Different biological effect of estrogen receptor-related receptor α in estrogen receptor-positive and -negative endometrial carcinoma

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Received June 17, 2008; Accepted September 15, 2008

DOI: 10.3892/mmr_00000050

Abstract. Estrogen receptor-related receptor α (ERRα) has been identified as a nuclear transfactor closely related to estrogen receptor α (ERα). ERRα interferes with ER-mediated signaling pathways through competition with ERα for the common DNA sites and coregulators. Thus, it may participate in the tumorigenesis of estrogen-related cancers. To elucidate the roles of ERRα in endometrial carcinogenesis and the crosstalk between ERα and ERRα, endometrial carcinoma Ishikawa and HEC-IA cells were treated with different concentrations of 17ß-E2 and/or the pure anti-estrogen drug ICI182,780. Using semi-quantitative RT-PCR and Western blot analysis, we found that 17ß-E2 down-regulated the expression of ERRα in ER-positive Ishikawa cells, while up-regulating the expression of ERRα in ER-negative HEC-IA cells. Down-regulation in Ishikawa cells was furthermore found to be largely abrogated by ICI182,780. Additionally, we constructed endometrial carcinoma cell lines with overexpression of ERRα by stable transfection, renaming these Ishikawa/ERRα and HEC-IA/ERRα, respectively. To investigate the effect of ERRα overexpression on the biological behavior of the cells, MTT assay and flow cytometry analysis were performed in the constructed cell lines. In ER-positive Ishikawa cells, the overexpression of ERRα inhibited cell growth in the presence of 17ß-E2, an inhibitory effect that might be due to a G2-G1 cell cycle arrest. In contrast, overexpression of ERRα stimulated cell proliferation in ER-negative HEC-IA cells independently of 17ß-E2. This accelerated action was associated with changes in cell cycle distribution. Our study demonstrates that, in addition to ER, ERRα seems to be an important regulator in endometrial carcinogenesis, playing different roles in estrogen-dependent and -independent endometrial carcinomas. ERRα might modulate the ER-mediated pathways via interference with ERα transcription in endometrial carcinoma cells.

Introduction

Estrogen plays a predominant role not only in the development and physiological function of female reproductive organs, but also in the proliferation and malignancy of estrogen-dependent tumors, such as breast cancer and endometrial carcinoma (1-3). Two classic nuclear receptors, human ERα and ERβ, mediate the actions of estrogen on growth, development and physiological function in different human tissues and organs (4-6). Acting as either homodimers or heterodimers, they modulate the transcription of target genes by binding to short DNA sequences referred to as estrogen responsive elements (EREs) within the promoters (4,7,8). The estrogen-ER complexes recruit coregulators to exert transcriptional activity in a ligand-dependent manner. By screening the cDNA library with the DNA binding domain (DBD) of ERα as a probe, a novel nuclear transcription factor subfamily was identified and named estrogen receptor-related receptor (ERR). The ERR subfamily, which includes the subtypes ERRα, ERRβ and ERRγ, displays a significant homology with ERα at the DBD (68% similarity of amino acids) (9,10). Despite being closely related to ERα, ERRs are not activated by any known natural estrogens or physiological ligands, and are therefore classified as orphan nuclear receptors (9-12). Similarly to ERα, ERRα can bind to the classical EREs, which are composed of two AGGTCA motifs arranged in a palindrome structure. Moreover, ERRα preferentially recognizes the consensus extended half-site TnAAGGTCA, referred to as ERR responsive element (ERRE) (13-15). This type of binding site is also recognized by steroidogenic factor-1, a regulator of the steroid biosynthesis pathway. ERR family members may therefore have extensive crosstalk with other nuclear receptors through common binding sites. As we know, ERRα may compete with ERα but not ERβ to bind to the same binding sites and share the same coregulatory proteins, reinforcing the notion that the two subtypes of related receptors share the same target genes (16-21). However, in contrast to

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Key words: estrogen receptor-related receptor α, estrogen receptor α, endometrial carcinoma, interaction
the ligand-dependent transcriptional manner of ERα, ERRα can constitutively activate the transcription of target genes containing EREs in the absence of exogenous ligands (16,17,19). Previously, ERRα was identified as a regulator of the human medium chain acyl coenzyme A dehydrogenase, human lactoferrin and aromatase genes (14,15,22). These observations offer the additional layer of regulatory complexity for ERRα to modulate the estrogen-ER-target networking signal pathway.

Recent studies have reported that ERRα is associated with hormone-related cancers, such as breast cancer, endometrial carcinoma, ovarian cancer, prostate cancer and colorectal cancer (23-28). Our previous study also found that ERRα may participate in the tumorigenesis of endometrial carcinoma, and that it plays different roles according to ERα status (29). In this study, we explored whether ERRα is involved in the classical ER-mediated pathways, and investigated the function of ERRα in endometrial carcinogenesis. We demonstrated the different estrogenic responses of ERRα in ER-positive versus ER-negative endometrial carcinoma cells. Furthermore, we constructed cell lines with ERRα overexpression by stable transfection and examined the effects of ERRα overexpression on cellular proliferation and cell cycle distribution.

Materials and methods

Reagents. Water-soluble 17β-estradiol (17β-E2) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Pure anti-estrogen drug ICI182,780 was purchased from Tocris, UK. G418 was purchased from Amresco Co. (USA).

Plasmids and plasmid construction. All recombinant DNA and plasmid construction experiments were performed according to standard procedures. The sequences and orientation of the DNA fragments inserted in the plasmid constructs were verified by standard DNA sequencing. Plasmid pSG-ERRα and pSG-ERα containing full-length cDNA fragments were generous gifts from Professor J.M. Vanacker (LBMC, Lyon, France). Eukaryotic expression vector PLXSN-ERRα, which contained a neomycin-resistant gene, was constructed as follows: the pSG-ERRα plasmid and PLXSN vector were digested with BamHI and EcoRI double-restricted endonuclease system (Promega, Mannheim, Germany); the full-length ERRα cDNA fragment was purified and directionally inserted into the appropriate sites in the eukaryotic expression vector PLXSN; the vectors were purified using the Wizard purification system (Promega).

Cell culture and drug treatment. Human ER-positive endometrial carcinoma Ishikawa and ER-negative endometrial carcinoma HEC-IA cells were obtained from American Type Culture Collection Center (ATCC) and cultured respectively in 90% RPMI-1640 medium or 90% DMEM (Invitrogen) supplemented with 10% fetal bovine serum (FBS) at 37°C in a 5% CO2 incubator. To determine whether 17β-E2 regulates ERRα expression at the mRNA and protein levels, a drug treatment assay was performed in Ishikawa and HEC-IA cells. Before treatment with the drug, cells were transfected into phenol red-free medium containing 1% serum replacement-2 (steroid-free and growth factor-free) for additional culturing

for at least 24 h. Cells were then treated with different concentrations of 17β-E2 (vehicle, 10^{-10}, 10^{-9}, 10^{-8} M) or 17β-E2 (10^{-8} M) plus pure anti-estrogen drug ICI182,780 (10^{-6} M) for 24 and 48 h, and were finally harvested for semi-quantitative RT-PCR and Western blot analysis.

Stable transfection. Ishikawa and HEC-IA cells were cultured in complete culture medium to achieve ~90% confluence. According to the manufacturer's instructions, transfection experiments in Ishikawa and HEC-IA cells were performed with Lipofectamine 2000 reagent (Invitrogen). PLXSN-ERRα vector (1 μg) or PLXSN basic vector (1 μg) was transfected into the Ishikawa and HEC-IA cells, respectively. After transfection for 48 h, the culture medium was replaced by fresh selective culture medium containing G418 (800 μg/ml in Ishikawa cells and 1 mg/ml in HEC-IA cells). Drug selection was sustained for 2 weeks, with selective culture medium containing G418 being changed every 3 days. Resistant clones were isolated and expanded in complete culture medium containing 300 μg/ml G418. Several G418-resistant stable clones were screened for the analysis of ERRα expression by semi-quantitative RT-PCR and Western blotting. The selected clones with ERRα overexpression were renamed Ishikawa/ERRα and HEC-IA/ERRα. Correspondingly, the clones transfected with PLXSN basic vector were named Ishikawa/vector and HEC-IA/vector.

cDNA transcription and primer sets. Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The quality and concentrations of RNA samples were assessed by the DNA Counter (Bio-Rad, Munich, Germany). Only samples with an OD260/280 ratio >1.7 were used in the experiments. In 20 μl of reaction mixture, cDNA was synthesized from 2 μg of DNase I-treated total RNA using random hexamer and M-MLV reverse transcriptase (Promega). Transcription was performed as follows: initial denaturation at 95°C for 5 min followed by 95°C for 1 min, annealing for 1 min at 59°C for ERRα (54°C for GADPH) and elongation at 72°C for 1 min, 35 cycles and a final extension step at 72°C for 10 min. A negative control was set to check for the possibility of exogenous contaminant DNA, and no amplified products were detected under this condition. The PCR products were electrophoresed on 2% agarose gel with ethidium bromide, and the image intensities were semi-quantitated with the ratio of ERRα mRNA and GAPDH mRNA. The special primer set sequences used were as follows: ERRα sense 5'-TGG TCC AGC TCC CAC TCG CT-3'; anti-sense 5'-TGG TCC AGC ACC GAT CCA TTC ACT G-3' (482 bp); ERβ sense 5'-CCG CTG GAT TCT TTT TCA AA-3'; anti-sense 5'-AAC GCT CAA ATG CCA AAT TG-3' (151 bp); ERß sense 5'-TGG ATG ACC TGC TCG TGG AG-3'; anti-sense 5'-TCA GCT GAC GTC CTC TGT GG-3' (178 bp); GADPH (as internal control) sense 5'-ACG CAT TTG GTC GTA TTG GG-3'; anti-sense 5'-TGG TTT TGG AGG GAT CTC GC-3' (230 bp).

Western blot analysis. Cell lysates were prepared by mammalian cell lysis/lysate extraction reagent (Sigma). Total soluble proteins were quantified by the Bradford method using a BCA...
protein assay reagent kit (Pierce, USA). Protein (100 μg) was loaded and separated on 10% SDS-polyacrylamide gel electrophoresis and electrophoretically transblotted onto polyvinylidene fluoride membranes. Blotted membranes were pre-blocked with TBS containing 0.1% Tween-20 and 5% nonfat dry milk for 2 h, then incubated with an anti-ERRα mouse monoclonal antibody (R&D, USA) at a dilution of 1:1000 overnight at 4°C. Subsequently, rabbit polyclonal IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and the ECL detection system (Pierce) were used to visualize the bands. All experiments were repeated three times.

**MTT assay.** Ishikawa/vector, Ishikawa/ERRα, HEC-IA/vector and HEC-IA/ERRα cells were respectively seeded at a density of 1x10⁴ cells/well onto 96-well plates and cultured in 90% RPMI-1640 or DMEM with 10% FBS for 24 h. This was replaced with phenol red-free medium containing 1% serum replacement-2, following which cells were cultured for an additional 24 h. Subsequently, 10⁻⁸ M of 17ß-E₂ or vehicle was added to the cells, which were treated for 24, 48, 72 or 96 h. Prior to the assay, the medium was replaced with 20 μl of MTT solution (5 mg/ml) per well. After incubation for 4 h, the medium was removed and formazan crystals were dissolved by shaking with 100 μl of DMSO for 30 min. Absorbance was measured at 490 nm with a micro-plate reader (Bio-Rad Inc., Richmond, CA). Six duplicate wells were used for each treatment, and experiments were repeated three times.

**Flow cytometry analysis.** Ishikawa/vector, Ishikawa/ERRα, HEC-IA/vector and HEC/IA-ERRα cells were cultured in phenol red-free medium with 1% serum replacement-2 for at least 24 h and were treated with 10⁻⁸ M of 17ß-E₂ or vehicle for 3 days. Cell cycle distribution was analyzed by flow cytometry. In brief, cells were harvested and washed with phosphate-buffered saline (PBS) twice, and fixed with ice-cold 80% ethanol overnight at 4°C. Cells were washed once again and incubated in PBS with ribonuclease A at 37°C for 30 min. After a final PBS wash, cells were incubated with propidium iodide (50 μg/ml) for 30 min at 4°C to protect them from light. Then, samples were processed using a FACSort flow cytometer (BD Biosciences) and analyzed using ModFit LT software (BD Biosciences). A minimum of 10,000 cells was counted per sample.

**Statistical analysis.** The independent samples t-test and ANOVA analysis were used to compare the parametric data with SPSS 10.0 software. A P-value <0.05 was considered statistically significant.

**Results**

Estrogen represses ERRα expression in ER-positive Ishikawa cells. Endometrial carcinoma Ishikawa cells were determined to be ER-positive and functional. In these cells, the results of semi-quantitative RT-PCR showed that the relative levels of ERRα mRNA were down-regulated after treatment with 10⁻¹⁰ to 10⁻⁴ M of 17ß-E₂ for 24 h. The maximally repressed effect (~3.6-fold decrease) was observed at the concentration of 10⁻⁸ M (Fig. 1A, lane 1-4). Moreover, this down-regulation was largely abrogated by 10⁻⁶ M of ICI182,780 (Fig. 1A, lane 5).

We also observed similar changes in protein levels by Western blot analysis. An ~6.6-fold decrease in ERRα protein was observed at the concentration of 10⁻⁸ M of 17ß-E₂, and a 2.0-fold decrease at the concentration of 10⁻⁶ M (Fig. 1B) after
After treatment for 24 h, 3.0- and 3.5-fold decreases of ERRα protein were observed at the concentrations of 10⁻⁶ and 10⁻⁸ M of 17ß-E₂ (Fig. 1C).

Estrogen stimulates ERRα expression in ER-negative HEC-IA cells. HEC-IA cells were determined to be ER-negative and non-functional. The opposite phenomena to that seen in Ishikawa cells was observed in these cells. After treatment with different concentrations of 17ß-E₂ for 24 h, mRNA expression of ERRα was extensively up-regulated. The maximal effect was found at the concentration of 10⁻⁸ M. Moreover, this up-regulation was not abrogated by ICI182,780 (10⁻⁶ M) (Fig. 2A). However, the results of Western blot analysis showed lagged protein changes. After treatment with 17ß-E₂ for 48 h, ERRα proteins were obviously increased compared with the control (Fig. 2C), but no significant changes were exhibited at 24 h after treatment with 17ß-E₂ (Fig. 2B).

Identification of ERRα overexpression in constructed cells. These experiments have revealed that, though 17ß-E₂ might modulate ERRα expression, it has the adverse effect in ER-positive and -negative endometrial carcinoma cells. To further clarify the biological roles of ERRα in ERα-positive and -negative endometrial carcinomas, we selected three clones of Ishikawa and HEC-IA cells with ERRα overexpression for further testing. The results of semi-quantitative RT-PCR and Western blotting indicated that the expression of ERRα mRNA and protein in the Ishikawa/ERRα and HEC-IA/ERRα cells was significantly higher than in untransfected cells and cells transfected with empty vector (Fig. 3), increasing ~3.6-fold in Ishikawa cells and 5.2-fold in HEC-IA cells compared with the controls. These two endometrial carcinoma cell lines...
with stable overexpression of ERRα, which we successfully constructed for the first time, provide good models for further research on the biological function of ERRα in endometrial carcinoma.

Effect of ERRα overexpression on ERα and ERβ mRNA expression. To determine whether functional crosstalk between ERRα and ERs is possible, we next examined the expression of ERα and ERβ mRNA in Ishikawa/ERRα, Ishikawa/vector and normal Ishikawa cells. The results showed that overexpression of ERRα reduced the transcriptional level of ERα but not ERβ in Ishikawa cells (Fig. 4).

Effect of ERRα overexpression on cell proliferation and the cell cycle. To ascertain whether the overexpression of ERRα alters cellular biological behavior, we analyzed cell proliferation and cell cycle distribution in Ishikawa and HEC-IA cells by MTT assay and flow cytometry. There were no significant differences in growth rate and cell cycle distribution between Ishikawa/ERRα and Ishikawa/vector cells in the absence of 17β-E2 (Fig. 5 and Table I). However, at 3 days post-treatment with 17β-E2, the growth rate of Ishikawa/ERRα cells was markedly inhibited compared to that of Ishikawa/vector cells. Moreover, the percentage of cells in the G0-G1 phase in Ishikawa/ERRα cells was significantly higher than in the Ishikawa/vector cells, and was accompanied by a decrease in S phase cells. However, the opposite results were observed in HEC-IA cells. The growth rate of HEC-IA/ERRα cells was obviously faster than that of the control cells in the presence or absence of 17β-E2 (Fig. 5). A marked increase in S phase cells and a decrease in G0-G1 phase cells in HEC-IA/ERRα cells were also observed as compared to the control cells (Table I). These findings suggest that the overexpression of ERRα might have a differential impact on cell growth and cell cycle distribution in ER-positive and -negative endometrial carcinomas.

Discussion

The action induced by estrogen involves a complicated process that involves not only ERs, target genes and cofactors, but also the closely related orphan nuclear receptors ERRs. Physiological ligands for ERRs have not yet been found, and some researches have demonstrated that they can constitutively interact with co-activators independently of any natural estrogen. However, ERRα can interfere with the estrogen-ER signaling pathway by competing with ERα for the same target gene sites and coregulators (13-16,30). In addition, ERα and ERRα co-expression has been reported in some estrogen-responsive tissues, such as that of the breast, ovary, uterus and bone (23-25,31-33). ERRα may therefore participate in the tumorigenesis of estrogen-related tumors.

To better understand the role of ERRα in estrogen-responsive tissues and endometrial carcinoma, we examined its estrogenic responsiveness and its functional relationship with ERα in ER-positive and -negative endometrial carcinoma cells, demonstrating its adverse effects on these cells. In ER-positive Ishikawa cells, the expression of ERRα was down-regulated by 17β-E2, an effect partially abrogated by the pure anti-estrogen drug ICI182,780. These findings imply that 17β-E2 may indirectly modulate the expression of ERRα via estrogen-ER signaling pathways. On the other hand, in ER-negative HEC-IA cells the expression of ERRα was up-regulated by 17β-E2, an effect which could not be abrogated by ICI182,780.

It has been suggested that 17β-E2 might regulate the expression of ERRα in HEC-IA cells, although via non-ER pathways. Liu et al (32) confirmed that the ERRα gene was a downstream target of ERα: multiple steroid hormone response element half-sites present in the ERRα promoter might be responsible for this estrogen-responsive activation. Therefore, in ER-positive cells, ERRα might be a direct transcriptional repressor of ERRα, and could inhibit its expression. However, in ER-negative cells, the mechanism by which 17β-E2...
regulates the expression of ERRα remains unclear. A previous study demonstrated that ERRα competed with ERα in binding to the ERE and repressed ERE-dependent transactivation in ER-positive MCF-7 cells, whereas in ER-negative HeLa cells it activated transcription (34). In light of these findings, we speculate that ERRα may play completely different roles in ER-positive and -negative endometrial carcinomas.

To further elucidate the roles and the molecular mechanism of ERRα in endometrial carcinogenesis, we constructed cell lines with an overexpression of ERRα by stable transfection.

Table I. Cell cycle distribution of post-transfected Ishikawa and HEC-IA cells.

<table>
<thead>
<tr>
<th></th>
<th>G0 (%)</th>
<th>P-value</th>
<th>G2/M (%)</th>
<th>P-value</th>
<th>S (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ishikawa/vector (E2-free)</td>
<td>55.8000±6.3074</td>
<td>0.602</td>
<td>4.8850±6.7104</td>
<td>0.834</td>
<td>39.3150±0.4031</td>
<td>0.561</td>
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<tr>
<td>Ishikawa/ERRα (E2-free)</td>
<td>59.0100±1.1314</td>
<td></td>
<td>3.5150±4.2921</td>
<td></td>
<td>37.4750±3.1608</td>
<td></td>
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<tr>
<td>Ishikawa/vector (E2)</td>
<td>43.2650±1.5768</td>
<td>0.010</td>
<td>9.3100±4.7659</td>
<td>0.347</td>
<td>42.4250±0.7283</td>
<td>0.018</td>
</tr>
<tr>
<td>Ishikawa/ERRα (E2)</td>
<td>58.5650±1.3930</td>
<td></td>
<td>4.1600±1.9940</td>
<td></td>
<td>37.2750±0.6010</td>
<td></td>
</tr>
<tr>
<td>HEC-IA/vector (E2-free)</td>
<td>51.6750±1.5203</td>
<td>0.035</td>
<td>16.5850±0.4596</td>
<td>0.963</td>
<td>31.7400±1.0607</td>
<td>0.014</td>
</tr>
<tr>
<td>HEC-IA/ERRα (E2-free)</td>
<td>41.6050±2.0153</td>
<td></td>
<td>16.4750±2.6658</td>
<td></td>
<td>41.9200±0.6505</td>
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<tr>
<td>HEC-IA/vector (E2)</td>
<td>52.3233±2.6364</td>
<td>0.014</td>
<td>11.3933±1.4561</td>
<td>0.059</td>
<td>36.2833±1.6257</td>
<td>0.001</td>
</tr>
<tr>
<td>HEC-IA/ERRα (E2)</td>
<td>43.5033±2.5727</td>
<td></td>
<td>5.0500±3.9262</td>
<td></td>
<td>51.4467±2.4800</td>
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</tr>
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Ishikawa and HEC-IA cells with overexpression of ERRα were cultured in the presence or absence of 17β-E2 for 3 days, and cell cycle distribution was measured. The numbers represent the mean ± SD of three independent experiments.

Figure 5. Effect of ERRα overexpression on cell proliferation. Cell proliferation was analyzed by the MTT assay. Ishikawa/vector, Ishikawa/ERRα, HEC-IA/vector and HEC-IA/ERRα cells were respectively seeded at a density of 1x10⁴ cells/well onto 96-well plates. Ishikawa (A) and HEC-IA cells (B) were treated with vehicle or 10⁻⁸ M of E₂ for 24, 48, 72 and 96 h as described in Materials and methods. Absorbance was measured at 490 nm. Six wells were used for each treatment. Each point represents the mean ± SD of three independent experiments.
The biological behaviors of these two cell lines were analyzed by MTT assay and flow cytometry. The results confirm that ERα plays a distinct role in ER-positive and -negative endometrial carcinomas. Through stable transfection and the MTT assay, we found that overexpression of ERα inhibited cell growth in the presence of 17β-E2 in ER-positive and functional Ishikawa cells. This inhibitory effect seemed to be due to a G0-G1 cell cycle arrest rather than induced apoptosis (data not shown). In contrast, the overexpression of ERα stimulated cell proliferation in ER-negative HEC-1A cells independently of 17β-E2. This accelerated cell growth was also found to be associated with changes in cell cycle distribution. These findings further confirm that ERα plays an entirely different role in ER-positive and -negative endometrial carcinoma. In ER-positive cells, it acts as the repressor of ER and competes to bind estrogen responsive genes. However, in ER-negative cells, it becomes the activator and constitutively activates the transcription of ERE-containing genes.

Previous studies have revealed extensive crosstalk between ERα and ERγ (18,21,30), and our stable transfection experiments confirm this complex interaction. The overexpression of ERα repressed the transcription of ERα but not ERβ in Ishikawa cells. The mechanism of this negative regulation is at present unclear.

Based on the current study, we hypothesize that the preponderant expression of ERα/ERγ in cells might be the key to endometrial carcinogenesis. Induced by estrogen or agonist, the E-ER complex might directly or indirectly inhibit the expression of ERα in both mRNA transcription and protein translation in ER-positive and functional cells, resulting in the competitive inhibition of ERα on ERα being extensively reduced, and in the estrogenic effect being indirectly magnified. This might partially explain why long-term estrogen stimulation induces hyperplasia and tumorigenesis of the endometrium (35,36). On the other hand, in ER-negative cells, estrogen up-regulated the expression of ERα. Moreover, ERα could constitutively transactivate estrogen responsive genes independently of the ERα function, leading to estrogenic effects (16,17,37,38).

In our previous study, we found increased expression of ERα in ERα-negative compared to ERα-positive endometrial cancer tissues (29). Therefore, we proposed that the classic E-ER signaling pathway might be a key factor leading to hormone-dependent endometrial carcinoma. However, in hormone-independent endometrial carcinoma, the ERα-mediated pathway may play a vital role. The change in the relative amount of ERα/ERα and the functional conversion of ERα from a repressor to an activator might be crucial steps in the development of endometrial carcinoma from the hormone-dependent to the hormone-independent type. The detailed mechanism by which this occurs remains to be further elucidated.

Results from this study suggest that ERα, a ligand-undefined nuclear receptor, may be the next target of endocrine therapy in endometrial carcinoma. It is possible that an ERα-specific inhibitor or therapeutically synthetic ligands will be valuable in the treatment of estrogen-independent endometrial carcinoma. Moreover, future endocrine therapeutic strategies for treating estrogen-related cancers should involve not only ERα, but ERα as well.

Acknowledgements

We thank Professor J.M. Vanacker for generously providing the plasmids. We also thank Drs C. Denkert, A. Mustea and D. Kösgen for their great support in this work. This research was supported by the National Nature Science Foundation of China (NSFC, serial no. 30371477).

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