade ($P = 0.0319$) were independent predictors of prognosis. Importantly, the combined loss of PTEN/p27<sup>kip1</sup> phenotype was also of independent prognostic relevance ($P = 0.019$).

### Discussion

PTEN inactivation is by far the most frequent molecular pathologic alteration in sporadic EEC. There is strong clinical and experimental evidence to suggest that PTEN alteration is both an early event in endometrial carcinogenesis and associated with the course of carcinomas that are less likely to develop an aggressive behavior. Despite the prevalence of endometrial carcinoma, the molecular basis for these changes during the development and progression of endometrial carcinoma are still poorly characterized (11, 33). This is due to the facts that endometrial carcinoma consists of two major groups of epithelial tumors and that the vast majority of them are hormone-dependent, which implies at least two pathways in endometrial tumorigenesis (7, 8).

Loss of PTEN expression has been previously correlated with both favorable and unfavorable clinical outcome (34, 35). These contradictory results suggest changing relationships between PTEN inactivation and other partly unknown molecular genetic alterations in different clinical stages that are responsible for different malignant phenotypes (36). Recently, p27 has been studied by Horree et al. (37) and showed a very heterogeneous expression in EEC. Although the tissue microarray approach has

### Table 1. Clinicopathologic and immunohistochemical features in endometrial carcinoma patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No.</th>
<th>% Survival (5 y)</th>
<th>PTEN expression, all cases/positive no. (% positive cases)</th>
<th>p27&lt;sup&gt;kip1&lt;/sup&gt; expression, all cases/positive no. (% positive cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>452</td>
<td>70</td>
<td>259/121 (47)</td>
<td>227/121 (53)</td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>295</td>
<td>80</td>
<td>156/68 (44)</td>
<td>134/75 (56)</td>
</tr>
<tr>
<td>II</td>
<td>75</td>
<td>72</td>
<td>51/30 (59)</td>
<td>43/21 (49)</td>
</tr>
<tr>
<td>III</td>
<td>67</td>
<td>30</td>
<td>37/19 (51)</td>
<td>35/15 (43)</td>
</tr>
<tr>
<td>IV</td>
<td>15</td>
<td>33</td>
<td>14/4 (29)</td>
<td>13/9 (49)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrioid</td>
<td>381</td>
<td>73</td>
<td>200/95 (48)</td>
<td>175/87 (49)</td>
</tr>
<tr>
<td>Nonendometrioid</td>
<td>71</td>
<td>51</td>
<td>50/25 (50)</td>
<td>43/28 (65)</td>
</tr>
<tr>
<td>Grading FIGO</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>I</td>
<td>194</td>
<td>80</td>
<td>116/46 (39)</td>
<td>99/43 (43)</td>
</tr>
<tr>
<td>II</td>
<td>145</td>
<td>67</td>
<td>81/44 (54)</td>
<td>74/44 (59)</td>
</tr>
<tr>
<td>III</td>
<td>113</td>
<td>55</td>
<td>58/31 (53)</td>
<td>49/31 (63)</td>
</tr>
<tr>
<td>IV</td>
<td>230</td>
<td>P &lt; 0.65</td>
<td>69/39 (57)</td>
<td>65/38 (58)</td>
</tr>
<tr>
<td>BMI &lt;25.0</td>
<td>77</td>
<td>70</td>
<td>77/35 (45)</td>
<td>65/34 (52)</td>
</tr>
<tr>
<td>25.0-29.9</td>
<td>86</td>
<td>68</td>
<td>56/17 (30)</td>
<td>48/17 (35)</td>
</tr>
<tr>
<td>BMI ≥30.0</td>
<td>67</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI subanalysis nonobese vs obese</td>
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</tr>
<tr>
<td>&lt;30.0</td>
<td>163</td>
<td>69</td>
<td>146/74 (51)</td>
<td>130/72 (55)</td>
</tr>
<tr>
<td>≥30.0</td>
<td>67</td>
<td>73</td>
<td>56/17 (30)</td>
<td>48/17 (35)</td>
</tr>
</tbody>
</table>

NOTE: $P$ values of the % survival column represent rank test results within the Kaplan-Meier analysis; all the other $P$ values were calculated following $\chi^2$ analysis.
limitations in studying heterogeneous biomarkers, we have recently shown that tissue microarrays can reliably detect genetic alterations in heterogeneous cell populations of endometrial carcinoma (32). In this study, we aimed to determine the direct relationship of PTEN and p27 in well-defined cell populations, which is possible by tissue microarrays. We investigated the PTEN/p27kip1 protein expression in relationship to patients BMI. We identified a group of PTEN/p27kip1-negative tumors with strong relationship to grade 1 tumors and presence of obesity. Within the group of EEC, PTEN/p27kip1-negative carcinomas had a significant better prognosis. The combined loss of PTEN/p27kip1 might be indicative for a better outcome particularly in grades 2 and 3 endometrioid carcinomas of FIGO stages I and II. Our findings are in line with previous reports that showed an association among PTEN inactivation with early stage, nonmetastatic disease, and more favorable outcome. Both Risinger et al. (34) and Salvesen et al. (35) showed that inactivated PTEN due to mutations is frequent in EEC and that this event defines a prognostically favorable subgroup in both early and advanced stages of endometrial carcinoma. Thus far, Risinger et al. found in patients with advanced-stage disease of endometrial carcinoma a more favorable prognosis due to the presence of PTEN mutations compared with patients with advanced-stage disease and poor prognosis due to the absence of PTEN mutations. However and nevertheless, recent data suggest that only PTEN mutations outside exons 5 to 7 might represent a molecular predictor of favorable prognosis independent of the clinical and pathologic characteristics of the tumors. In addition, PTEN inactivation due to promoter hypermethylation has recently been found to be associated with more advanced stages in endometrial carcinoma. Furthermore, loss of PTEN expression followed by Akt phosphorylation has been considered a poor prognostic factor for patients with EEC (8). These recent reports may contribute to an understanding of our findings of PTEN expression loss in 50% of NEEC. Moreover, the results of the previously cited immunohistochemical investigations led to the proposal that the immunoexpression level of the PTEN protein might be a surrogate for PTEN inactivation by mutation, deletion, or promoter hypermethylation (8). Endometrial carcinoma is characterized by disruption of PTEN or p53 tumor suppressor pathways, respectively. PTEN inactivation is characteristic for the EEC and p53 inactivation for NEEC (29). Deletions or mutations of PTEN are early events in EEC but have also been reported for NEEC. Our finding of PTEN negativity in ~52% of EEC represents a relatively high frequency. This is consistent with the reported PTEN mutation rate of 37% to 83% of EEC (9, 38). In addition, recent data are strongly suggestive that the PTEN gene is a target for mutations in endometrial cancers that have deficiencies in DNA repair (34). Thus, in endometrial carcinomas with microsatellite instability, the PTEN gene is mutated at a much higher frequency compared with EEC that do not exhibit microsatellite instability (27-32%). On the other hand, Mutter et al. (38) found similar PTEN mutation rates of 83% in microsatellite-stable as well as unstable carcinomas, proposing a combined effect of their mutation detection and sample selection methods. In our analysis, we did not determine the microsatellite instability status. Our data show an absence of PTEN expression in half of nonendometrioid carcinomas. This prevalence is slightly higher than reported in other studies. This discrepancy may be result of different antibodies or our PTEN scoring on tissue microarrays. However, other mechanisms than mutation of PTEN inactivation may also occur in NEEC. In early-stage endometrial carcinomas, the complete as well as incomplete loss of the PTEN function has been described (39). According to classic models of malignant transformation, the loss or mutation of only one allele can result in selective proliferative and survival advantage due to impaired apoptosis. PTEN haploinsufficiency could be an important factor in early tumor cell selection and expansion and might therefore occur predominantly in early-stage EEC (39). At later stages in tumor

### Table 2. Phenotype analysis of PTEN and p27kip1 expression in relation to the FIGO stage, histologic type and grade, and BMI

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PTEN-/p27kip1-</th>
<th>PTEN-/p27kip1+</th>
<th>PTEN+/p27kip1-</th>
<th>PTEN+/p27kip1+</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>% Survival (n = 233)</td>
<td>84 (n = 75)</td>
<td>59 (n = 54)</td>
<td>65 (n = 40)</td>
<td>55 (n = 64)</td>
<td>P = 0.01*</td>
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<td>FIGO stage</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>51</td>
<td>33</td>
<td>19</td>
<td>42</td>
<td>P &lt; 0.1†</td>
</tr>
<tr>
<td>II</td>
<td>12</td>
<td>7</td>
<td>9</td>
<td>13</td>
<td></td>
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<td>Histology</td>
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<tr>
<td>Endometrioid</td>
<td>63</td>
<td>41</td>
<td>35</td>
<td>50</td>
<td>P &lt; 0.1†</td>
</tr>
<tr>
<td>Nonendometrioid</td>
<td>12</td>
<td>13</td>
<td>5</td>
<td>14</td>
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<tr>
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<td>19</td>
<td>18</td>
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<td>13</td>
<td>12</td>
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</tr>
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<td>BMI (for n = 193)</td>
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<td>25.0-29.9</td>
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<td>22</td>
<td>14</td>
<td>20</td>
<td>PTEN-/p27kip1- vs all other phenotypes: P &lt; 0.005</td>
</tr>
<tr>
<td>≥30.0</td>
<td>27</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

*P value following rank test within Kaplan-Meier analysis.
†P values following χ² analysis.
progression, the complete loss of PTEN function due to loss of both alleles could exacerbate the course of the disease toward a more aggressive phenotype and metastatic growth. Therefore, we suggest that inactivated PTEN in NEEC is characterized by the complete loss of PTEN function due to biallelic loss.

In the present study, the 5-year survival in surgically staged EEC of FIGO stage I is 80%. This percentage is comparatively low with regard to recently published outcome data (40). Creasman et al. reported a 5-year survival rate of 89.6% in FIGO stage I for patients treated in 1999 to 2001. Presumably, varying treatment modalities in the academic and nonacademic setting and over the period of 14 years influenced negatively our survival rate. On the other hand, and more than one decade ago, the reported 5-year survival rates in endometrial carcinoma of FIGO stage I ranked from 56% to 92%, the average value being 78.4% (41).

There is now striking evidence that increased BMI is associated with significantly higher risk for endometrial carcinoma. Recently, Reeves et al. (5) showed by analyzing the cancer incidence and mortality in relation to BMI in the Million Women Study that as many as half of all endometrial carcinoma cases in postmenopausal women are attributed to overweight or obesity. Clearly, excessive body weight in an increasing part of the society reflects unfavorable changes of lifestyle factors in developed countries. It represents therefore a major modifiable risk factor. On the other hand, our data indicate a clear association between a higher BMI and the more frequent occurrence of EEC that are confined to the uterine corpus, suggesting that BMI > 30 would be associated with a more favorable overall survival. Paradoxically, a significant decrease of excessive body weight in women would increase relatively the percentage of nonendometrioid tumors with more unfavorable clinical courses.

Besides the epidemiologic data, the mechanisms that link excess weight and cancer risk are not fully understood. Three hormonal systems are the most studied candidates: the insulin and insulin-like growth factor axis, sex steroids, and adipokines. All three systems are interlinked through insulin. However, their role might vary between cancer types (5, 6).

More than one system might affect the risk of endometrial cancer: increased estradiol not only increases endometrial cell proliferation and inhibits apoptosis but also might stimulate the local synthesis of insulin-like growth factor-I in endometrial tissue. Furthermore, chronic hyperinsulinemia might promote tumorigenesis in estrogen-sensitive tissues, because it reduces blood concentrations of sex hormone-binding globulin and, in turn, increases bioavailable estrogen. Adiponectin is the most abundant adipokine. It is secreted mainly from visceral fat adipocytes and is inversely correlated with BMI (6).

More recently, a study on sex-specific diet-induced obesity showed a critical interaction between PTEN and adipocyte enhancer binding protein 1, a transcriptional factor with carboxypeptidase activity that is vital for transcriptional repression function (42). In 2005, Zhang et al. (43) presented the first report of a signal-transducing molecule being involved with the regulation of transcription during adipogenesis by describing the role of the directly involved adipocyte enhancer binding protein 1. They provided evidence that adipocyte enhancer binding protein 1 negatively regulates the PTEN function by protein-protein interaction with PTEN, thus
promoting its degradation. In addition, they further suggested that adipocyte enhancer binding protein 1 might be a novel effector of estrogen action that is specific for pathways related to the regulation of obesity (43, 44). Their data provide new insights into a further pathway related to the alteration of the PTEN function in overweight and obese women, suggesting a mechanism leading to the inactivation of the tumor suppressor function of PTEN due to increased BMI. This supports the notion that avoidance of overweight and obesity remains the main priority for endometrial carcinoma prevention (3).

In summary, our results show a clear association between loss of PTEN function and a less aggressive disease progression in EEC. Although there was a statistically significant association between certain staining patterns and better outcome, the assessment of the PTEN/p27 phenotypes is not likely to become part of a clinically useful immunohistochemical panel at the present time because of the varying expression of both proteins in neoplastic and nonneoplastic endometrium. The data further underline the central role of the Akt pathway in obesity-driven cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Combined PTEN and p27kip1 Protein Expression Patterns Are Associated with Obesity and Prognosis in Endometrial Carcinomas

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