Detection of PTEN Immunoreactivity in Endometrial Hyperplasia and Adenocarcinoma

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Objective: *To investigate PTEN (phosphatase and tensin homolog deleted on chromosome 10) expression in endometrial hyperplasia and adenocarcinoma as analyzed by immunohistochemistry.*
Material and Method: *PTEN protein expression was evaluated by immunohistrochemical study of 70 paraffin-embedded curettage endometrial tissue samples (10 normal endometrium, 55 endometrial hyperplasia, and 15 endometrial adenocarcinomas) selected from surgical pathology files of the Division of Gynecologic Pathology, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, from 2001 to 2004. Intensity of epithelial staining of PTEN immunoreactivity in different histologic types was determined.*
Results: *Absence of PTEN protein expression was detected in 60% of endometrial carcinoma, 60% of atypical endometrial hyperplasia, and 24% of typical endometrial hyperplasia. In endometrial hyperplasia without atypia group, the majority of cases revealed moderate to strong PTEN expression, with 70% in simple hyperplasia and 47% in complex hyperplasia. There is a significant statistical difference of PTEN immunoreactivity among proliferative endometrium, endometrial hyperplasia and endometrial carcinoma group (p = 0.004).*
Conclusion: *Complete loss of PTEN protein expression was most commonly found in endometrial carcinoma and hyperplasia with cytologic atypia.*

Keywords: *PTEN, Endometrial hyperplasia, Endometrial carcinoma*

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PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a tumor suppressor gene, mapped on chromosome 10q23.3[1]. The function of PTEN is to inhibit phosphatidylinositol 3-kinase/Akt signaling pathway by preventing phosphorylation, and thus PTEN plays an important role in controlling cellular survival, growth, and apoptosis[2]. Mutations of PTEN are frequently detected in several cancers e.g. breast, brain, prostate, and endometrium[3]. In all histologic subtypes of endometrial adenocarcinoma, endometrioid subtype is reported to have the highest frequency (34-80%) of PTEN mutations[4,5]. Endometrial hyperplasia is a well-known precursor lesion for endometrial carcinoma. Interestingly, PTEN mutations have been reported in 13-55% of premalignant endometrial lesions[6,7]. The present study was conducted to evaluate PTEN expression in endometrial hyperplasia and adenocarcinoma in Thai women as analyzed by immunohistochemistry.

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Material and Method

Tissue samples

Seventy paraffin-embedded endometrial tissue samples diagnosed as normal endometrium, endometrial hyperplasia, or endometrial adenocarcinoma were selected from surgical pathology files of the Division of Gynecologic Pathology, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, from 2001 to 2004. As most cases of endometrial hyperplasia were submitted from curettage, so all the selected samples, including normal endometrium and endometrial adenocarcinoma, in the present study

were curettage specimens. Clinical data were obtained from medical records. Hematoxylin-eosin-stained sections from each case were reviewed to confirm the histological diagnosis. The most representative paraffin block for each case was then selected for immunohistochemical analysis.

Immunohistochemical study

Sections were deparaffinized and subjected to immunohistochemical staining, with standard streptavidin-biotin-peroxidase techniques, and diaminobenzidine (DAB) as the chromogen. Sections of 4 to 5 micron in thickness underwent antigen retrieval by microwave. Sections were incubated with monoclonal antibody 6H2.1 (dilution 1:100 in phosphate buffer, Cascade Bioscience, Winchester, Massachusetts, USA) for 1 hour at room temperature, washed and incubated with secondary biotinylated horse anti-mouse immunoglobulin G. PTEN expression was detected by sequential addition of avidin peroxidase and diaminobenzidine. Negative control was performed by substituting the primary antibody with nonimmune serum. Positive and negative controls were conducted simultaneously. Slides were then evaluated under light microscope. Only brown staining in nucleus was accepted as presence of immunoreactivity. The intensity of the epithelial staining was scored as 0 (absent), 1 (mild), 2 (moderate), and 3 (strong).

Statistical analysis

Statistical analysis was performed with SPSS for Windows software (version 13; SPSS Inc, Chicago, IL, USA). Data were reported as mean $\pm$ standard deviation. The chi-square test and Fisher's exact test were used. Statistical significance is when p-value < 0.05.

Results

Of 70 selected cases included in the present study, samples were 10 normal endometrial tissues (5 proliferative endometrium and 5 secretory endometrium), 55 endometrial hyperplasia, and 15 endometrial carcinoma. Among the endometrial hyperplasia group, 10 cases were simple hyperplasia without atypia, 15 complex hyperplasia without atypia, 6 simple hyperplasia with atypia, and 14 complex hyperplasia with atypia. The mean age $\pm$ SD of endometrial hyperplasia without atypia, atypical endometrial hyperplasia and endometrial carcinoma groups was 46.68 $\pm$ 9.92, 47.50 $\pm$ 9.18 and 59.60 $\pm$ 12.57 years, respectively.

Complete loss of PTEN immunoreactivity was found in 60% of endometrial carcinoma (Fig. 1A) and 60% of endometrial hyperplasia with cytologic atypia (Fig. 1B). In the endometrial without atypia group, the majority of cases revealed moderate to strong PTEN expression, with 70% in simple hyperplasia (Fig. 1C) and 47% in complex hyperplasia (Fig. 1D). Moderate to strong PTEN immunoreactivity was also detected in all cases of proliferative endometrium (Fig. 1E), whereas the majority of secretory endometrium group displayed absent to weak staining (Fig. 1F). Details of PTEN immunoreactivity in each group are shown in Table 1. There is a significant statistical difference in PTEN immunoreactivity between normal endometrium, endometrial hyperplasia and the endometrial carcinoma group ($p = 0.004$). In the endometrial hyperplasia group, there is also a significant statistical difference in PTEN immunoreactivity between endometrial hyperplasia without atypia and atypical endometrial hyperplasia ($p = 0.031$). Although absence of PTEN immunoreactivity was more often detected in complex architecture than simple architecture in both typical and atypical endometrial hyperplasia, however, no significant statistical difference was demonstrated in both groups ($p = 0.345$ and 0.642 in typical hyperplasia and atypical hyperplasia respectively).

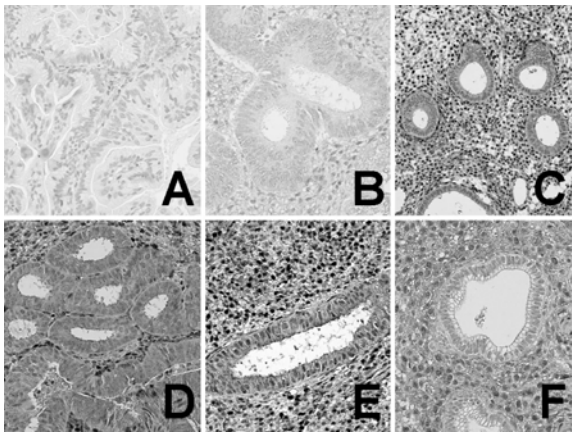

Fig. 1 A) Absent PTEN immunohistochemical staining in endometrial adenocarcinoma,
B) Weak PTEN immunohistochemical staining in complex hyperplasia with atypia,
C) Strong PTEN immunohistochemical staining in simple hyperplasia without atypia,
D) Moderate PTEN immunohistochemical staining in complex hyperplasia without atypia,
E) Strong PTEN immunohistochemical staining in normal proliferative endometrium,
F) Weak PTEN immunohistochemical staining in normal secretory endometrium

Table 1. Immunohistological assessment of PTEN immunoreactivity in endometrial tissue samples (n = 70)

Endometrial tissue diagnosis	No. of samples	PTEN immunohistochemical assessment			
		Absent	Mild	Moderate	Strong
Proliferative endometrium	5	0	0	3 (60%)	2 (40%)
Secretory endometrium	5	2 (40%)	3 (60%)	0	0
Simple hyperplasia without atypia	10	1 (10%)	2 (20%)	4 (40%)	3 (30%)
Complex hyperplasia without atypia	15	5 (33%)	3 (20%)	6 (40%)	1 (7%)
Simple hyperplasia with atypia	6	3 (50%)	3 (50%)	0	0
Complex hyperplasia with atypia	14	9 (64%)	5 (36%)	0	0
Endometrial carcinoma	15	9 (60%)	6 (40%)	0	0

Discussion

Endometrial carcinoma is the most common invasive neoplasm of the female genital tract in the United States and is the fifth most common cancer of women worldwide[8]. Based on clinicopathologic observations, Bokhman proposed two types of endometrial carcinomas: type 1 endometrial carcinoma representing an estrogen-related tumor, usually arising in the background of endometrial hyperplasia, and type 2 endometrial carcinoma that is unrelated to estrogen[9]. Endometrioid subtype comprises over 80% of endometrial carcinomas and most endometrioid tumors are type 1 endometrial carcinomas.

Endometrial hyperplasia is currently classified by World Health Organization (WHO) into four groups: simple hyperplasia without atypia, complex hyperplasia without atypia, simple hyperplasia with atypia, and complex hyperplasia with atypia[10]. In the past, endometrial hyperplasia is indicated as a precursor lesion for endometrial adenocarcinoma. The risk of progression of endometrial hyperplasia into endometrioid adenocarcinoma is closely related to the presence of cytologic atypia and architectural crowding[11]. Currently, there is a discussion to replace WHO classification of the precursors of type 1 endometrial carcinoma by the term endometrial hyperplasia without specification and the term endometrial intraepithelial neoplasia (EIN)[12].

The pathogenesis of endometrial carcinoma and its transition from normal to neoplasm is complex and involves many molecular disturbances, such as microsatellite instability, K-ras mutation, and beta-catenin inactivation[13-15]. PTEN is a tumor suppressor gene that also plays an important role in the pathogenesis of endometrial adenocarcinoma, especially endometrioid subtype[4]. PTEN gene is found to be mutated in 34-80% of endometrioid endometrial adenocarcinoma and 13-55% in endometrial hyperplasia[4-7]. In the present study, absence of PTEN immunoreactivity was detected in the majority of endometrial carcinoma and atypical endometrial hyperplasia, but only in 24% of typical endometrial hyperplasia and none in normal proliferative endometrium. There is a significant statistical difference of PTEN immunoreactivity among groups of normal endometrium, endometrial hyperplasia, and endometrial carcinoma. However, further study in a larger population is necessary.

In a review of the literature, Mutter GL et al[16] examined the expression of PTEN protein in endometrial tissue samples. Absent PTEN expression was found in 61% of endometrial cancer, 75% of EIN, and 29% of unopposed estrogen effect endometrium. Cirpan et al[17] reclassified endometrial hyperplasia cases, examined PTEN protein immunoreactivity in cases with endometrial adenocarcinoma and, surprisingly, found that none of the 10 cases of endometrial carcinomas showed absent PTEN expression. In addition, complete loss of PTEN immunoreactivity was found in only 1 out of 24 cases with EIN. Difference in these results with the present study may be due to the source of primary antibody: Cirpan et al used mouse monoclonal antibody 28H6 clone whereas mouse monoclonal antibody 6H2.1 clone was used in the present study.

PTEN gene expression in normal human endometrium changes throughout the different phases of the normal menstrual cycle, due to fluctuating physiological levels of steroid hormones. Mutter et al[18] reported that high PTEN expression was observed in the proliferative phase, while loss of PTEN protein in epithelial cells and increased PTEN expression of stromal cells were seen in the secretory phase. In the present study, although only 10 normal endometrial samples were selected for examination, the results correlated with published studies[18,19], namely, moderate to strong PTEN immunoreactivity in proliferative

endometrium and absent or mild PTEN expression was in the secretory endometrium.

In summary, decreased PTEN expression tended to associate with neoplastic features of endometrium with significant statistical difference of PTEN immunoreactivity between groups of normal endometrium, endometrial hyperplasia, and endometrial carcinoma. Complete loss of PTEN protein expression was most commonly found in endometrial carcinoma and endometrial hyperplasia with cytologic atypia.

References

1. Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, et al. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 1997; 15: 356-62.
2. Mutter GL. Pten, a protean tumor suppressor. Am J Pathol 2001; 158: 1895-8.
3. Bonneau D, Longy M. Mutations of the human PTEN gene. Hum Mutat 2000; 16: 109-22.
4. Risinger JI, Hayes AK, Berchuck A, Barrett JC. PTEN/MMAC1 mutations in endometrial cancers. Cancer Res 1997; 57: 4736-8.
5. Tashiro H, Blazes MS, Wu R, Cho KR, Bose S, Wang SI, et al. Mutations in PTEN are frequent in endometrial carcinoma but rare in other common gynecological malignancies. Cancer Res 1997; 57: 3935-40.
6. Orbo A, Kaino T, Arnes M, Kopp M, Eklo K. Genetic derangements in the tumor suppressor gene PTEN in endometrial precancers as prognostic markers for cancer development: a population-based study from northern Norway with long-term follow-up. Gynecol Oncol 2004; 95: 82-8.
7. Latta E, Chapman WB. PTEN mutations and evolving concepts in endometrial neoplasia. Curr Opin Obstet Gynecol 2002; 14: 59-65.
8. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007; 57: 43-66.
9. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol 1983; 15: 10-7.
10. Silverberg SG, Kurman RJ, Nogales F, Mutter GL, Kubik-Huch RA, Tavassoli FA. Tumors of uterine corpus. In: Tavassoli FA, Devilee P, editors. Pathology and genetics of tumours of the breast and female genital organs. World Health Organization classification of tumours. Lyon, France: IARC Press; 2003: 217-32.
11. Horn LC, Meinel A, Handzel R, Einenkel J. Histopathology of endometrial hyperplasia and endometrial carcinoma: an update. Ann Diagn Pathol 2007; 11: 297-311.
12. Mutter GL, Zaino RJ, Baak JP, Bentley RC, Robboy SJ. Benign endometrial hyperplasia sequence and endometrial intraepithelial neoplasia. Int J Gynecol Pathol 2007; 26: 103-14.
13. Enomoto T, Inoue M, Perantoni AO, Buzard GS, Miki H, Tanizawa O, et al. K-ras activation in premalignant and malignant epithelial lesions of the human uterus. Cancer Res 1991; 51: 5308-14.
14. Machin P, Catasus L, Pons C, Munoz J, Matias-Guiu X, Prat J. CTNNB1 mutations and beta-catenin expression in endometrial carcinomas. Hum Pathol 2002; 33: 206-12.
15. Burks RT, Kessis TD, Cho KR, Hedrick L. Microsatellite instability in endometrial carcinoma. Oncogene 1994; 9: 1163-6.
16. Mutter GL, Lin MC, Fitzgerald JT, Kum JB, Baak JP, Lees JA, et al. Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. J Natl Cancer Inst 2000; 92: 924-30.
17. Cirpan T, Terek MC, Mgoyi L, Zekioglu O, Iscan O, Ozsaran A. Immunohistochemical evaluation of PTEN protein in patients with endometrial intraepithelial neoplasia compared to endometrial adenocarcinoma and proliferative phase endometrium. Eur J Gynaecol Oncol 2006; 27: 389-92.
18. Mutter GL, Lin MC, Fitzgerald JT, Kum JB, Eng C. Changes in endometrial PTEN expression throughout the human menstrual cycle. J Clin Endocrinol Metab 2000; 85: 2334-8.
19. Guzeloglu-Kayisli O, Kayisli UA, Al Rejjal R, Zheng W, Luleci G, Arici A. Regulation of PTEN (phosphatase and tensin homolog deleted on chromosome 10) expression by estradiol and progesterone in human endometrium. J Clin Endocrinol Metab 2003; 88: 5017-26.

การศึกษา PTEN ในเยื่อบุโพรงมดลูกที่หนาผิดปกติและมะเร็งเยื่อบุโพรงมดลูก

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วัตถุประสงค์: *เพื่อสำรวจการแสดงออกของ PTEN ในเยื่อบุโพรงมดลูกที่หนาผิดปกติและมะเร็งเยื่อบุโพรงมดลูก*

วัสดุและวิธีการ: *ศึกษาการแสดงออกของ PTEN โดยวิธีการย้อม immunohistochemistry ในชิ้นเนื้อเยื่อบุโพรงมดลูกจากบล็อกพาราฟินที่ได้จากการขูดมดลูกที่โรงพยาบาลจุฬาลงกรณ์ในช่วงปี พ.ศ. 2544-2547 โดยคัดเลือกจำนวน 70 ราย ซึ่งประกอบด้วย เนื้อเยื่อบุโพรงมดลูกปกติ 10 ราย, เยื่อบุโพรงมดลูกที่หนาผิดปกติ 55 ราย และมะเร็งเยื่อบุโพรงมดลูก 15 ราย โดยประเมินจากความเข้มของการติดสีย้อม*

ผลการศึกษา: *ไม่พบการติดสีย้อมของ PTEN ใน 60% ของมะเร็งเยื่อบุโพรงมดลูก, 60% ของเยื่อบุโพรงมดลูกที่หนาผิดปกติชนิดมีเซลล์ผิดปกติ (atypia) และ 24% ของเยื่อบุโพรงมดลูกที่หนาผิดปกติชนิดไม่มีเซลล์ผิดปกติ ส่วนใหญ่ในกลุ่มเยื่อบุโพรงมดลูกที่หนาผิดปกติชนิดไม่มีเซลล์ผิดปกติพบมีการติดสีเข้ม โดยพบ 70% ของ simple hyperplasia และ 46.67% ของ complex hyperplasia จากการคำนวณพบว่าการแสดงออกของ PTEN มีความ แตกต่างระหว่างเนื้อเยื่อบุโพรงมดลูกปกติ, เยื่อบุโพรงมดลูกที่หนาผิดปกติ และมะเร็งเยื่อบุโพรงมดลูก อย่างมีนัยสำคัญทางสถิติ (p = 0.004)*

สรุป: *ส่วนใหญ่ของมะเร็งเยื่อบุโพรงมดลูกและเยื่อบุโพรงมดลูกที่หนาผิดปกติชนิดมีเซลล์ผิดปกติ (atypia) ไม่พบการแสดงออกของ PTEN*