Correlation between Loss of PTEN Expression and Akt Phosphorylation in Endometrial Carcinoma

Yasunobu Kanamori, Junzo Kigawa, Hiroaki Itamochi, Muneaki Shimada, Masakuni Takahashi, Syunji Kamazawa, Shinya Sato, Ryoji Akeshima, and Naoki Terakawa

Department of Obstetrics and Gynecology, Tottori University School of Medicine, Yonago 683-8504, Japan

ABSTRACT

The tumor suppressor PTEN acts as a lipid phosphatase, regulates the phosphatidylinositol 3-kinase (PI3K)/Akt-signaling pathway, and modulates cell cycle progression and cell survival. Somatic mutations of PTEN have been reported in a variety of cancers, especially in endometrial carcinoma. To clarify whether and how PTEN and the PI3K/Akt pathway relates to endometrial carcinoma, we examined the expression of those pathway-related proteins in patients with endometrial carcinoma. Of 103 endometrial carcinomas, 37 (36%) showed negative immunohistochemical staining of PTEN. Western blotting revealed that the expression of PTEN in PTEN-negative cases was significantly lower compared with that in positive cases. In contrast, phospho-Akt level in negative cases was significantly higher. We found a significant inverse correlation between PTEN and phospho-Akt ($r = -0.796$). The expression of phospho-Bad was greater in negative cases, suggesting that Bad might be a target for Akt. The present study demonstrates the phosphorylation of Akt accompanied by the loss of PTEN in clinical specimens of endometrial carcinomas.

INTRODUCTION

Endometrial carcinoma, one of the most common malignancies of the female genital tract, is the fourth most common cancer of women in the United States (1). Despite its prevalence, the molecular mechanisms of endometrial carcinogenesis have been poorly understood. Alterations of the K-ras and p53 genes were reported in endometrial carcinoma, but the extent of these alterations is limited (2). PTEN is a tumor suppressor gene located on 10q23, and alterations of this gene have been identified in a large fraction of cancers including endometrial carcinoma (3). The incidence of PTEN mutations (30–50%) in endometrial carcinoma is one of the highest among analyzed tumors (4, 5). PTEN is the most commonly mutated gene identified in endometrial carcinoma (4). Additionally, the mutations were also seen in about 20% of cases of endometrial hyperplasia, a precursor of endometrial carcinoma (6, 7). Accordingly, inactivation of PTEN is considered to be an early event in endometrial carcinogenesis.

PTEN is a lipid phosphatase dephosphorylating the 3-position of phosphatidylinositol 3, 4, 5-triphosphate, a second messenger of PI3K (3, 8). PTEN antagonizes PI3K activity and negatively regulates its downstream-target, the serine/threonine kinase Akt (9). Phosphorylated and activated Akt modulates the activity of a variety of downstream-proteins that relate to cell survival and proliferation (3). It is known that activated Akt phosphorylates and then inactivates the pro-apoptotic factor Bad, which suppresses apoptosis and promotes cell survival (10, 11). On the other hand, overexpression of PTEN inhibits cell growth and induces a G1 arrest with an increase in the cell cycle kinase inhibitor p27 (12–14), suggesting that PTEN inactivation may result in progression of the cell cycle through down-regulation of p27.

Those previous studies suggest that the loss of PTEN function with subsequent activation of PI3K/Akt signaling pathway contributes to carcinogenesis. However, these findings have not been confirmed in patients with endometrial carcinoma. To clarify whether and how PTEN and the PI3K/Akt pathway relates to endometrial carcinoma, we examined the expression of pathway-related proteins such as PTEN, Akt, Bad, and p27 in clinical specimens of endometrial carcinomas.

MATERIALS AND METHODS

Specimen Collection. Paraffin-embedded specimens were collected from 103 patients with endometrial carcinoma who underwent hysterectomy at Tottori University Hospital, Yonago, Japan, between 1990 and 1999. The histological type was endometrioid in all subjects. The number of histological grades included 51 grade 1, 34 grade 2, and 18 grade 3 specimens. Specimens of normal endometrium were obtained from six patients with benign tumor (three in the proliferative phase and three in the secretory phase). All patients gave informed consent before collection of the specimens, according to our institutional guideline.

Immunohistochemistry. A 4-μm section was cut from the paraffin blocks of endometrial carcinoma and normal endometrium. Each section was mounted on a silane-coated glass slide,
deparaffinized, and soaked for 15 min at room temperature in 0.3% H$_2$O$_2$/methanol to block endogenous peroxidase. A mouse monoclonal anti-PTEN antibody, PTEN A2B1 (Santa Cruz Biotechnology, Santa Cruz, CA), was applied for 2 h at 37°C. The primary antibody was visualized using the Histofine Simple Stain PO(M) kit (Nichirei, Tokyo, Japan) according to the instruction manual. The slide was counterstained with hematoxylin.

**Western Blot Analysis.** The tumor specimen was obtained at the time of surgery, snap-frozen, and stored at −80°C. The frozen specimen was homogenized in a lysis buffer containing 50 mM Tris-HCl (pH 7.6), 150 mM NaCl, 0.1% SDS, 1 mM DTT, 10 mM NaF, 2 mM Na$_3$VO$_4$, and 1× Complete Protease Inhibitor Cocktail (Boehringer Mannheim, Ingelheim, Germany). The lysate was centrifuged, and the supernatant was prepared. Protein concentration of the supernatant was measured by Bradford’s assay (15).

Seventy µg of each protein sample was separated by 14% SDS-PAGE, blocked in 2.5% skim milk/TPBS (1× PBS; 0.1% Tween-20) for PTEN and p27, or in 5% BSA/TPBS for phospho-Akt and phospho-Bad. Those samples were probed with each primary antibody overnight at 4°C. The source of anti-PTEN is described above; anti-p27 was obtained from Santa Cruz Biotechnology; and anti-phospho-Akt (Ser473) and anti-phospho-Bad (Ser136) were obtained from New England Biolabs (Beverly, MA). After incubation with horseradish peroxidase-conjugated secondary antibody, protein signals were detected using enhanced chemiluminescence (Amersham, Buckinghamshire, United Kingdom). The

**Fig. 1** PTEN expression in endometrial tissue. **A,** normal endometrium. **B,** positive staining for PTEN in endometrial carcinoma (a positive case). **C,** heterogeneous staining pattern in endometrial carcinoma (a mixed case). **D,** negative staining for PTEN in endometrial carcinoma (a negative case).
immunoblots were quantitated using a public domain NIH image program (written by Wayne Rasband, NIH3).

Statistical Analysis. We used an unpaired *t* test to analyze the differences in expression level of proteins. A Pearson's correlation test was performed to examine the relationship.

RESULTS

In all normal endometria, PTEN staining was observed in both epithelial and stromal cells (Fig. 1A; PTEN was stained in cytoplasm). In carcinoma specimens, stromal cells were stained with PTEN, similarly to normal endometrium, then PTEN-staining in stromal cells was used as the positive control in each case. The status of staining in endometrial carcinoma was evaluated according to the following criteria: (a) a positive case was defined as all of the tumor cells showing staining; (b) a mixed case was defined as the presence of both staining and nonstaining cells; and (c) a
negative case was defined as no staining of any tumor cells. As a result, there were 50 (49%) PTEN-positive cases (Fig. 1B), 16 (15%) mixed cases (Fig. 1C), and 37 (36%) negative cases (Fig. 1D).

According to the immunohistochemical analysis, we selected PTEN-positive or PTEN-negative endometrial carcinomas. Twenty of the protein samples of tumors (10 positive and 10 negative) were examined for the expression of PTEN, phospho-Akt, phospho-Bad, and p27 by Western blotting. Fig. 2 shows the expression of those proteins according to the difference of PTEN-staining status. The level of PTEN expression in negative cases was significantly lower than in the positive cases (Fig. 3A). In contrast, the phospho-Akt level in negative cases was significantly higher (Fig. 3B). A significant inverse correlation between PTEN and phospho-Akt expression was observed (Fig. 3C). The phospho-Bad level in negative cases was significantly higher (Fig. 3D). The expression level of p27 did not differ between positive and negative cases.

DISCUSSION

Immunohistochemical analysis showed that PTEN was expressed in normal endometrium and tumor stromal cells. In contrast, PTEN-negative staining was seen in 37% of endometrial carcinomas, and mixed pattern was seen in 16% of cases. As a result, loss or decrease of PTEN expression was seen in over 50% of endometrial carcinomas. In a previous study, the mutations of PTEN were observed in 50% of endometrial carcinomas (4). These results support the involvement of PTEN in the development and/or progression of endometrial carcinoma in over 50% of cases.

The loss of PTEN expression is considered to reflect the loss of PTEN function induced by a variety of mechanisms such as homozygous deletion, nonsense mutation with LOH, and promoter methylation (16). However, there are no reports concerning PTEN function in patients with endometrial carcinoma. In the present study, we first examined the expression of the molecules relating to the Akt pathway according to the status of PTEN function in clinical samples of endometrial carcinoma. The present study showed that Akt was significantly phosphorylated in tumor tissue with a loss of PTEN expression, and that phospho-Akt expression was negatively correlated with PTEN expression. This finding supports the basic evidence that Akt activation accompanied by PTEN inactivation is a key step in the development and/or progression of carcinomas (3, 9).

Interestingly, Bad, a proapoptotic factor, was more phosphorylated in PTEN-negative tumors, but not extremely so, suggesting that Bad may be a target for Akt phosphorylation. Bad inactivation appears to be involved in part in endometrial carcinogenesis.

In a recent study (17), increased growth activity accompanied by the down-regulation of p27 was observed in PTEN null ES cells. This effect could be dependent on phosphorylation and activation of Akt. The present study failed to find a relationship between PTEN and p27. The Akt pathway leading to p27 is not known in detail, although posttranscriptional regulation was shown to be involved in p27 expression (18). Additional studies are necessary to address the potential roles of p27 in endometrial carcinogenesis.

Mechanisms other than the loss of PTEN function may also contribute to the development and/or progression of endometrial carcinoma, because the expression of PTEN was observed in one-half of endometrial carcinomas. The results of our investigation suggest that, in at least one-half of endometrial carcinomas, the loss of PTEN function relates to carcinogenesis. Activation of Akt caused by the loss of PTEN may be involved in the mechanism of carcinogenesis by preventing apoptosis by Bad inactivation in patients with endometrial carcinoma.

REFERENCES

Clinical Cancer Research

Correlation between Loss of PTEN Expression and Akt Phosphorylation in Endometrial Carcinoma

Yasunobu Kanamori, Junzo Kigawa, Hiroaki Itamochi, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/7/4/892

Cited articles
This article cites 17 articles, 12 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/7/4/892.full.html#ref-list-1

Citing articles
This article has been cited by 9 HighWire-hosted articles. Access the articles at:
/content/7/4/892.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.