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Estrogen Activates Telomerase

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Abstract

Telomerase activity is present in most malignant tumors and provides a mechanism for the unlimited potential for division of neoplastic cells. Although telomerase is known to be a regulated enzyme, the factors and mechanisms involved in telomerase regulation are not well understood. In the present study, we examined the effects of estrogen on telomerase activity. Telomerase activity in estrogen receptor (ER)-positive MCF-7 cells was up-regulated by the treatment with 17β-estradiol. This activation accompanied up-regulation of the telomerase catalytic subunit, hTERT mRNA. Gel shift assays revealed that the imperfect palindromic estrogen-responsive element in the hTERT promoter specifically binds to ER. Transient expression assays using luciferase reporter plasmids containing various fragments of hTERT promoter showed that this imperfect palindromic estrogen-responsive element is responsible for transcriptional activation by ligand-activated ER. We also found that estrogen activates c-Myc expression in MCF-7 cells and that E-boxes in the hTERT promoter that bind c-Myc/Max play additional roles in estrogen-induced transactivation of hTERT. Estrogen thus activates telomerase via direct and indirect effects on the hTERT promoter. These findings may help elucidate the mechanisms of hormonal control of telomerase activity and aid understanding of the roles of sex steroids in cellular senescence and aging as well as estrogen-induced carcinogenesis.

Introduction

Telomerase is a cellular reverse transcriptase which catalyzes the synthesis and extension of telomeric DNA (1, 2). This enzyme is specifically activated in most malignant tumors but is usually inactive in normal somatic cells, with the result that telomeres shorten progressively with cell division (3, 4). A mechanism to maintain telomere stability is required for cells to overcome replicative senescence, and telomerase activation may therefore be a rate-limiting or critical step in cellular immortality and oncogenesis (5).

Although telomerase is known to be a regulated enzyme, the factors and mechanisms involved in telomerase regulation are not well understood. There is mounting evidence that hormones may regulate telomerase activity in estrogen target tissues (6–9). In particular, human endometrium expresses telomerase activity despite its somatic origin, and this activity is tightly regulated in a menstrual phase-dependent manner, suggesting the presence of controlling mechanisms of telomerase by sex steroids (6, 7).

Recent studies have identified three major components of human telomerase: the template RNA (hTR),[2] telomerase-associated protein, and the reverse transcriptase subunit (hTERT; Refs. 10–14). hTR and hTERT are known to be sufficient to reconstitute telomerase activity *in vitro* (15, 16). There is a good correlation between expression of hTERT mRNA and the presence of telomerase activity in extracts from tissue culture cells and normal and cancer tissues, whereas hTR is expressed constitutively in both cancer and normal cells, irrespective of the status of telomerase expression (17–19). In a previous study, down-regulation of telomerase activity by treatment with differentiating agents accompanied repression of hTERT mRNA expression but not that of hTR in leukemia cells (14). These findings suggest that hTERT is a rate-limiting determinant of telomerase activity.

Recent success in cloning hTERT promoter enabled us to investigate mechanisms controlling the transcriptional activity of hTERT (20–23). We previously found that the proximal 181-bp region of the hTERT promoter, which contains E-boxes that bind c-Myc/Max, is essential for transactivation in telomerase-positive cells (22). However, few findings have been obtained concerning other *cis*-elements that regulate hTERT transcription. In the present study, we examined the effects of estrogen on hTERT transcription as well as telomerase activity, using the estrogen-receptor-positive MCF-7 cell line, and demonstrated that estrogen activates telomerase via direct and indirect effects on hTERT promoter.

Materials and Methods

Cell Lines. MCF-7 cells and SiHa cells were obtained from the American Type Culture Collection (Rockville, MD) and were grown in DMEM with 10% FCS (with insulin added at 10 μg/ml for MCF-7 cells) in the presence of 5% CO_2 at 37°C. NHKs were purchased from Clonetics (San Diego, CA) and were grown according to the manufacturer's protocol. In estrogen-induction assays, cells were grown in phenol red-free media containing 10% dextran-coated charcoal-treated FCS for 48 h prior to treatment with estrogen.

Stretch PCR Assay. For quantitative analyses of telomerase activity, stretch PCR assays were performed using the Telochaser system according to the manufacturer's protocol (Toyobo, Tokyo, Japan; Refs. 24, 25). The PCR products were electrophoresed on a 7% polyacrylamide gel and visualized with SYBR Green I Nucleic Acid Gel Stain (FMC BioProducts, Rockland, ME). To monitor the efficacy of PCR amplification, 10 ng of internal control from λ phage DNA sequence (Toyobo) together with 50 pmol of specific primers (Toyobo) were added to the PCR mixture per reaction. Relative telomerase activity was determined by measuring band intensities of telomerase ladders and comparing them with those of internal standards. Band intensity was measured using NIH Image picture analyzing soft.

RNA PCR Analysis. The expression of hTERT mRNA and c-Myc mRNA was analyzed by semi-quantitative RT-PCR amplification as described previously (14, 26). Briefly, hTERT and c-Myc mRNAs were amplified using the primer pairs: 5′-CGGAAGAGTGTCTGGAGCAA-3′ (LT5) and 5′-GGATGAAGCGGAGTCTGGA-3′(LT6) for hTERT and 5′-AAGTCCTGCGCCTCGCAA-3′ and 5′-GCTGTGGCCTCCAGCAGA-3′ for c-Myc. Total RNA was isolated from the tissues using Isogen (Nippon Gene, Japan) according to the manufacturer's protocol, and cDNA was synthesized from 1 μg of RNA using the RNA PCR kit version 2 (TaKaRa, Ohtsu, Japan) with random primers. Serially diluted cDNA reverse-transcribed from 1 μg of RNA (corresponding to 50 ng to 1 μg) was first subjected to RT-PCR to obtain standard curves. Band intensity was counted with NIH Image picture analyzing soft. A linear correlation was confirmed between band intensity and dose of cDNA templates under the conditions described below. Typically, 2-μl aliquots of the reverse-transcribed cDNA were subjected to 28 cycles of PCR in 50 μl of 1× buffer [10 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, 50 mM KCl] containing 1 mM each of dATP, dCTP, dGTP, and dTTP; 2.5 units

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[2] The abbreviations used are: hTR, human telomerase RNA; hTERT, human telomerase reverse transcriptase subunit; NHK, normal human foreskin keratinocyte; RT-PCR, reverse transcription-PCR; LUC, luciferase; ERE, estrogen-responsive element; ER, estrogen receptor; E2, 17β-estradiol; TM, 4-hydroxytamoxifen.

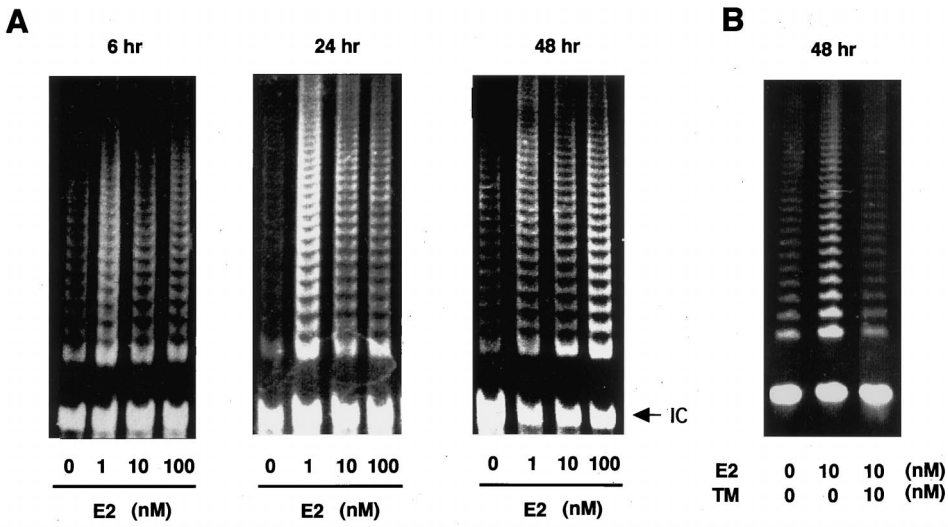

Fig. 1. Estrogen activates telomerase activity in MCF-7 cells. *A*, MCF-7 cells were treated with or without E2 at different concentrations. *B*, MCF-7 cells were also treated with TM in the presence of E2. Cell pellets were collected at different time points and subjected to quantitative stretch PCR assays. *IC*, internal standard to verify the amplification efficiency of PCR.

of Taq DNA polymerase (TaKaRa, Japan); and 0.2 μM each of specific primers. Each cycle consisted of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 90 s. PCR products were electrophoresed in 7% polyacrylamide gel and stained with SYBR green I (FMC BioProducts). The efficiency of cDNA synthesis from each sample was estimated by PCR with glyceraldehyde-3-phosphate dehydrogenase-specific primers as described previously (16).

Plasmid Construction. The structures of hTERT promoter-LUC constructs are shown in Fig. 3. Various lengths of DNA fragments upstream of the initiating ATG codon of the *hTERT* gene were PCR-amplified and inserted into LUC reporter vector pGL3-Basic, a promoter- and enhancer-less vector (Promega) in sense orientation relative to the LUC coding sequence at *Mlu*1 and *Bgl*II sites. As a positive control plasmid, pGL3-Control (Promega) was used, in which the *LUC* gene is driven by the SV40 LT enhancer/promoter. pGL3-ERE-promoter and p-Sp1/ER-promoter were constructed by inserting the head-in-tail tetramers of potential ERE at −2677 or Sp1/ER site at −873 into enhancer-less vector pGL3-promoter (Promega) upstream of SV40 promoter sequences. The c-Myc promoter-LUC reporter plasmid (p-MycP-LUC) containing 877 bp of p1 promoter and upstream Alu sequences was kindly provided by Dr. H. Ariga (Hokkaido University, Sapporo, Japan). ERα expression vector (pSG5-HEO) was a gift from P. Chambon (Institut de Genetique et de Biologie Moleculaire et Cellulaire, Strasbourg, France). Reporter plasmid p-181 MycMT contains substitution mutations in two c-Myc binding sites at −165 and +44, which were introduced by PCR-based site-specific mutagenesis. E-box consensus sequences (CACGTG) were altered to TTTGTG at −165 and CACAAG at +44.

LUC Assay. Transient transfection of LUC reporter plasmids was performed using Lipofect AMINE (Life Technologies, Gaithersburg, MD) according to the protocols recommended by the manufacturer. LUC assays were performed using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI), in which *Renilla* LUC plasmids were cotransfected as a control plasmid to standardize transcription efficiency. All experiments were performed at least three times for each plasmid, and results represent the average relative LUC activity.

Gel Shift Assay. Recombinant ER protein (α) produced by a baculovirus-expression system (TaKaRa, Ohtsu, Japan) was used for the gel shift assay. Two to 10 pmol of ER proteins were incubated with 0.5 μg of poly(dI·dC) in the presence or absence of unlabeled competitors on ice for 20 min in a 25-μl reaction volume containing 10% glycerol, 25 mM HEPES (pH 7.9), 50 mM KCl, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM DTT, and 4 mg/ml BSA. After incubation, 10,000 cpm of ^{32}P end-labeled oligonucleotide probes (5′-TGTTGGTCAGGCTGATCTCAAA-3′) containing the potential ERE in the core promoter at −2678 were added, and the reaction was incubated at 4°C for an additional 20 min. The underlined sequences of the probe were altered to CTCA in the mutated oligonucleotides for competition assay. After electrophoresis on a 4% polyacrylamide gel, the gel was dried and subjected to autoradiography. Consensus oligonucleotides for ERE (GTCCAAAGTCAGGTCACAGTGACCTGATCAAAGTT) from the *Xenopus* vitellogenin *A2* gene (21) and for c-Myc (GGAAGCAGACCACGTGGTCTGCTTCC; Santa-Cruz; catalog number sc-2509) or antibodies specific against ERα (SantaCruz; catalog number sc-786x) were added to the binding reactions before incubation with labeled probes for competition and supershift assays, respectively.

Results

To examine the effect of estrogen on telomerase activity, ER-positive MCF-7 breast cancer cells were cultured in the absence or presence of E2 at various concentrations, and telomerase activity was examined using a quantitative telomerase assay (Fig. 1*A*; Refs. 24, 25). When E2 was added at ≥1 nM, telomerase activity was up-regulated within 6 h and persisted until at least 48 h. Maximal activation (more than 6-fold *versus* control) was observed at 24 h after treatment. This effect was largely abrogated by the addition of TM, an antagonist of ER (Fig. 1*B*; Refs. 27, 28). Cell growth properties were monitored during treatment with E2, but no significant differences were observed between cells with or without treatment with E2 (data not shown).

Semiquantitative RT-PCR assays were performed to examine whether activation of telomerase by estrogen was due to up-regulation of expression of hTERT. Treatment of MCF-7 cells with E2 led to up-regulation of hTERT mRNA (Fig. 2*A*). An increase in hTERT

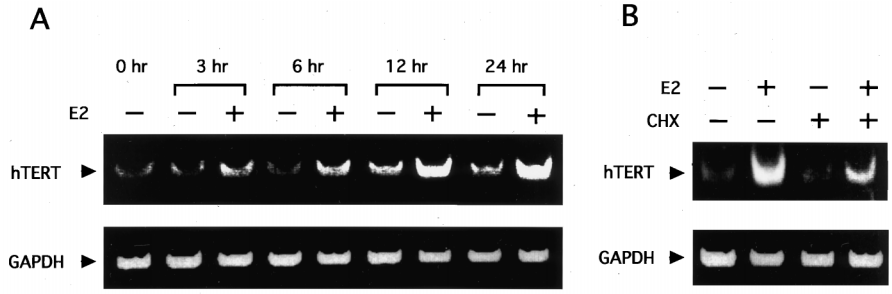

Fig. 2. Estrogen activates hTERT mRNA expression in MCF-7 cells. Semiquantitative RT-PCR assays to detect hTERT mRNA are shown. *A*, MCF-7 cells were treated with or without E2 at 1 nM, and RNAs were extracted at different time points. *B*, MCF-7 cells were treated with cycloheximide (*CHX*) at 10 μg/ml for 1h prior to treatment with E2. RNAs were extracted 9 h after treatment with E2 at 1 nM. *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

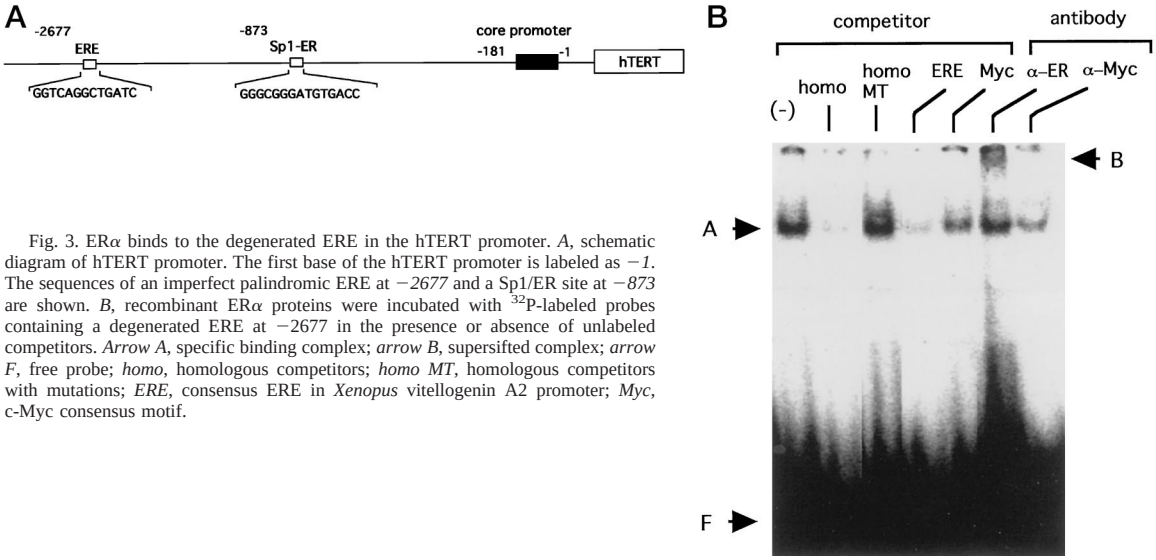

Fig. 3. ERα binds to the degenerated ERE in the hTERT promoter. *A*, schematic diagram of hTERT promoter. The first base of the hTERT promoter is labeled as *−1*. The sequences of an imperfect palindromic ERE at *−2677* and a Sp1/ER site at *−873* are shown. *B*, recombinant ERα proteins were incubated with ^{32}P-labeled probes containing a degenerated ERE at −2677 in the presence or absence of unlabeled competitors. *Arrow A*, specific binding complex; *arrow B*, supersifted complex; *arrow F*, free probe; *homo*, homologous competitors; *homo MT*, homologous competitors with mutations; *ERE*, consensus ERE in *Xenopus* vitellogenin A2 promoter; *Myc*, c-Myc consensus motif.

mRNA was observed within 3h after treatment of E2 and peaked at 12 h after treatment. This effect was not blocked by treatment with cycloheximide, although the extent of activation was slightly reduced (Fig. 2*B*). The rapid kinetics of hTERT activation by estrogen as well as its independence of new protein synthesis are consistent with the direct effect of estrogen on hTERT transcription.

We then cloned 3.3 kb of the 5′ flanking sequence of the *hTERT* gene (22). A computer-assisted homology search revealed a degenerate ERE in the hTERT promoter at −2677 (the first base in the mRNA is labeled as +1, and the first base of the promoter is labeled as −1). This sequence (GGTCAGGCTGATC) is only one base different from the consensus ERE (GGTCANNNTGACC; Fig. 3*A*). In addition, we found another potential ERE at −873, in which a SP1-binding site (GGGCGGG) is adjacent to an ER half site (TGACC: reverse). Similar SP1/ER sites previously have been reported to function as EREs in some estrogen responsive-gene promoters (29–32). To examine whether ER binds to these sites, gel shift analyses were performed (Fig. 3*B*). Recombinant ERα proteins were incubated with oligonucleotide probes spanning each site. A specific band was observed with probes containing the putative ERE at −2677, which was competed by homologous competitors but not by its mutant oligonucleotides in which GGTCA palindromic sequences were abrogated by substitution mutations. This band was also competed by ERE consensus sequences in the *Xenopus* vitellogenin *A2* gene promoter (33) but not by unrelated oligonucleotides such as the c-Myc consensus motif. Furthermore, the band was supershifted by addition of ERα antibody but not by that of c-Myc. In contrast, we failed to observe a specific ER band when we used the Sp1/ER site at −873, although Sp1 binding was detected with recombinant Sp1 proteins (data not shown). These findings suggest that ER can bind directly to the hTERT promoter through the degenerated ERE motif.

To examine the effect of estrogen on the transcriptional activity of the hTERT promoter, LUC assays were performed in which the hTERT-promoter reporter plasmids were transfected into a variety of cell types. When reporter plasmids with 3.3 kb of hTERT promoter (pGL3-3328) were transfected into ER-positive MCF-7 cells, transcriptional activity was significantly increased by E2 (Fig. 4*A*). Addition of tamoxifen largely inhibited this activation. In contrast, E2 treatment did not activate promoter activity of hTERT in ER-negative SiHa (from cervical cancer cells) or normal foreskin NHK cells. However, once ERα expression vectors were introduced into these cells, estrogen significantly activated the hTERT promoter (Fig. 4*B*).

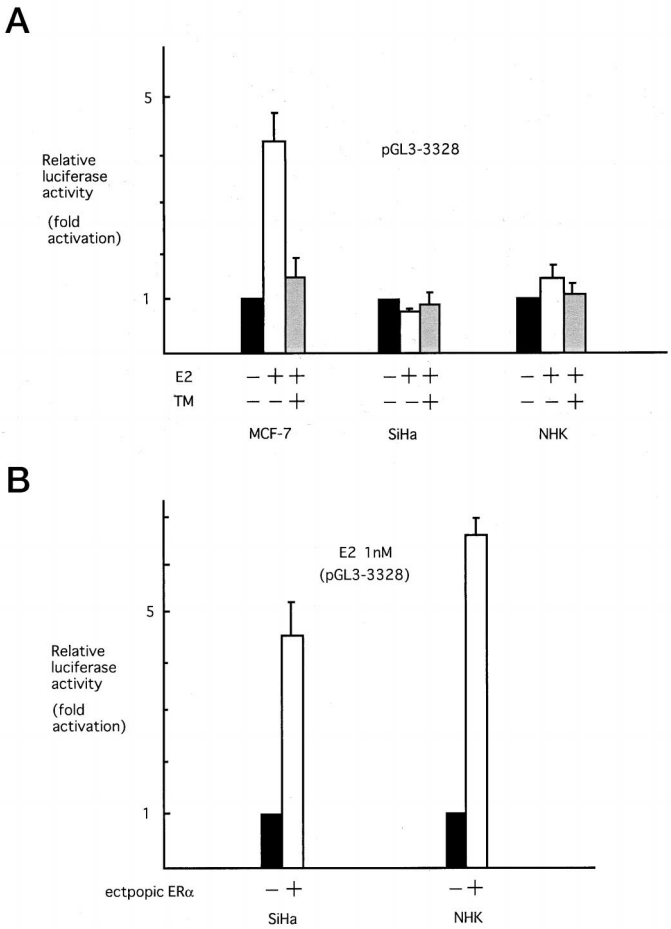

Fig. 4. Estrogen activates hTERT promoter. *A*, MCF-7, SiHa, and NHKs were transfected with pGL3-3328 LUC reporter plasmids, treated with or without E2 at 1 nM for 48 h, and LUC assays were performed. TM was also added to the medium at 10 nM in the presence of E2. *Fold activation* is shown as LUC activity relative to that of E2-untreated samples. *B*, pGL3-3328 was transfected into SiHa and NHK cells together with ERα expression vectors. Cells were treated with E2 at 1 nM for 48 h, and LUC assays were performed. *Fold activation* is shown as LUC activity relative to that of control samples transfected with blank vectors.

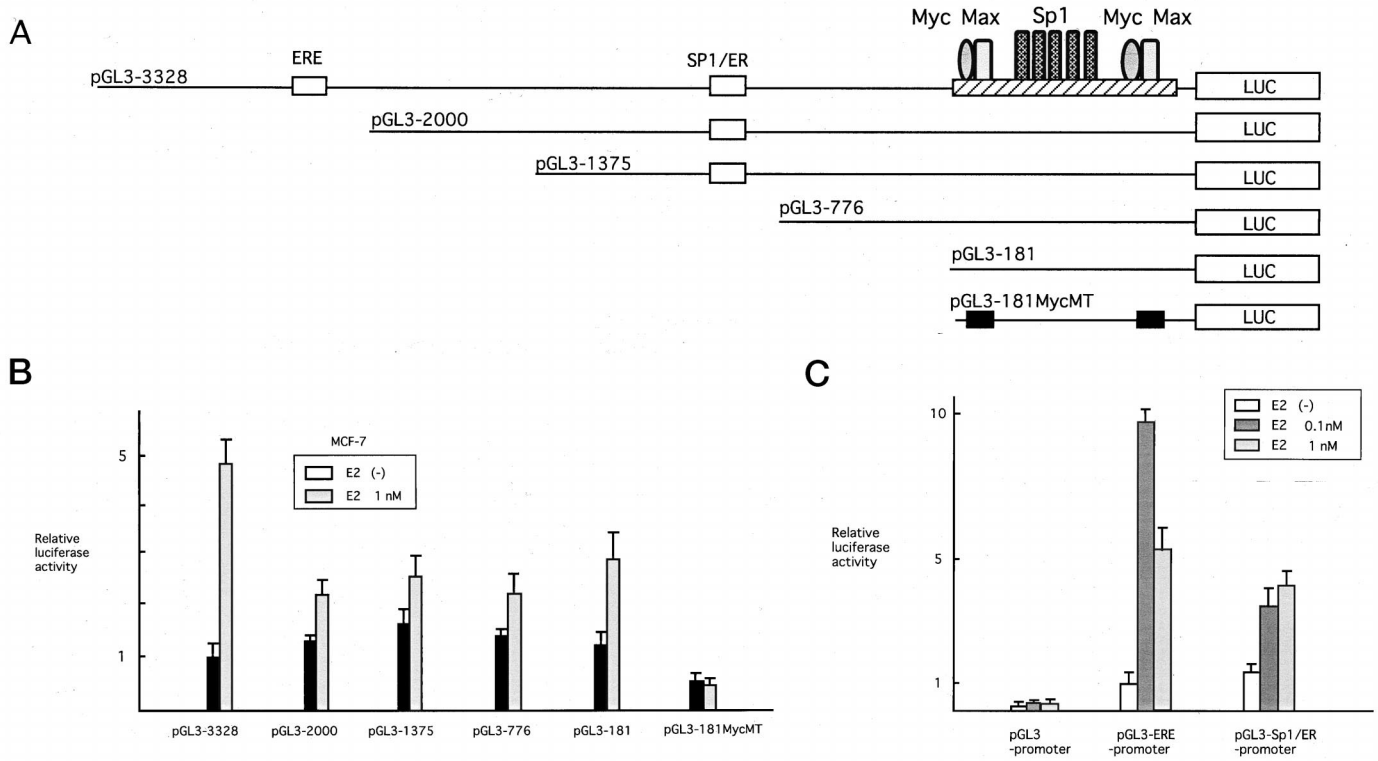

Fig. 5. Identification of ERE in the hTERT promoter. *A*, schematic diagram of LUC reporter constructs. Binding sites for c-Myc (*shaded ovals*) and Sp1 (*cross-hatched columns*) in the core promoter (*hatched box*) are shown. An imperfect palindromic ERE at −2677 and an Sp1/ER site at −873 are shown in the *open boxes. B*, various deletion mutants of hTERT-promoter reporter plasmids were transfected into MCF-7 cells, which were treated with or without E2 at 1 nM for 48 h, and LUC assay were performed. *Relative luciferase activity* in each reporter plasmid is shown. *C*, effects of putative EREs on estrogen-induced transactivation of SV40 promoter. The imperfect palindromic ERE at −2677 and the Sp1/ER site at −873 were each cloned upstream of SV40 promoter as head-to-tail tetramers in LUC reporter plasmids, pGL3-promoter. MCF-7 cells were transfected with these plasmids, treated or not with E2 at 0.1 or 1 nM for 48 h, and LUC assays were performed. *Relative luciferase activity* for each reporter plasmid is shown.

These findings suggest that the effects of estrogen on hTERT promoter depend on ER expression and are not specific to MCF-7 cells.

To further confirm the specificity of the EREs in the hTERT promoter, various deletion mutants in reporter plasmids were tested by LUC assays using MCF-7 cells (Fig. 5*A*). Five-fold activation of transcription was observed with E2 treatment in pGL3-3328, but a deletion mutant, pGL3-2000, lacking the imperfect palindromic ERE, exhibited a 70% decrease in transactivation (Fig. 5*B*). Furthermore, when this putative ERE was cloned upstream of the SV40 promoter in a LUC reporter plasmid (pGL3-ERE-promoter), ~10-fold transcriptional activation was observed with E2 treatment (Fig. 5*C*). These findings further support that this site functions as an authentic ERE. In contrast, deletion of the Sp1/ER site (pGL3-776) did not significantly affect the responsiveness to estrogen (Fig. 5*B*). When this site was cloned upstream of the SV40 promoter in a LUC reporter plasmid (pGL3-Sp1/ER-promoter), 2–3-fold activation was observed with E2 treatment (Fig. 5*C*). Thus, the role of the Sp1/ER site as an ERE appears to be less significant. In examining other promoter deletions, we found that additional EREs may exist within the proximal 181-bp region because 2–3-fold activation was observed with E2 treatment in pGL3-181. This region is known to function as a core promoter essential for basal transcription of hTERT. Of particular interest, this region lacks a typical ERE but contains two binding sites for c-Myc, which is a direct activator of hTERT transcription (20, 21). We found that E2 activation of the core promoter was completely eliminated when the c-Myc sites were abrogated by mutations (pGL3-181 MycMT; Fig. 5*B*). We also confirmed that E2 significantly activates the c-Myc promoter in LUC assays using c-Myc promoter-reporter plasmids (p-MycP-LUC; Fig. 6*A*). RT-PCR assays also revealed that E2 activates c-Myc mRNA expression in MCF-7 cells (Fig. 6*B*). These findings suggest that c-Myc mediates estrogen-induced transcriptional activation of hTERT, demonstrating the presence of indirect ER activation pathways.

Discussion

To our knowledge, this is the first study showing that the *hTERT* gene is a target of estrogen. Our findings provide evidence for a direct interaction of activated ER with an imperfect ERE in the hTERT promoter, leading to estrogen-dependent transactivation of hTERT. The role of the Sp1/ER site in estrogen-induced transcriptional activation was, however, less significant than that of the imperfect ERE, although similar Sp1/ER motifs have been confirmed to function as EREs in some estrogen-inducible gene promoters, such as c-Myc, cathepsin D, retinoic acid receptor α, and Hsp 27 (29–32). A weak interaction of estrogen-ER with the ERE half site is known to be

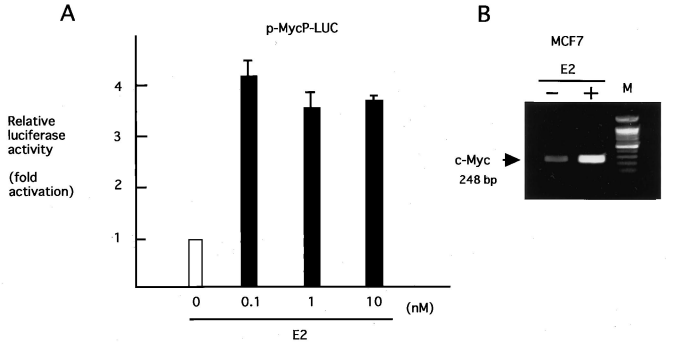

Fig. 6. Estrogen activates c-Myc expression. *A*, MCF-7 cells were transfected with c-Myc promoter LUC reporter plasmid, p-MycP-LUC, treated or not with E2 at various concentrations for 48 h, and LUC assays were performed. *Fold activation* is shown as LUC activity relative to that of E2-untreated samples. *B*, MCF-7 cells were treated or not with E2 at 10 nM for 48 h, and RT-PCR assays were performed to detect c-Myc mRNA. *M*, DNA size marker (100-bp ladder).

stabilized by an interaction with an adjacent bound Sp1 or Sp1-like factor (29). Protein-protein interaction of ER and Sp1 enhances DNA binding of Sp1 to GC-rich sequences. Thus, the effects of the Sp1/ER site may depend on the status of Sp1 expression in cells (34).

The findings presented have important implications concerning hormone-dependent telomerase activation and help explain the molecular mechanisms by which telomerase activity in human endometrium is regulated in a menstrual phase-dependent manner. Telomerase activity in the endometrium is weak or undetectable during the menstruation period (6, 7). However, once the proliferative phase begins, telomerase is dramatically up-regulated, with maximal activity in late proliferative phase or preovulatory phase and subsequent reduction in activity in the secretory phase. Both circulating and local levels of estrogen increase during the proliferative phase, with a peak level in the preovulatory phase, followed by a drastic decrease in the secretory phase, consistent with the profile of telomerase expression during the menstrual cycle. Interestingly, c-Myc in endometrium exhibits similar changes in expression during the menstrual cycle that are probably regulated by estrogen, based on the present finding that estrogen activates c-Myc. Estrogen may thus play a causative role in regulating telomerase in human endometrium.

Prolonged exposure to estrogen is one of the risk factors for development of endometrial cancer. There is accumulating evidence that estrogen administration without progesterone treatment increases the frequency of occurrence of endometrial cancer (35). Although the effects of estrogen on the proliferation of endometrial cells are complex, activation of hTERT and subsequent telomerase activation may contribute to estrogen-induced endometrial carcinogenesis. Continuous administration of estrogen has been used as hormone replacement therapy to prevent osteoporosis and other estrogen-deficient syndromes, termed climacteric disorders. An increasing number of postmenopausal patients have been treated with hormone replacement therapy. Of particular interest is whether patients treated with estrogen have extended life spans, a possibility suggested by the present observation that estrogen activates telomerase. A long-term prospective study with a large number of patients will be necessary to answer this important issue.

In summary, the present study demonstrated that hTERT is both a direct and indirect target of estrogen, implying the existence of hormone-dependent mechanisms of control of telomerase activity. Our findings also suggest that sex steroids may play the important roles in cellular aging and cancer. Further analysis of the hormonal control of telomerase may provide insights into the molecular mechanisms of aging as well as carcinogenesis of hormone-dependent tumors.

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References

1. Greider, C. W., and Blackburn, E. H. Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. Cell, *43:* 405–413, 1985.
2. Greider, C. W., and Blackburn, E. H. A telomeric sequences in the RNA of *Tetrahymena* telomerase required for telomere repeat synthesis. Nature (Lond.), *337:* 331–337, 1989.
3. Kim, N. W. Piatyszek, M. A., Prowse, K. R., Harley, C. B., West, M. D., Ho, P. L., Coviello, G. M., Wright, W. E., Weinrich, S. L., and Shay, J. W. Specific association of human telomerase activity with immortal cells and cancer. Science (Washington DC), *266:* 2011–2015, 1994.
4. Shay, J. W. A survey of telomerase activity in human cancer. Eur. J. Cancer, *33:* 787–791, 1997.
5. Harley, C. B. Telomeres and telomerase in aging and cancer. Curr. Opin. Genet. Dev., *5:* 249–255, 1995.
6. Kyo, S., Takakura, M., Kohama, T., and Inoue, M. Telomerase activity in human endometrium. Cancer Res., *57:* 610–614, 1997.
7. Tanaka, M., Kyo, S., Takakura, M., Kanaya, T., Sagawa, T., Yamashita, K., Okada, Y., Hiyama, E., and Inoue, M. Expression of telomerase activity in human endometrium is localized to epithelial granular cells and regulated in a menstrual phase-dependent manner correlated with cell proliferation. Am. J. Pathol., *153:* 1985–1991, 1998.
8. Meeker, A. K., Sommerfeld, H. J., and Coffey, D. S. Telomerase is activated in the prostate and seminal vesicles of the castrated rats. Endocrinology, *137:* 5743–5746, 1996.
9. Bednarek, A. K., Chu, Y., and Aldaz, C. M. Constitutive telomerase activity in cells with tissue-renewing potential from estrogen-regulated rat tissues. Oncogene, *16:* 381–386, 1998.
10. Feng, J., Funk, W. D., Wang, S. S., Weinrich, A. A. A., Chiu, C. P., Adams, R. R., Chang, E., Allsopp, R. C., Yu, J., Le, S., West, M. D., Harley, C. B., Andrew, W. H., Greider, C. W., and Villeponteau, B. The RNA component of human telomerase. Science (Washington DC), *269:* 1236–1241, 1995.
11. Harrington, L., McPhail, T., Mar, V., Zhou, W., Oulton, R., Amgen E ST Program, Bass, M. B., Arruda I., and Robinson, M. O. A mammalian telomerase-associated protein. Science (Washington DC), *275:* 973–977, 1997.
12. Nakayama, J., Saito, M., Nakamura, H., Matsuura, A., and Ishikawa, F. *TLP1*: a gene encoding a protein component of mammalian telomerase is a novel member of WD repeats family. Cell, *88:* 875–884, 1997.
13. Nakamura, T. M., Morin, G. B., Chapman, K. B., Weinrich, S. L., Andrews, W. H., Lingner, J., Harley, C. B., and Cech, T. R. Telomerase catalytic subunit homologs from fission yeast and human. Science (Washington DC), *277:* 955–959, 1997.
14. Meyerson, M., Counter, C. M., Eaton, E. N., Ellisen, L. W., Steiner, P., Caddle, S. D., Ziaugra, L., Beijersbergen, R. L., Davidoff, M. J., Liu, Q., Bacchetti, S., Haber, D. A., and Weinberg, R. A. *hEST2*, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. Cell, *90:* 785–795, 1997.
15. Weinrich, S. L., Pruzan, R., Ma, L., Ouellette, M., Tesmer, V. M., Holt, S. E., Bodnar, A. G., Lichtsteiner, S., Kim, N. W., Trager, J. B., Taylor, R. D., Carlos, R., Andrews, W. H., Wright, W. E., Shay, J. W., Harley, C. B., and Morin, G. B. Reconstitution of human telomerase with template RNA component hTERC and the catalytic protein subunit hTERT. Nat. Genet., *17:* 498–502, 1997.
16. Nakayama J., Tahara H., Tahara E., Saito M., Ito K., Nakamura H., Nakanishi T., Tahara E., Ide T, and Ishikawa, F. Telomerase activation by hTRT in human normal fibroblasts and hepatocellular carcinomas. Nat. Genet., *18:* 65–68, 1998.
17. Takakura, M., Kyo, S., Kanaya, T., Tanaka, M, and Inoue, M. Expression of human telomerase subunits and correlation with telomerase activity in cervical cancer. Cancer Res., *58:* 1558–1561, 1998.
18. Kyo, S., Kanaya, T., Takakura, M., Tanaka, M., and Inoue, M. Human telomerase reverse transcriptase as a critical determinant of telomerase activity in normal and malignant endometrial tissues. Int. J. Cancer, *80:* 60–63, 1999.
19. Kyo, S., Kanaya, T., Takakura, M., Tanaka, M., Yamashita, A., Inoue, H., and Inoue, M. Expression of human telomerase subunits in ovarian malignant, borderline and benign tumors. Int. J. Cancer, *80:* 804–809, 1999.
20. Wu, K. J., Grandori, C., Amacker, M., Simon-Vermot, N., Polack, A., Lingner, J., and Dalla-Favera, R. Direct activation of TERT transcription by c-MYC. Nat. Genet., *21:* 220–224, 1999.
21. Greenberg, R. A. O'Hagan, R. C., Deng, H., Xiao, Q., Hann, S. R., Adams, R. R., Lichtsteiner, S., Chin, L., Morin, G. B., and DePinho, R. A. Telomerase reverse transcriptase gene is a direct target of c-Myc but is not functionally equivalent in cellular transformation. Oncogene, *18:* 1219–1226, 1999.
22. Takakura, M., Kyo, S., Kanaya, T., Hirano, H., Takeda, J., Yutsudo, M., and Inoue, M. Cloning of human telomerase catalytic subunit (*hTERT*) gene promoter and identification of proximal core promoter sequences essential for transcriptional activation in immortalized and cancer cells. Cancer Res., *59:* 551–557, 1999.
23. Cong, Y. S., Wen, J., and Bacchetti, S. The human telomerase catalytic subunit *hTERT*: organization of the gene and characterization of the promoter. Hum. Mol. Genet., *8:* 137–142, 1999.
24. Tatematsu, K., Nakayama, J., Danbara, M., Shionoya, S., Sato, H., Omine, M, and Ishikawa, F. A novel quantitative 'stretch PCR assay,' that detects a dramatic increase in telomerase activity during the progression of myeloid leukemias. Oncogene, *13:* 2265–2274, 1996.
25. Kyo, S., Takakura, M., Tanaka, M., Kanaya, T., and Inoue, M. Telomerase activity in cervical cancer is quantitatively distinct from that in its precursor lesions. Int. J. Cancer, *79:* 66–70, 1998.
26. Yeilding, N. M., Rehman, M. T., and Lee, W. M. Identification of sequences in c-Myc mRNA that regulate its steady-state levels. Mol. Cell. Biol., *16:* 3511–3522, 1996.
27. Thomas, M., Monet, J. D., Brami, M., Dautigny, N., Assailly, J., Ulmann, A., and Bader, C. A. Comparative effects of 17 β-estradiol, progestin R5020, Tamoxifen and RU38486 on lactate dehydrogenase activity in MCF-7 human breast cancer cells. J. Steroid Biochem., *32:* 271–277, 1989.
28. DeFriend, D. J., Anderson, E., Bell, J., Wilks, D. P., West, C. M., Mansel, R. E., and Howell, A. Effects of 4-hydroxytamoxifen and a novel pure antioestrogen (ICI 182780) on the clonogenic growth of human breast cancer cells in vitro. Br. J. Cancer, *70:* 204–211, 1994.
29. Klein-Hitpass, L., Schorpp, M., Wagner, U., and Ryffel, G. U. An estrogen-responsive element derived from the 5′-flanking region of the *Xenopus* vitellogenin *A2* gene functions in transfected human cells. Cell, *46:* 1053–1061, 1986.
30. Dubik, D., and Shiu, R. P. C. Mechanism of estrogen activation of c-*myc* oncogene expression. Oncogene, *7:* 1587–1594, 1992.
31. Krishnan, V., Wang, X., and Safe, S. Estrogen receptor-Sp1 complexes mediate estrogen-induced cathepsin D gene expression in MCF-7 human breast cancer cells. J. Biol. Chem., *269:* 15912–15917, 1994.
32. Rishi, A. K., Shao, Z. M., Baumann, R G., Li, X. S., Sheikh, M. S., Kimura, S., Bashirelahi, N., and Fontana, J. A. Estradiol regulation of the human retinoic acid receptor αgene in human breast carcinoma cells is mediated via an imperfect half-palindromic estrogen response element and Sp1 motifs. Cancer Res., *55:* 4999–5006, 1995.
33. Porter, W., Wang, F., Wang, W., Duan, R., and Safe, S. Role of an Sp1/ERE in E2-induced Hsp-27 gene expression. Mol. Endocrinol., *10:* 1371–1378, 1996.
34. Porter, W., Saville, D., Hoivik, D., and Safe, S. Functional synergy between the transcription factor Sp1 and the estrogen receptor. Mol. Endocrinol., *11:* 1569–1580, 1997.
35. Sutton G. Hormonal aspects of endometrial cancer. Curr. Opin. Obstet. Gynecol., *2:* 69–73, 1990.