INTRODUCTION

Urinary stones in humans occur more commonly in adult men than adult women. Although there are some racial differences, men are affected about twice as frequently as women. However, the occurrence of urinary stones increases in women in their fifties and sixties once menopause occurs [1-3]. The lower incidence of urinary stones in premenopausal women is thought to be due to a protective effect of estrogen [4-6]. Although the exact mechanism remains unclear, this effect is thought to be similar to that of anti-arteriosclerosis [7]. On the other hand, osteopontin (OPN) is a well-known stone matrix protein, although whether it functions as an inhibitor or promoter in stone formation is unclear [8-12].

A recent report on the interaction of estrogen and OPN indicates that estrogen appears to inhibit stone formation. Although the exact mechanism remains unclear, this effect is thought to be similar to that of anti-arteriosclerosis [7]. On the other hand, osteopontin (OPN) is a well-known stone matrix protein, although whether it functions as an inhibitor or promoter in stone formation is unclear [8-12].

The Interaction between Female Sex Hormone Receptors and Osteopontin in a Rat Hyperoxaluric Model

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Summary: It is well known that the incidence of urinary stones is higher in men than women. Although it is believed that the lower incidence of urinary stones in women is due to a protective effect of estrogen, the mechanisms remain unclear. To clarify the relation between female sex hormones and stone matrix protein, we examined the interaction of estrogen receptor-α (ERα), estrogen receptor-related receptor-α (ERRα), and stone matrix protein osteopontin (OPN) in a rat hyperoxaluric model and in primary cultured rat kidney cells. Adult female Wistar rats were divided into 6 groups. Groups 1 and 4 consisted of normal females, Groups 2 and 5 consisted of ovariectomized females, and Groups 3 and 6 consisted of ovariectomized females receiving female sex hormone supplements. Groups 1-3 were administered distilled water, while groups 4-6 were administered 0.5% ethyleneglycol (EG). Moreover, rat kidney primary cultured cells were examined after treatment with female sex hormones under various conditions. The expressions of ERα, ERRα and OPN-mRNA in whole kidney and primary cultured cells were examined using Real-Time PCR. The expressions of OPN and ERRα-mRNA were suppressed by ovariectomy. Supplementation with female sex hormones increased the expression of OPN and ERRα-mRNA. In contrast, the expression of ERα-mRNA was increased by ovariectomy and suppressed by supplementation with female sex hormones. The results of the mRNA expression in primary cultured cells matched those in the hyperoxaluric model rats. Although the reason for the difference in expression between ERα and ERRα-mRNA is unclear, estrogen may regulates OPN expression through ERα and/or ERRα, either independently or in combination. Moreover, the decrease of OPN induced by removal of estrogen may increase urinary stones in postmenopausal women.

Key words estrogen, estrogen receptor-α, estrogen receptor-related receptor-α, osteopontin, urinary stone
formation by increasing OPN expression in the kidneys [13]. Other reports have indicated that estrogen receptor-related receptor-α (ERRα) interacts with OPN, which is closely related to estrogen receptor-α (ERα). Moreover, estrogen interacts with OPN through ERRα in osteoblast differentiation [14-16].

In the present study, we examined the interaction of ERα, ERRα and OPN-mRNA expression in a rat hyperoxaluric model. Moreover, we performed additional experiments using rat primary cultured cells. Kidney cells obtained from rats raised under various female sex hormone conditions were used to generate primary cell cultures that preserved their in vivo characteristics. The interactions of ERα, ERRα and OPN were investigated in these cultured cells after exposure to oxalate.

MATERIALS AND METHODS

Rat hyperoxaluric model

Nine-week old female Wistar rats (190 to 200g) were used in this study. Hyperoxaluric status was induced by administration of 0.5% ethyleneglycol (EG). Distilled water was given as the control. These rats were fed a standard commercial CE-2 diet. Each group consisted of 5 rats. Groups 1 and 4 consisted of normal females, Groups 2 and 5 consisted of ovariectomized females, and Groups 3 and 6 consisted of ovariectomized females receiving female sex hormone supplements (estrogen in the form of estradiol dipropionate and progesterone in the form of hydroxyprogesterone capronate [Mochida Pharm., Tokyo, Japan]). These sex hormones were injected intramuscularly in the femoral region at 0.5 mg and 6.25 mg, respectively, once a week for three weeks. Groups 1, 2 and 3 were treated with distilled water. Groups 4, 5 and 6 were treated with 0.5% EG.

Primary cultured cells

Nine-week old female Wistar rats were divided into three groups: normal-females (A), ovariectomized-females (B) and ovariectomized-females given female sex hormone supplements under the same conditions described above (C). The rats were sacrificed after three weeks of distilled water treatment, and the kidneys were removed. Single cell suspensions were obtained by enzyme digestion (collagenase and deoxyribonuclease 1 [SIGMA chemical Co., St. Louis, USA]) and Ficoll-Hypaque solution (Pharmacia Biotech). These cells were used to develop primary cultured kidney cells by incubation in a 25 cm² flask with 10 ml of RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). When the cultured cells were ready for study, the above media were removed and the cells were exposed to 0.5 mM potassium oxalate (KOx) or PBS (as a control) for one hour. PBS-exposed cells were divided into three groups: A (PBS), B (PBS) and C (PBS). KOx-exposed cells were also divided into three groups: A (KOx), B (KOx) and C (KOx). Experiments were performed three times.

Blood analysis

A blood sample was collected from each rat at sacrifice. Plasma calcium, creatinine and estradiol were determined (SRL Inc., Tokyo, Japan).

Urine analysis

24-hour urine was collected from each rat during the third week of the experiment, and urine volume and excretion of ionic components, including calcium, magnesium and oxalate (SRL Inc., Tokyo, Japan) were determined in each specimen. The urinary excretion of oxalate was measured under acidic conditions according to the SRL’s protocol. Blood and urine data were presented as mean ± standard deviation (SD).

Primers and probes for Real-Time PCR

Primers and probes used were from TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA, USA) as follows, Glyceraldehyde-3 phosphate dehydrogenase (GAPDH) (assay ID: Rn 99999916_s1) ; OPN (assay ID: Rn 01449972_m1) ; ERα (assay ID: Rn 00664737_m1) ; ERRα (assay ID: Rn 01479215_g1).

Reverse transcriptase for Real-Time PCR

The mRNA levels of GAPDH, OPN, ERα and ERRα in whole kidney and primary cultured cells were determined using Real-Time PCR. Total RNA was isolated from kidney and primary cultured cells using TRIZOL Reagent (invitrogen) according to the manufacturer’s protocol. Five micrograms total RNA from whole kidney and two micrograms total RNA from primary cultured cells were reverse-transcribed to cDNA. In brief, 10 μl reactions contained 5 μg or 2 μg total RNA, 1 μl of Random primers (50 ng/μl) (invitrogen), 1 μl of 10 mM dNTPs (invitrogen), and DEPC water. This mixture was incubated 10 min at 70 °C, and cooled for 2 min on ice. Next, 2 μl of 10xRT buffer (QIAGEN), 4 μl of 25 mM Mgcl2 (QIAGEN), 2 μl of 0.1 M DTT (invitrogen), 1 μl of RNase inhibitor (Promega) and 1 μl of Super Script III (invitrogen) were added. That mixture (total 20 μl) was incubated
for 50 min at 42 °C, and again for 15 min at 70 °C.

**Real-Time quantitative PCR**

PCR products were directly monitored by TaqMan PCR assay methods (Applied Biosystems, Foster City, CA, USA). The threshold cycle (Ct) was defined as the fractional cycle number at which fluorescence exceeded the threshold level. The relative quantity of mRNA was calculated from a standard curve that used dilution methods with a reference sample. The quality of cDNA in each sample was confirmed using GAPDH as an internal reference. All PCR reactions were performed using a 7300 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). For each PCR run, the 20 μl reaction mixtures contained 10 μl of TaqMan 2X PCR Master Mix, 1 μl of TaqMan Gene Expression Assays, 8 μl of DEPC water and 1 μl of cDNA. Real-time PCR was performed according to the manufacturer’s protocol.

**Statistical analyses**

Data are presented as mean ± SD. Statistical analyses were performed by one-factor analysis of variance with a post hoc test (Fisher’s protected least-significant difference test). A result of p<0.05 was considered to be statistically significant.

**RESULTS**

**Serum and urinary data**

Serum estradiol levels in Groups 3 and 6 were significantly higher than those in the other four groups. Moreover, the mean estradiol level of the ovariectomized groups was lower than that of the non-ovariectomized groups, although the difference was not significant. The mean serum calcium level and urinary excretion of calcium tended to be higher in the groups receiving female sex hormone (Groups 3,6) regardless of whether the rats received distilled water or EG.

The urinary excretion of magnesium in Groups 4 and 6 was significantly higher than in Groups 1 and 2. The urinary excretion of oxalate in Group 4 was significantly higher than in Groups 1 and 6 (Table 1,2).

**Real-Time quantitative PCR**

Figure 1 shows the relative quantities of OPN-mRNA expression. The relative quantity of OPN-mRNA was significantly lower in Group 2 (0.27±0.24)

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**TABLE 1. Serum concentration of estradiol, calcium and creatinine**

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum estradiol (pg/ml)</th>
<th>Serum calcium (mg/gl)</th>
<th>Serum creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.2±15</td>
<td>10±0.3</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>2</td>
<td>5.9±2.6</td>
<td>10.1±0.5</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>3</td>
<td>301±221.7</td>
<td>10.5±0.3</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>4</td>
<td>28.4±17.5</td>
<td>10.5±0.3</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>5</td>
<td>5.7±2.1</td>
<td>10.5±0.5</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>6</td>
<td>226.7±152.8</td>
<td>11.1±1.1</td>
<td>0.4±0.1</td>
</tr>
</tbody>
</table>

**Table 2. P Value**

- <0.01 Group 3 versus 1, 2, 4 and 5
- <0.01 Group 6 versus 1, 2 and 5
- <0.05 Group 4 versus 6
- <0.05 Group 6 versus 1 and 2
- <0.05 Group 3 versus 2 and 4
- <0.05 Group 4 versus 1 and 2
- <0.05 Group 6 versus 1 and 2

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**TABLE 2. Urinary concentration of calcium, magnesium and oxalate**

<table>
<thead>
<tr>
<th>Group</th>
<th>Urinary calcium (mg/day)</th>
<th>Urinary magnesium (mg/day)</th>
<th>Urinary oxalate (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5±0.2</td>
<td>1.1±1.3</td>
<td>0.2±0.2</td>
</tr>
<tr>
<td>2</td>
<td>0.3±0.04</td>
<td>0.9±0.6</td>
<td>0.4±0.3</td>
</tr>
<tr>
<td>3</td>
<td>1.2±0.5</td>
<td>2.3±0.7</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>4</td>
<td>0.5±0.3</td>
<td>2.6±2.5</td>
<td>0.5±0.4</td>
</tr>
<tr>
<td>5</td>
<td>0.3±0.1</td>
<td>2.5±1.7</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>6</td>
<td>1.1±0.5</td>
<td>3.8±1.5</td>
<td>0.1±0.03</td>
</tr>
</tbody>
</table>

**Table 2. P Value**

- <0.01 Group 6 versus 1, 2, 4 and 5
- <0.01 Group 3 versus 1 and 5
- <0.05 Group 3 versus 2 and 4
- <0.05 Group 4 versus 1 and 2
- <0.05 Group 6 versus 1 and 2
- <0.05 Group 4 versus 1 and 6
than in Group 1 (0.67 ± 0.25), and was significantly lower in Group 5 (0.5 ± 0.22) than in Group 4 (1.1 ± 0.75). Administration of 0.5% EG significantly boosted OPN-mRNA expression in Group 4 as compared with Group 1. In addition, the relative quantities of OPN-mRNA in Groups 3 (0.59 ± 0.5) and 6 (0.8 ± 0.18) were increased significantly by supplementation with female sex hormones, as compared with Groups 2 and 5. Figure 2 shows the relative quantities of ERα-mRNA expression. ERα-mRNA levels were significantly higher in the groups given 0.5% EG than in the groups given distilled water {Group 4 (0.41 ± 0.21) versus Group 1 (0.25 ± 0.07), Group 5 (0.6 ± 0.27) versus Group 2 (0.34 ± 0.31)}. Ovariectomy resulted in a significant increase in the relative quantity of ERα-mRNA in Group 5 as compared with Group 4. In contrast, supplementation with female sex hormones produced a significant decrease in the relative quantity of ERα-mRNA in Group 3 (0.14 ± 0.06) as compared with Group 2, and in Group 6 (0.17 ± 0.05)

Fig. 1. Relative quantity of OPN-mRNA.
Group 1: normal female rat + distilled water
Group 2: ovariectomized rat + distilled water
Group 3: ovariectomized rat + distilled water + female sex hormones
Group 4: normal female rat + 0.5% EG
Group 5: ovariectomized rat + 0.5% EG
Group 6: ovariectomized rat + 0.5% EG + female sex hormones
The expression of OPN-mRNA in Groups 2 and 5 were significantly lower than that in Groups 1 and 4. The expression of OPN-mRNA was significantly higher in Group 4, which was given 0.5% EG, than in Group 1 (the control). The expression of OPN-mRNA were significantly higher in Groups 3 and 6, which received female sex hormone supplements, than in Groups 2 and 5.

Fig. 2. Relative quantity of ERα-mRNA.
Administration of 0.5% EG significantly increased the expression of ERα-mRNA in Groups 4 and 5 as compared with Groups 1 and 2. Ovariectomy, significantly increased the expression of ERα-mRNA in Group 5 as compared with Group 4. On the other hand, the expression of ERα-mRNA was significantly lower in Groups 3 and 6, which received female sex hormone supplements, than in Groups 2 and 5.

Fig. 3. Relative quantity of ERRα-mRNA.
Ovariectomy significantly decreased expression of ERRα-mRNA in Groups 2 and 5, as compared with Groups 1 and 4. Administration of 0.5% EG significantly increased the expression of ERRα-mRNA in Group 4 compared to Group 1. The expression of ERRα-mRNA was significantly higher in Group 3, which received female sex hormone supplements, than in Group 2.
as compared with Group 5. Figure 3 shows the relative quantities of ERRα-mRNA expression. ERRα-mRNA levels were significantly lower in ovariectomized Group 2 (0.18±0.12) than in Group 1 (0.34±0.06), and were also significantly lower in ovariectomized Group 5 (0.25±0.09) than in Group 4 (0.42±0.09). Administration of 0.5% EG significantly boosted ERRα-mRNA expression in Group 4 as compared with Group 1. In addition, the relative quantity of ERRα-mRNA was significantly higher in C (KOx), which received female hormone supplements, than in B (KOx).

Figure 4 shows the relative quantities of OPN-mRNA expression in primary cultured cells. The expression of OPN-mRNA in B (KOx) (0.52±0.17) was significantly lower than in A (KOx) (1.14±0.13). The expression of OPN-mRNA was significantly higher in A (KOx) and C (KOx) (1.06±0.32), which were exposed to KOx, than in A (PBS) (0.61±0.25) and C (PBS) (0.45±0.2). The expression of OPN-mRNA was significantly higher in C (KOx), which received female hormone supplements, than in B (KOx).

Figure 5 shows the relative quantities of ERα-mRNA expression in primary cultured cells. The relative quantities of ERα-mRNA expression in each group were A (PBS): (0.3±0.13); B (PBS): (0.38±0.12); C (PBS): (0.26±0.09); A (KOx): (0.36±0.1); B (KOx): (0.53±0.28); and C (KOx): (0.41±0.37). The changes in mean relative quantities of ERα-mRNA expression, although not statistically significant, resembled those of the ERα-mRNA expression in the rat hyperoxaluric model. In addition, the mean relative quantities of ERα-mRNA were increased by exposure to KOx.

Figure 6 shows the relative quantities of ERRα-mRNA expression in primary cultured cells. The relative quantities of ERRα-mRNA expression in each group were: A (PBS): (0.81±0.49); B (PBS): (0.66±0.23); C (PBS): (0.74±0.21); A (KOx): (1.0±0.18); B (KOx): (0.99±0.12); and C (KOx): (1.1±0.54). The changes in mean relative quantities of ERRα-mRNA expression in primary cultured cells, although not statistically significant, resembled those of the ERRα-mRNA expression rat hyperoxaluric model.
in Group B (KOx) (0.52±0.17) as compared with Group A (KOx) (1.14±0.13). In contrast, supplementation with female sex hormones produced a significant increase in the relative quantity of OPN-mRNA in Group C (KOx) (1.06±0.32) as compared with Group B (KOx). OPN-mRNA expression was boosted significantly by exposure to KOx in Group A (KOx) as compared with Group A (PBS) (0.61±0.25), and in Group C (KOx) versus Group C (PBS) (0.45±0.2).

Figure 5 shows the relative quantities of ERα-mRNA expression in primary cultured cells. The changes in mean relative quantities of ERα-mRNA expression although not significant, were similar to the changes in ERα-mRNA expression in the rat hyperparathyroidic model.

Figure 6 shows the relative quantities of ERRα-mRNA expression in primary cultured cells. As was the case with ERα, the changes in mean relative quantities of ERRα-mRNA expression in the primary cultured cells resembled those of ERRα-mRNA expression in the rat hyperparathyroidic model.

**DISCUSSION**

Calcium oxalate (CaOx) stones are the most common type of urinary stone in humans, and their occurrence has been increasing in recent years. Although formation of upper urinary tract CaOx stones is related to lifestyle and dietary habits, the mechanism underlying the formation of these stones remains unclear. However, upper tract urinary stones occur more commonly in men than women by a ratio of about 2:1 [1-3]. In women, however, the occurrence of CaOx stones increases after menopause and peaks in the fifth to sixth decades of life. From these findings, it has been suggested that women are protected against stone formation by the effect of estrogen in the premenopausal state. Indeed, according to recent reports, urinary excretion of calcium, oxalate and uric acid was lower in women than men. Moreover, compared with men, urinary calcium was lower in women until the age of 50 years, when it equalled that of men [4]. Citrate was equal in both genders until the age of 60 after which it tended to decrease in women [17]. On the other hand, surgical menopause is associated with an increased risk of stone formation. The sudden loss of ovarian production of estrogens and androgens in women with surgical menopause leads to a more rapid bone loss compared with women experiencing natural menopause [5,6].

In the present study, the mean urinary excretion of calcium was lower in the ovariectomized Groups 2 and 5 than in the non-ovariectomized Groups 1 and 4, although the differences were not significant. Moreover, the addition of female sex hormones was found to increase calcium excretion. Although these findings differ from those of previous reports on human urinary stones, they are partially compatible with findings in studies of postmenopausal women and in rat urinary stone models [13,18,19]. It is unclear why urinary calcium excretion was increased by supplementation with female sex hormones. The supplementary dose of female sex hormones in this study was approximately five time the normal level in human females, so over supplementation of female sex hormones may have induced increased calcium absorption in the gut [17]. Moreover, these reports indicated that higher urinary calcium excretion due to estrogen supplementation did not increase the risk of calcium renal stone formation. In addition, animal studies have shown that decreases in estrogen affect urinary oxalate levels. In ovariectomized rats, the administration of estrogen has been shown to decrease urinary oxalate excretion by over 50% [18]. In the present study, urinary excretion of oxalate decreased significantly in Group 6 as compared with Group 4.

On the other hand, as regards the interaction between sex hormones and stone matrix protein, estrogen appears to inhibit stone formation by increasing OPN expression in the kidneys [13]. Although OPN is a well-known stone matrix protein, whether it functions as an inhibitor or promoter in stone formation is unclear. Some reports suggest that OPN protects renal epithelial cells from anti CaOx crystal injury [8-10]. But other reports have shown that CaOx attachment was inhibited in OPN antisense transfected renal epithelial cells [11,12].

However, ERα in the kidney regulates estrogen, which may protect against renal epithelial cell damage. Estrogen similarly protects against epithelial cell damage in arteries. The interaction of estrogen and OPN in urinary stone formation is probably similar in mechanism to that of arteriosclerosis or aortic calcification [7].

In addition, ERRα is an orphan nuclear receptor that regulates the OPN gene, and ERRα is closely related to ERα. ERRα is expressed in a variety of adult and embryonic tissues, and interacts with OPN in osteoblast differentiation [14-16].

In our study, the expressions of OPN and ERRα-mRNA were decreased significantly in ovariectomized rats (Groups 2 and 5) as compared with non-ovariectomized rats (Groups 1 and 4), regardless of whether they were administered distilled water or 0.5% EG.
Further, these expressions were increased by supplementation with female sex hormones (Groups 3 and 6). Thus, there is a possibility that OPN and ERRα-mRNA expression were controlled by estrogen. Previous reports have shown that although estrogen is not an ERRα-ligand, it stimulates ERRα expression in uterus or breast tissue [20-22]. Moreover, the expression of OPN and ERRα-mRNA in groups receiving 0.5% EG tended to be higher than in the groups given distilled water, and the changes in OPN-mRNA were similar to those in ERRα-mRNA expression. These findings indicate a possibility that ERRα expression stimulated ERα target genes in the absence of estrogen. Previous reports have in fact shown that ERRα can stimulate ERα target genes in the absence of estrogen. In addition, over expression of ERRα can stimulate ERE (estrogen-responsive element) dependent transcription in ER-negative cells [20,23].

On the other hand, in our study, ERα-mRNA expression was increased by ovariectomy, despite a decrease in OPN and ERRα-mRNA expression. Moreover, supplementation with female sex hormones produced a significant decrease in the expression of ERRα-mRNA. Although more investigation is necessary to explain these results, similar findings were reported previously. According to these reports, ER positivity was significantly higher in the subepithelial tissues of the vagina in postmenopausal women not receiving hormone replacement therapy than in either premenopausal women or women receiving estrogen supplementation. Similar results were seen in rats [24,25].

Moreover, it was reported that ovariectomy upregulates expression of ERs in porcine platelets [26]. Therefore, there are currently two different models of the relationship between estrogen and ER signaling. In model-1, estrogen exposure causes a rapid down-regulation of ER-mRNA and protein, and up-regulation in the absence of estrogen. In this model, the primary response to an estrogenic stimulus is a feedback inhibition of ER-mRNA levels. Conversely, in model-2, estrogen exposure causes an increase in the ER-mRNA level and protein [27]. ER expression in renal epithelial cells, which maintain rapid cell proliferation to prevent cell injury, may be regulated by a mechanism of this model-1.

Moreover, a study of ER-levels in MCF-7 human breast cancer cells grown in short-term or long-term absence of estrogens reported that while cells grown in the short-term absence of estrogens have ER-levels similar to those of control cells, cells grown in the long-term absence of estrogens have ER-levels three times higher than controls. In addition, these latter cells maintained high sensitivity to estrogen despite a long-term absence of estrogen [28]. These findings suggest that high levels of expression and sensitivity are maintained in renal epithelial cells despite a long-term absence of estrogen.

Although we examined the interaction of estrogen and OPN through ERα and ERRα expression, the findings of the present study do not necessarily apply to human urinary stones. Moreover, in rat hyperoxaluric models, results differ depending on the conditions of stone formation [29]. As a result, the present investigation of changes in female sex hormone receptors in such unusual states may not produce meaningful results.

Therefore, we carried out another experiment using primary cultured cells developed from kidney cells of rats raised under various female sex hormone conditions. Our results showed that the changes of OPN, ERα, and ERRα-mRNA expression after exposure to PBS or KOx were similar to those in the rat hyperoxaluric model.

In summary, though it is unclear whether ERRα or ERα function independently or together in regulation of target genes in various cells, it is apparent that ERRα modulates the ERα mediated signaling pathway both positively and negatively [21,23,30]. On the other hand, ERRα can stimulate ERα target genes in the absence of estrogen. In the interaction of ERα, ERRα and OPN, ERRα is a direct target of ERα activity, and ERα induces ERRα expression in the breast. Moreover, ERRα stimulates the estrogen-responsive gene, OPN [20]. Our current findings are in agreement with the above reports.

Although it is unclear why there was a negative correlation between ERα and ERRα-mRNA expression in the present study, estrogen may regulates OPN expression through ERRα or ERRα both in combination. Moreover, the decrease of OPN induced by removal of estrogen may increase the incidence of urinary stones in postmenopausal women.

REFERENCES

11. Vanacker JM, Delmarre C, Guo X, and Laudet V. Transcriptional targets shared by estrogen receptor-related receptors (ERRα) and estrogen receptor (ER) α, but not by ERβ. The EMBO Journal 1999; 81:4270-4279.