The PTEN, BAX, and IGFIIR Genes Are Mutated in Endometrial Atypical Hyperplasia

Kousuke Yoshinaga,1,3 Hironobu Sasano,2 Toru Furukawa,1 Hiromitsu Yamakawa,1,3 Michihiro Yuki,1,3 Shinji Sato,3 Akira Yajima3 and Akira Horii1,4

Departments of 1Molecular Pathology, 2Anatomic Pathology and 3Obstetrics and Gynecology, Tohoku University School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai, 980-8575

To pursue the pathogenesis of endometrial carcinogenesis, we investigated microsatellite instability, mutations in the PTEN, TGFβRII, IGFIIR, and BAX genes, and LOHs on 10q in 18 putative endometrial premalignant lesions (11 endometrial atypical hyperplasias (ATHs), 2 complex hyperplasias, and 5 simple hyperplasias) as well as 8 endometrial cancers (ECs). In the ATH cases, MSIs as well as LOHs at 10q were observed at frequencies similar to those in ECs. Mutations in PTEN, BAX, and IGFIIR were observed only in ATHs and ECs. These results suggest that (1) PTEN, BAX, and IGFIIR are already mutated in ATHs, and (2) ATH is one of the precursor lesions which could lead to EC.

Key words: Endometrial hyperplasia — PTEN — IGFIIR — BAX — MSI

EH, including ATH, SH, and CH, are thought to be premalignant lesions that can lead to EC. It was reported that nearly 20% of EHs develop to ECs.1,2) Proliferation of the normal endometrium is regulated by estrogens and overgrowth can be caused by excessive estrogen exposure. Some of the hyperproliferative foci are reversible, and some are not; the latter form a group of high-risk lesions. It is clinically very important to discriminate the high-risk lesions from the others. However, the genetic pathways relevant to ECs are not yet well understood. To date, frequent microsatellite instability has been reported in ECs,3,4) and mutations in specific genes such as PTEN have been reported in endometrioid ECs.5,6) Frequent LOH was also observed in chromosome 10q in ECs.5,6) PTEN was cloned and mapped to 10q23.3,6,7) and frequent somatic mutations were reported in ECs.5,6) However, detailed analysis of the LOH studies suggested that there exists a 100-kb region of frequent allelic loss between D10S587 and D10S1723 on chromosome 10q25.3-q26.1 in ECs.15,16) We have now extended our study to premalignant lesions of the endometrium.

We first analyzed five microsatellite markers including two mononucleotide repeats (BAT25 and BAT26)13) and three dinucleotide repeats in 10q (D10S587 and D10S1723 at the critical region in 10q25.3-q26.115,16) and D10S2492 at the PTEN locus).14) The study was conducted with twenty-one paired tumors and corresponding normal tissues (11 ATHs, 5 SHs, 2 CHs), as well as 8 ECs (five of which were accompanied by ATHs) obtained at endometrial curettage from Japanese patients with endometrial tumors at the Clinical Laboratory of Sendai City Medical Association and NTT Tohoku Hospital (Sendai). These samples were fixed in formalin and embedded in paraffin. DNAs were extracted according to methods described previously.15) Histopathological diagnosis and clinical staging were done according to the criteria of WHO16) and FIGO.17) respectively.

Microsatellite analyses were performed according to methods described previously.3) Examples are shown in Fig. 1. LOH was obvious in ATH case 1638; the intensity of the lower band, indicated by a thin arrow, was significantly reduced at D10S587 and D10S1723 loci (case 8694). Interestingly, the accompanying ATH lesion in this case retained both PTEN alleles. All of these results are summarized in Tables I through III; the incidences of LOHs at the PTEN and D10S587/D10S1723 loci in ATH were quite similar to those in ECs.
Table I. Summary of Microsatellite Analyses

| Case  | Histological diagnosis | MSI\(^a\) | BAT25 | BAT26 | PTEN | D10S587 | D10S1723 |
|-------|------------------------|----------|-------|-------|-------|---------|---------|
| 7649  | SH                     | –        | H     | H     | ND    | ○       | ○       |
| 6012  | SH                     | –        | ND    | ND    | ○     | ○       | ND      |
| 7233  | SH                     | –        | H     | H     | H     | ○       | ○       |
| 7480  | SH                     | –        | H     | H     | H     | ○       | ○       |
| 7257  | SH                     | –        | H     | H     | H     | ○       | ○       |
| 7083  | CH                     | –        | H     | H     | H     | ○       | ○       |
| 7806  | CH                     | –        | H     | H     | H     | ○       | ○       |
| 7830  | ATH                    | +/-      | H     | H     | ○     | ○       | ○       |
| 4483  | ATH                    | +/-      | H     | H     | ○     | ○       | ○       |
| 9193  | ATH                    | +        | MSI   | MSI   | H     | MSI     | MSI     |
| 10058 | ATH                    | +        | MSI   | MSI   | H     | MSI     | MSI     |
| 7626  | ATH                    | –        | ND    | ND    | H     | ○       | ●       |
| 1638  | ATH                    | –        | H     | H     | H     | ○       | ●       |
| *8694 | ATH                    | +        | H     | MSI   | ○     | H       | MSI     |
| *6013 | ATH                    | –        | H     | H     | ○     | H       | ●       |
| *2993 | ATH                    | –        | H     | H     | H     | ●       | ●       |
| *1465 | ATH                    | –        | H     | H     | H     | ○       | ○       |
| *241  | ATH                    | –        | H     | H     | ○     | ○       | ○       |
| *8694 | EC                     | +        | H     | MSI   | ●     | H       | MSI     |
| *6013 | EC                     | –        | H     | H     | ○     | H       | ●       |
| *2993 | EC                     | –        | H     | H     | H     | ●       | ●       |
| *1465 | EC                     | –        | H     | H     | H     | ○       | ○       |
| *241  | EC                     | –        | H     | H     | ○     | ○       | ○       |
| 10087 | EC                     | –        | H     | H     | H     | ●       | ○       |
| 5854  | EC                     | +        | MSI   | ○     | ○     | MSI     | MSI     |
| 3698  | EC                     | –        | H     | H     | ○     | H       | ●       |

SH, simple hyperplasia; CH, complex hyperplasia; ATH, atypical hyperplasia; EC, endometrial cancer. Asterisks denote samples of ATHs accompanied by ECs. Closed and open circles denote loss and retention of heterozygosity, respectively. H, homozygosity; ND, not determined due to the poor yield of PCR products; MSI, microsatellite instability.

\(^a\) Tumors in which altered sized bands were observed at two or more of the five microsatellite loci were defined as MSI+, and that at only one locus, as MSI+/-.

\(^b\) Previously reported 5-bp insertion/deletion polymorphism in intron 4 of PTEN.\(^10\)

Fig. 1. Examples of allelotype study by the use of microsatellite markers in chromosome 10q. A, Ca, and N denote endometrial atypical hyperplasia, endometrial cancer, and normal tissues, respectively. Note that these two markers D10S587 and D10S1723 are within a 200-kb region on chromosome 10q25.3-q26.1.\(^12\) In cases 1638, 6013, and 2993, LOHs (indicated by thin arrows) are clearly observed. In cases 10058 and 4483, MSIs (indicated by thick arrows) are clearly seen.
MSIs were also observed in this experiment. ATH case 10058 gained one or two CA dinucleotide repeats, as indicated by thick arrows on the left (see Fig. 1). Similarly, in case 4483, gain of one CA repeat was clearly observed. Our results are summarized in Table I; MSI was observed in four (36%) of 11 ATHs. Among these four, two tumors showed MSIs at all five microsatellite loci examined. We
previously observed MSIs in 26% (26/100) of endometrial cancers.3,4) In this study, we also analyzed ECs and found that two (25%) of eight cases showed MSIs. The overall frequencies of MSI+ in ATHs and ECs were almost equivalent. Our results suggested that MSI is one of the early events in endometrial carcinogenesis, in good agreement with the ideas of Mutter et al.19)

We then searched for mutations in the \( TGF\beta RI \), \( IGFIIR \), and \( BAX \) genes using a PCR-based assay.3) The poly(A)\(_{10} \) tract in the \( TGF\beta RI \) gene and the poly(G)\(_{8} \) tract within the coding regions were analyzed. Primers used for mutational analyses were described previously.3,4) Typical results are shown in Fig. 2. ATH case 7830 (MSI−) had a 2-bp deletion in the \( BAX \) gene, and ATH case 10058 (MSI+) had a 1-bp deletion in the \( IGFIIR \) gene. These mutations were confirmed by nucleotide sequencing (data not shown).

In our previous study,3,4) \( IGFIIR \) and \( BAX \) were mutated in MSI+ ECs at the frequencies of 15.4% (4/26) and 11.5% (3/26), respectively. Therefore, these two are thought to be the target gatekeeper genes in ECs. In the present study, although the number of tumors analyzed was not large, 25% (1/4) of the MSI+ ATHs harbored mutations in \( IGFIIR \) (see Tables I and II). Mutation in the \( BAX \) gene was also observed in one ATH case that did not show the MSI phenotype. On the other hand, no mutations in \( TGF\beta RI \) were observed, in good agreement with previous observations in ECs.20) These results suggest that...
the BAX and IGFIIIR genes, but not the TGFβRII gene, are the gatekeepers at the early stage of endometrial carcinogenesis.

Mutation of the PTEN gene has frequently been observed in endometrial cancer, especially in MSI+ cases. Since the amount of DNA was limited and the samples were formalin-fixed and paraffin-embedded tissues, it was not possible to survey the entire coding region of the gene; we only surveyed the mutational hot spots of the PTEN gene in ECs using a PCR-based assay. Thus, we analyzed a poly(A)₆ stretch at codon 265–267 in exon 7 and a poly(A)₆ stretch with two direct repeats as well as a palindromic structure spanning codons 316–323 in exon 8. Nucleotide sequences of the primers for the target region in exon 7 are 5′-CAATGACAAGGAA-3′ and 5′-TTTA TTTGC-3′, and those in exon 8 are 5′-AAGTACA-3′ and 5′-TCCCAATGAAAGTAGTA-AAGTACA-3′ and those in exon 8 are 5′-CGTGCAG-ATAATGACAAGGAA-3′ and 5′-CGTGCAG-ATAATGACAAGGAA-3′. Typical examples of the results are shown in Fig. 3A. Two somatic mutations were observed inATH case 10058: a 1-bp deletion in exon 7 and a 4-bp deletion in exon 8. The nucleotide sequence of the antisense strand of exon 8 is shown in Fig. 3B: a 4-bp deletion at the direct repeat spanning codons 317–320 was clearly seen. The nucleotide sequence in exon 7 was also analyzed, and confirmed a 1-bp deletion in the poly(A)₆ stretch (data not shown). We could not determine whether a two-hit mutation had occurred in this ATH tumor. Histopathological features of this tumor are also shown in Fig. 3C and 3D. Atypical hyperplasia with no sign of cancer was observed. Recently, mutations of PTEN in endometrial hyperplasias were reported by Maxwell et al., in good agreement with the results of our PTEN mutation analysis.

In this study, we analyzed the “premalignant lesions” of the endometrium and found mutations in the BAX, IGFIIIR, and PTEN genes as well as frequent LOH at 10q25.3-q26.1 and MSI in ATHs, but not in SHs or CHs. Genetic alterations observed in ATHs were similar to those observed in ECs. Although we could not examine a large number of the “premalignant lesions,” our results support the idea that ATHs are at high risk for cancer development, but SHs and CHs are not. Further studies are needed to understand the multistep carcinogenesis of the endometrium.

We thank Dr. Nobuyoshi Ozawa (NTT Tohoku Hospital, Sendai) for providing specimens, and Fumiko Date, Keiko Abe, and Katsuhiko Ono (Tohoku University School of Medicine, Sendai) for their assistance in preparation of the samples. This work was supported by grants from the Ministries of Education, Science, Sports and Culture, and Health and Welfare, Japan.

(Received July 10, 1998/Revised September 2, 1998/Accepted September 8, 1998)

REFERENCES

1) Kurman, J. R., Kaminiski, P. F. and Norris, H. J. The behavior of endometrial hyperplasia. Cancer, 56, 403–412 (1985).
2) Terakawa, N., Kigawa, J., Taketani, Y., Yoshikawa, H., Yajima, A., Noda, K., Okada, H., Kato, Y., Yakusbijii, M., Tanizawa, O., Fujimoto, F., Nozawa, S., Takahashi, T., Hasumi, T., Furuhashi, N., Aono, T., Sakamoto, A., Furusato, M. and the Endometrial Hyperplasia Study Group. The behavior of endometrial hyperplasia: a prospective study. J. Obstet. Gynaecol. Res., 23, 223–230 (1997).
3) Ouyang, H., Shiwaku, H. O., Hagiwara, H., Miura, K., Abe, T., Kato, Y., Ohtani, H., Shiiba, K., Souza, R. F., Meltzer, S. J. and Horii, A. The insulin-like growth factor II receptor gene is mutated in genetically unstable cancers of the endometrium, stomach, and colorectum. Cancer Res., 57, 1851–1854 (1997).
4) Ouyang, H., Furukawa, T., Abe, T., Kato, Y. and Horii, A. The BAX gene, the promoter of apoptosis, is mutated in genetically unstable cancers of the colorectum, stomach, and endometrium. Clin. Cancer Res., 4, 1071–1074 (1998).
5) Nagase, S., Sato, S., Tetzuka, F., Wada, Y., Yajima, A. and Horii, A. Detection mapping on chromosome 10q25-q26 in human endometrial cancer. Br. J. Cancer, 74, 1979–1983 (1996).
6) Steck, P. A., Pershouse, M. A., Jasser, S. A., Yang, A. W. K., Lin, H., Ligon, A. H., Langford, L. A., Baumgard, M. L., Hattier, T., Davis, T., Frye, C., Hu, R., Swedlund, B., Teng, D. H. F. and Tavtigian, S. V. Identification of a candidate tumor suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat. Genet., 15, 356–362 (1997).
7) Li, J., Yen, O., Liaw, D., Podospanina, K., Bose, S., Wang, S. I., Puc, J., Miliareis, C., Rodgers, L., McCombie, R., Bigner, S. H., Giovanna, B. C., Ittmann, M., Tygko, B., Hibshoosh, H., Wigler, M. H. and Parsons, R. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science, 275, 349–1947 (1997).
8) Kong, D., Suzuki, A., Zou, T.-T., Sakurada, A., Bose, S., Wang, S. I., Puc, J., Miliareis, C., Rodgers, L., McCombie, R., Bigner, S. H., Giovanna, B. C., Ittmann, M., Tygko, B., Hibshoosh, H., Wigler, M. H. and Parsons, R. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science, 275, 349–1947 (1997).
9) Tashiro, H., Blazes, M. S., Wu, R., Cho, K. R., Bose, S., Wang, S. I., Li, J., Parsons, R. and Ellenson, L. H. Mutations in PTEN are frequent in endometrial carcinoma but
rare in other common gynecological malignancies. *Cancer Res.*, **57**, 3935–3940 (1997).

10) Risinger, J. I., Hayes, A. K., Berchuck, A. and Barrett, J. C. *PTEN/MMAC1* mutations in endometrial cancers. *Cancer Res.*, **57**, 4736–4738 (1997).

11) Nagase, S., Yamakawa, H., Sato, S., Yajima, A. and Horii, A. Identification of a 790-kilobase region of common allelic loss in chromosome 10q25-q26 in human endometrial cancer. *Cancer Res.*, **57**, 1630–1633 (1997).

12) Yamakawa, H., Nagase, S., Yuki, M., Shiwaku, H. O., Yoshinaga, K., Soeda, E., Hoshi, M., Hayashi, Y., Sato, S., Yajima, A. and Horii, A. Identification of a 100-kb region of common allelic loss on chromosome bands 10q25-q26 in human endometrial cancer. *Genes Chromosom. Cancer*, **23**, 74–77 (1998).

13) Dietmaier, W., Wallinger, S., Bocker, T., Kullmann, F., Fishel, R. and Ruschoff, J. Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. *Cancer Res.*, **57**, 4749–4756 (1997).

14) Cairns, P., Okami, K., Halachmi, S., Halachmi, N., Esteller, M., Herman, J. G., Jen, J., Isaacs, W. B., Bova, S. G. and Sidransky, D. Frequent inactivation of *PTEN/MMCA1* in primary prostate cancer. *Cancer Res.*, **57**, 4997–5000 (1997).

15) Goelz, S., Stanley, R., Hamileon, S. and Vogelstein, B. Purification of DNA from formaldehyde fixed and paraffin embedded human tissue. *Biochem. Biophys. Res. Commun.*, **130**, 118–126 (1985).

16) World Health Organization. “Histological Typing of Female Genital Tract Tumors,” 2nd Ed. (1994). Springer-Verlag, Heidelberg.

17) International Federation of Gynecology and Obstetrics, FIGO Stages — 1988 Revision. *Gynecol. Oncol.*, **35**, 125–127 (1989).

18) Sakurada, A., Suzuki, A., Sato, M., Yamakawa, H., Orikasa, K., Uyeno, S., Ono, T., Ohuchi, N., Fujimura, S. and Horii, A. Infrequent genetic alterations of the *PTEN/MMCA1* gene in Japanese patients with primary cancers of the breast, lung, pancreas, kidney, and ovary. *Jpn. J. Cancer Res.*, **88**, 1025–1028 (1997).

19) Mutter, G. L., Boynton, K. A., Faquin, W. C., Ruiz, R. E. and Javanovic, A. S. Allelotype mapping of unstable microsatellites establishes direct lineage continuity between endometrial precancers and cancer. *Cancer Res.*, **56**, 4481–4486 (1996).

20) Abe, T., Ouyang, H., Migitia, T., Kato, Y., Kimura, M., Shiiba, K., Sumamura, M., Matsuo, S. and Horii, A. The somatic mutation frequency of the *transforming growth factor β receptor type II* gene varies widely among different cancers with microsatellite instability. *Eur. J. Surg. Oncol.*, **22**, 474–477 (1996).

21) Maxwell, L. G., Risinger, J. I., Gumbs, C., Shaw, H., Bentley, R. C., Barrett, C. J., Berchuck, A. and Futreal, A. P. Mutation of the *PTEN* tumor suppressor gene in endometrial hyperplasia. *Cancer Res.*, **58**, 2500–2503 (1998).