Estrogen Up-Regulates Mismatch Repair Activity in Normal and Malignant Endometrial Glandular Cells

Tsutomu Miyamoto, Tanri Shiozawa, Hiroyasu Kashima, Yu-Zhen Feng, Akihisa Suzuki, Miyuki Kurai, Toshio Nikaido, and Ikuo Konishi

Department of Obstetrics and Gynecology (T.M., T.S., H.K., Y.-Z.F., A.S., M.K., I.K.), Shinshu University School of Medicine, Matsumoto 390-8621, Japan; Department of Obstetrics and Gynecology (Y.-Z.F.), The Third Hospital, HeBei Medical University, Shijiazhuang 050051, China; and Department of Regenerative Medicine (T.N.), Toyama University Faculty of Medicine, Toyama 939-0398, Japan

Impaired mismatch repair (MMR) is reportedly crucial in the early stages of endometrial carcinogenesis. Although estrogen exposure is considered an important risk factor for endometrial carcinoma, the relationship between estrogen and MMR activity remains undetermined. The present study was undertaken to elucidate the effect of estrogen on MMR activity in normal and malignant endometrial cells. The expression of MMR proteins, hMLH1 and hMSH2, and its correlation with estrogen was examined using immunohistochemical and immunofluorescent techniques. The effect of estradiol (E2) on the expression of hMLH1/hMSH2 protein/mRNA and in vitro MMR activity using two types of heteroduplex (G/T mismatches, 2-base insertion-deletion loops) was examined in cultured normal endometrial glandular cells and estrogen receptor-positive endometrial carcinoma Ishikawa cells. Immunohistochemical expression of hMLH1 and hMSH2 in normal endometrial glands was positively correlated with the serum E2 levels. The expression of hMLH1/hMSH2 protein and mRNA was increased in normal endometrial glandular and Ishikawa cells by E2 treatment. In vitro MMR activity was up-regulated by E2 in both types of cell and heteroduplex. Immunofluorescent analysis demonstrated that E2 enhanced proliferation and hMLH1/hMSH2 expression in both cells; however, proliferating cells without hMLH1/hMSH2 expressions implying high-risk cells were more frequently observed under low E2 concentrations. Collectively, the E2-induced up-regulation of MMR activity in endometrial cells suggests that high estrogen levels act as an intrinsic defense against endometrial carcinogenesis, whereas the imbalance between cell growth and MMR under low E2 environment as seen at postmenopause is vulnerable to carcinogenesis. (Endocrinology 147: 4863–4870, 2006)
Materials and Methods

Immunohistochemistry

Twenty-six samples of normal endometrium were obtained from women with regular menstrual cycles, who underwent a hysterectomy for leiomyomas or endometrial curettage for cytological abnormality of undetermined significance. Histologically, all of these samples showed normal proliferative phase endometria: secretory phase endometria were excluded from the study. The patients ranged in age from 26 to 55 yr, and their serum estrone (E1) and E2 levels were measured on the day of tissue extraction. An indirect immunostaining was performed as previously described (8) using antibodies against hMLH1 (BD Bioscience, Franklin Lakes, NJ), hMSH2 (Calbiochem, Dernstadt, Germany), and Ki-67 (Dako, Glostrup, Denmark). Each result was described as a positivity index (PI), which was calculated by multiplying the intensity of nuclear staining [strongly positive (3), positive (2), weakly positive (1), negative (0)] with the matched percentage of nuclear-positive cells among 300 cells (maximum score 300). Correlations between PI and serum E1 and E2 levels were evaluated with Spearman’s rank correlation. A correlation coefficient greater than 0.4 (positive) or less than −0.4 (negative) was considered to reflect a strong correlation. Each tissue sample was used with the approval of the Ethics Committee of Shinshu University after obtaining written consent from the patients.

Cell culture

Cultured normal endometrial glandular (NEG) cells were prepared as described previously (12). An ER-positive endometrial cancer Ishikawa cell line was kindly provided by Dr. Nishida (Department of Obstetrics/Gynecology, Tsukuba University). Both cells were serum starved (0.1% charcoal-filtered fetal bovine serum) with rapamycin (500 ng/ml) for 2 d. The medium was renewed with serum-starved medium and E2 and 1IC182.780 (Sigma, St. Louis, MO) were added at various concentrations every 24 h. After 2 d culture, cells were harvested and used for Western blotting, MMR assay, and semiquantitative RT-PCR.

Immunofluorescent staining

NEG cell and Ishikawa cells were cultured on collagen-coated chamber slides. When these cells became 50% confluent, serum was starved for 24 h. Various concentrations of E2 were then added and cultured 48 h. After the culture, slides were washed by PBS and stored at −80 C until experiments. For staining, cells were fixed in acetone at −20 C for 15 min, and endogenous peroxidase activity was blocked by methanol for 15 min. After washing by PBS, anti-hMLH antibody (mouse; BD-PharMingen, San Diego, CA) or goat anti-hMSH2 antibody (mouse; Oncogene, Boston, MA) was added together with anti-phosphorylated (p) histone H3 antibody (rabbit; Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at room temperature. After washing, fluorescent (green)-conjugated goat antimmunoglobulin antibody (Alexa Fluor 488, Oncogene) and fluorescent (red)-conjugated goat antibiotin antibody (Alexa Fluor 594) were added for 30 min at room temperature. Nuclear staining was performed using 4,6-diamino-2-phenylindole (Calbiochem). After washing, cells were mounted, and the reactivity was observed using laser confocal microscope (TCS SP2 AOBS; Leica, Wetzlar, Germany). The result of staining for hMLH1 and hMSH2 was described as PI as used in immunohistochemistry of this section, and that for p-histone H3 was described as the percentage of the positive cells among 500 cells.

Western blotting and RT-PCR

Western blotting was performed using proteins of total fractions and the antibodies for hMSH2 and hMLH1 used in the immunostaining as previously described (12). RT-PCR was performed as reported (13) using the primers listed below. The bands detected in Western blotting and RT-PCR were analyzed using densitometry, and the results were given relative to loading controls.

Primer sets included: hMLH1, sense, 5′-tcactcttcacacctagca-3′, antisense, 5′-gctgtttggttcttcc-3′; hMSH2 sense, 5′-cactgtagttggtgtc-3′, antisense, 5′-aggcttgttatcagc-3′; and glycerol-3-phosphate dehydrogenase sense, 5′-agccccattgtacgc-3′, antisense, 5′-ggctctacgccaatgtga-3′.

In vitro MMR assay

The wild-type M13mp2 phage and two mutated type M13mp2 phages containing a mutation (single base mutation changing 89G to 89A) or deletion (deletion of 90G and 91A) in the Lac Z sequence (14) were kindly provided by Dr. Thomas A. Kunkel (National Institutes of Health, Bethesda, MD). The wild-type and two mutated types of M13mp2 were hybridized, and two types of heteroduplexes [named as G/T mismatches and 2-base insertion-deletion loops (IDLs)] were generated for the MMR assay. The MMR assay was performed as previously described (14). In brief, 50 µg of cytoplasmic protein were used to repair 5 ng of each heteroduplex. The repaired heteroduplex was introduced into 50 µl of BMH 71–18 (TaKaRa), 5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside (TaKaRa), and JM109 (TaKaRa) as a host cell. When the (+) strand of the heteroduplex was repaired, a dark blue plaque formed. When the (+) strand of the heteroduplex was repaired, a white (colorless) plaque formed. When the heteroduplex was not repaired, a mixed colored plaque formed. Each type of plaque was counted, and MMR activity was calculated as follows: MMR activity = 100 × (1 − percent of mixed colored plaques with protein extracts/percent of mixed colored plaques without extracts). Each result indicates a mean value for three independent experiments. The statistical analysis was conducted using the Kruskal-Wallis test. P < 0.05 was considered significant.

Results

hMLH1 and hMSH2 expression and serum estrogen levels

The expression of hMLH1 and hMSH2 was observed in the nucleus, with a PI of 155.4 ± 59.9 (mean ± sd) for hMLH1 and 125.3 ± 52.0 for hMSH2. Women with relatively low estrogen levels (E2 < 100 pg/ml) showed weak and limited expression of hMLH1 and hMSH2 with a small number of Ki-67-positive cells (Fig. 1, case 1). In contrast, women with elevated estrogen levels (E2 ≥ 100 pg/ml) showed diffuse and strong expression of both proteins with increased expression of Ki-67 (Fig. 1, case 2). The Pls of hMLH1/hMSH2 and serum levels of E2 and E1 in 26 women showed a strong correlation (E2 and hMLH1, P = 0.0058, rho = 0.552; E2 and hMSH2, P = 0.0050, Fig. 2). The Pl of hMSH2 and E1 level also showed a significant correlation (P = 0.0540, rho = 0.388). The Pl of hMLH1 and E1 level tended to be correlated (P = 0.054, rho = 0.388). The expression of Ki-67 significantly and strongly correlated with that of hMLH1 (P < 0.0001, rho = 0.806) and hMSH2 (P < 0.0037, rho = 0.581).

Effect of E2 on the expression of hMLH1 and hMSH2

Western blotting showed that the expression of both hMLH1 and hMSH2 proteins was increased by treatment with 10−6, 10−8, and 10−10 M of E2 in NEG cells (Fig. 3A). The expression of hMLH1 protein showed an increase in a dose-dependent fashion with a peak ratio of 2.3 at 10−8 M of E2. In Ishikawa cells, the expression of both hMLH1 and hMSH2 proteins was increased by E2 treatment with a peak of hMLH1 at 10−8 M (2.1) and hMSH2 at 10−10 M (3.0) (Fig. 3B). The E2-induced up-regulation of these proteins was suppressed by the addition of ICI182,780, with up to 49% suppression for hMLH1 and 42% for hMSH2 at 10−10 M (Fig. 3B). The RT-PCR revealed that the expression of hMLH1 and hMSH2 mRNAs was increased by E2 treatment, with a peak expression of both hMLH1 (2.4) and hMSH2 (2.3) at 10−8 M in Ishikawa cells (Fig. 3C).
Effect of estradiol on MMR activities

Typical blue, colorless, and mixed plaques are shown in Fig. 4A. In NEG cells, the MMR activity for both G/T mismatches and 2-base IDLs was increased significantly by E2 treatment at 10^{-10}, 10^{-8}, and 10^{-10} M (Fig. 4, B and C), with a peak of efficiency at 10^{-10} M for G/T mismatches (relative ratio 1.5) and 10^{-8} M for 2-base IDLs (2.8). In Ishikawa cells, G/T mismatch repair activity was increased by E2 treatment at 10^{-10} and 10^{-8} M with a peak at 10^{-8} M (1.4) (Fig. 4D). The repair activity for 2-base IDLs was also significantly up-regulated in a dose-dependent fashion by E2 treatment with a peak at 10^{-8} M (1.5) (Fig. 4E). The increased MMR activity for both G/T mismatches and 2-base IDLs at 10^{-8} M of E2 in Ishikawa cells was suppressed by the addition of ICI182.780 at 10^{-7} M (Fig. 4, F and G). These findings indicate that E2-induced increased expression of MMR proteins are correlated well with in vitro MMR activity in normal and malignant endometrial glandular cells.

Correlation between the expression of MMR protein and growth activity in vivo

As shown in Fig. 1, when the serum E2 level was high as seen in case 2, the expression of both hMLH1 and hMSH2 was diffusely and strongly positive with minimal intercellular heterogeneity of staining. There were many Ki-67-positive proliferating cells; however, these Ki-67-positive cells were always associated with strong MMR proteins expression. In contrast, as shown in case 1, when the serum E2 level was low, the expression of MMR proteins was diffusely weak or negative. Interestingly, however, there were still several proliferating cells, even under the low E2 level, and these proliferating cells were devoid of MMR protein expression. The Ki-67-positive/ MMR protein-negative cells were more frequently observed in patients under low serum E2 levels. We consider the Ki-67-positive/MMR-negative cells may have an imbalance between MMR function and cell growth, and these cells are high-risk cells in terms of endometrial carcinogenesis.
Correlation between the expression of MMR protein and growth activity in vitro

To further examine the presence or absence of such high-risk cells in vitro, the expression of MMR protein was examined under various E2 concentrations and was simultaneously compared with growth activity as indicated by phosphorylated (p)-histone H3 protein in NEG cells and Ishikawa cells using immunofluorescent staining. Figure 5 exemplifies a result of immunofluorescence for the expression of hMLH1, p-histone H3 protein in different E2 concentrations. When E2 concentration was high (10^{-8} M, lower panel), the hMLH1 expressions were diffusely positive, and almost all of the cells were p-histone H3 positive. In contrast, when E2 concentration was low (10^{-12} M, upper panel), the expression of hMLH1 was diffusely weak and the number of p-histone H3-positive cells was drastically decreased in number. However, there were several cells with positive staining for H-histone H3 along with weak or negative for hMLH1 under low E2 concentration as indicated by arrows. We considered that the p-histone H3-positive/hMLH1-negative cells indicate the possible imbalance between cell growth and MMR function, suggesting high-risk cell as also observed in vivo.

Results of immunofluorescent staining for hMLH1, hMLH2, and p-histone H3 in NEG cells are summarized in Fig. 6A. The expression of hMLH1 and hMSH2 was increased in higher E2 concentrations (10^{-10}, 10^{-8} M), compared with that in 10^{-12} M with significant differences (P < 0.05), and the number of p-histone H3-positive cells were also tended to be increased under higher E2 concentrations. Treatment with ICI182.780 suppressed the expression of both MMR proteins and p-Histone H3. The percentage of high-risk NEG cells with p-histone H3-hMLH1 expression or with p-histone H3-hMSH2 expression was higher in lower E2 concentration (10^{-12} M) than that in higher E2 concentrations (10^{-10}, 10^{-8} M) with significant differences (P < 0.05) (Fig. 6B). Treatment with ICI182.780 increased the number of cells with p-histone H3-hMLH1 expression with significant difference (P < 0.05). In Ishikawa cells (Fig. 7), the expression of hMLH1 and hMSH2 was increased under higher E2 concentrations (10^{-9}, 10^{-8} M) than that under low E2 concentration (10^{-12} M) in a dose-dependent fashion with a peak at 10^{-9} M with significant differences (P < 0.05), and the number of p-histone H3-positive cells were in parallel with MMR protein expressions. Treatment with ICI182.780 suppressed the expression of both MMR proteins and p-histone H3 (Fig. 7A). The percentage of the cells with p-histone H3-hMLH1 expression or with p-histone H3-hMSH2 expression was also lower in higher E2 concentrations (10^{-10}, 10^{-8} M), compared with that in low E2 concentration (10^{-12} M) with significant differences (P < 0.05). Treatment with ICI182.780 also increased the number of the cell with p-histone H3>MMR protein expressions (Fig. 7B).

Discussion

In the DNA mismatch repair system, hMSH2 forms hMutSα and hMutSβ complexes with hMSH6 and hMSH3, respectively, whereas hMLH1 and hPMS2 form a hMutLα heterodimer. The hMutSα and hMutLα complexes together correct single-base mismatches and IDLs, whereas the hMutSβ and hMutLα complexes correct mainly loops (11). In the present study, the immunohistochemical expression of
hMLH1 and hMSH2 was observed in normal proliferative-phase endometria, being consistent with previous reports (7, 8). In addition, it was significantly correlated with the serum E2 level and Ki-67 expression, implying that endometrial glandular cells with increased growth activity under high-level estrogen environment have elevated levels of both hMLH1 and hMSH2 proteins. Our *in vitro* data obtained using Western blotting and RT-PCR also demonstrated that the expression of these MMR proteins was up-regulated by E2 treatment in both normal glandular cells and endometrial carcinoma Ishikawa cells. All of these findings indicate that E2-induced growth of normal and malignant endometrial glandular cells is usually associated with increased expression of hMLH1 and hMSH2. The amount of MMR proteins is likely to be controlled at the transcriptional level because the mRNA expression of both hMLH1 and hMSH2 was increased by E2 treatment in Ishikawa cells. The expression of hMLH1 and hMSH2 has reportedly been augmented in proliferating cells (15). The hMLH1 promoter has a hemiestrogen-responsive element (16), and the hMSH2 promoter has an activator protein-1-like sequence (17), suggesting the presence of an estrogen-mediated transcriptional activation of MMR proteins.

Our study also demonstrated that E2 treatment increases the MMR activity for both G/T mismatches and 2-base IDLs in cultured normal glandular cells and endometrial carcinoma Ishikawa cells. The *in vitro* assay used in the present study is suitable for the analysis of MMR activities in which hMSH2 and hMLH1 are involved because the addition of recombinant hMutS/hMutL complexes containing hMSH2/hMLH1 was shown to increase markedly the efficiency of repair (13). It is also noteworthy that the MMR activity for both heteroduplexes was significantly increased at an E2 concentration of $10^{-10}$ M, which is within the physiological range of its concentration. In Ishikawa cells, the G/T mismatches repair activity was significantly increased at $10^{-10}$ M of E2 with a peak at $10^{-8}$ M. This pattern correlated well with that of increased expression of hMLH1 and hMSH2 protein under E2 treatment, suggesting that these proteins primarily contribute to the repair of G/T mismatches. In contrast, strong MMR activity for 2-base IDLs was observed at $10^{-6}$ M of E2 in Ishikawa cells. The discrepancy between

---

**FIG. 4.** Results of *in vitro* MMR assay for activity to repair G/T mismatches and 2-base IDLs in cultured NEG cells (B and C) and Ishikawa cells (D–G). A, Mixed, colorless, and blue plaques are shown. B–G, E2 induced an elevation of MMR activity for both G/T mismatches and 2-base IDLs in each of the cell types. Each result shows the mean value for three independent experiments. Addition of ICI182.780 suppressed the E2-induced up-regulation of MMR activity for both.* $P < 0.05$
the patterns of MMR activity and hMLH/hMSH2 protein expression suggest the involvement of other types of repair proteins, and we recently observed the estrogen-induced up-regulation of hMSH6 and hPMS2 mRNA expression in Ishikawa cells (data not shown). In addition, the E2-induced elevation of MMR activity was suppressed by ICI182.780 treatment, indicating that the effect of E2 on MMR activity was mediated by signaling through ER. To our knowledge, this is the first report of E2-induced activation of MMR in human endometrial glandular cells. In the normal glandular cells, however, the expression of hMLH1 protein peaked at 10^{-6} M of E2 and that of hMSH2 at 10^{-10} M, whereas MMR activity increased at 10^{-8} and 10^{-10} M. Therefore, it is still unclear which of the two proteins predominantly contributes

**FIG. 5.** Result of immunofluorescent staining for hMLH1 (A and D), p-histone H3 protein (B and E; a growth marker) and 4',6'-diamino-2-phenylindole (C and F; a nuclear marker) in NEG cells (A–C, and D–F are same cells). In low E2 concentration (E2: 10^{-12} M, upper panel), the expression of hMLH1 was weak and the number of p-histone H3 cells was small. In high E2 concentration (E2: 10^{-8} M, lower panel), the expression of hMLH1 and p-histone H3 was increased. Note that there are a few cells with positive staining for p-histone H3 (B, arrows) and weak or negative staining for hMLH1 (A, arrows), suggesting high-risk cells at low E2 concentration.

**FIG. 6.** Result of immunofluorescent staining for hMLH1 and hMSH2 in NEG cells at various E2 concentrations. A, PI of hMLH1/hMSH2 and the percentage of p-histone H3-positive cells. The expression of hMLH1, hMSH2, and p-histone H3 was increased in a dose-dependent fashion of E2, with significant differences (P < 0.05). The addition of ICI182.780 significantly reduced the expression of MMR proteins (P < 0.05). B, Percentage of high-risk NEG cells positive for p-histone H3 but weak or negative for hMLH1/hMSH2 expression as shown by arrows in Fig. 5. The percentage of high-risk NEG cells with p-histone H3>hMLH1 expression was significantly lower in higher E2 concentrations. The addition of ICI182.780 opposed the effect of E2. The same tendency was observed in NEG cells with p-histone H3>hMSH2 expression. *1, Significantly different from that of 10^{-12} M of E2; *2, Significantly different from that of 10^{-8} M of E2 with ICI182.780.
to the MMR in normal endometrium. With regard to other factors affecting the efficiency of repair, an oxidative stress caused by inflammation has been reported to inactivate MMR in human erythroleukemic cells (13).

The present study suggested that the MMR activity in normal endometrium is caused by estrogen itself but not by the association with the cells growth. Because although both the growth of normal glandular cells and the expression of MMR protein were stimulated by E2 treatment, the expression pattern was quite different between MMR and growth markers. The expression of MMR protein showed diffuse staining pattern with minimal heterogeneity, i.e. when E2 level was high, most of the cells were diffusely positive, and when E2 level is low, they were diffusely weak or negative. In contrast, growth markers such as Ki-67 or p-histone H3 showed sporadic staining pattern, and the growth activity was indicated by the number of positive cells. The difference of expression pattern between MMR protein and growth markers may be due to the difference of molecular mechanisms. We have recently shown an indirect mechanism of E2-induced cyclin-D1 transcription, i.e. via c-Jun and activator protein-1 sequence, in the growth of normal endometrial glandular cells (12).

The most striking finding both in vivo and in vitro was that such imbalance between the cell proliferation and the MMR proteins expression was more frequently seen in relatively low E2 concentrations. Immunohistochemical staining of hMLH1 and hMSH2 in the normal endometrium in vivo showed that the glandular cells positive for Ki-67 and negative for MMR proteins were more frequently present in lower E2 levels. Our in vitro study using immunofluorescent analysis in normal glandular cell also revealed that the number of cells of growth marker-positive and MMR proteins-negative was higher in lower E2 concentrations.

It should be emphasized that proliferating cells still exist, even under lower E2 levels. It is therefore suggested that when E2 level is physiologically high, endometrial glandular cells vigorously proliferate and the elevated MMR activities compensate for the increase of duplication-related DNA errors, resulting in the prevention against carcinogenesis. In contrast, when E2 level is relatively low as seen in peri- or postmenopausal women, small number of proliferating cells do exist, but these cells may have limited or negative MMR activities. Such discrepancy between the cell proliferation and DNA repair may be advantageous for the accumulation of DNA errors and vulnerable to endometrial carcinogenesis. In addition, the chronic exposure of low serum estrogen levels or unopposed estrogen status, often observed in women with oligomenorrhea or anovulation, implies infrequent exposures to progesterone. Progestins are known to suppress the proliferation of endometrial glandular cells (2), and thus, they act as an antioncogenic factor. We previously reported that the progestin-induced growth suppression of endometrial epithelial cells is mediated by a tumor suppressor, p27 (18). Therefore, the advantage of low serum estrogen

![Fig. 7. Result of immunofluorescent staining for hMLH1 and hMSH2 in Ishikawa cells at various E2 concentrations. A, PI of hMLH1/hMSH2 and the percentage of the p-histone H3-positive cells. The expression of hMLH1, hMSH2, and p-histone H3 was increased in a dose-dependent fashion of E2 with a peak at 10^{-8} M, with significant differences (P < 0.05). The addition of ICI182.780 significantly reduced the expression of MMR proteins and p-histone H3 (P < 0.05). B, Percentage of Ishikawa cells positive for p-histone H3 but weak or negative for hMLH1/hMSH2 expression. The percentage of (high risk) Ishikawa cells with p-histone >hMLH1 expression was significantly lower in higher E2 concentrations, with a nadir at 10^{-8} M of E2. The addition of ICI182.780 opposed the effect of E2. The same tendency was observed in Ishikawa cells with p-histone H3 >hMSH2. *1, Significantly different from that of 10^{-12} M of E2; *2, Significantly different from that of 10^{-8} M of E2 with ICI182.780; *3, Significantly different from that of 10^{-10} M of E2.](https://academic.oup.com/endo/article-abstract/147/10/4863/2500623)
levels in endometrial carcinogenesis may actually be enhanced by the lack of progesterins. These findings of MMR deficiency under low E2 levels may be correlated with the clinical observation of the frequent occurrence of endometrial carcinoma in women with relatively low serum E2 levels.

Generally, estrogen is regarded as an important carcinogen. Estrogens have reportedly caused renal cancer via ER signaling in a Syrian hamster model, which was associated with overexpression of c-myc and c-fos (19). In human endometrial carcinomas, however, overexpression of c-myc is rare (5). Evans et al. (20) reported that E2 treatment suppressed nucleotide excision repair activity in ER-positive keratinocytes and thus contributes to skin carcinogenesis. However, the involvement of abnormal NER, as seen in xeroderma pigmentosum, is not evident in endometrial carcinomas. Recently catechol estrogens have been reported to evoke breast and endometrial cancers in animal models (21–23). Sasaki et al. (24) reported the presence of a silencing methylation of the caten-O-methyltransferase gene in human endometrial carcinoma. However, actual tissue concentrations of these metabolites have not been elucidated in normal and carcinomatous endometria, and levels of estrogen metabolites are shown to be generally low when the parent estrogen level is low (3). Further studies are needed to clarify the presence or absence of the actual effect of estrogen metabolites on endometrial carcinogenesis.

In summary, the present study demonstrated that estrogen increases the expression of HLH1 and hMSH2 and enhances MMR activity in normal and malignant endometrial glandular cells in dose-dependent fashion. Thus, possible increase in replication errors in actively proliferating endometrial cells in dose-dependent fashion. Thus, the possible increase in replication errors in actively proliferating endometrial glandular cells is likely be compensated for by the increase in MMR activity under high-estrogen-level conditions. Conversely, chronic exposure of low levels of estrogen was associated with the presence of small number of proliferating cells lacking MMR proteins expression, and such an imbalance between the cell proliferation and MMR function is likely to be related to endometrial carcinogenesis most frequently seen in women with relatively low estrogen levels.

Acknowledgments

Received May 11, 2006. Accepted June 23, 2006.

Address all correspondence and requests for reprints to: Tanri Shiozawa, M.D., Associate Professor, Department of Obstetrics and Gynecology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan. E-mail: tanri@hsp.md.shinshu-u.ac.jp.

This work was supported by a Grant-in-Aid for Scientific Research 06454468 and 07807154 from the Ministry of Education, Science, and Culture, Tokyo. Japan. E-mail: tanri@hsp.md.shinshu-u.ac.jp.

Disclosure statement: authors have nothing to disclose.

References


11. Li GM 2003 DNA mismatch repair and cancer. Front Biosci 8:997–1017


Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.