ACADEMIC PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Biochemical and Biophysical Research Communications 303 (2003) 130–136

BBRC

www.elsevier.com/locate/ybbrc

Inhibition of human telomerase reverse transcriptase gene expression by BRCA1 in human ovarian cancer cells

Chenyi Zhou and Jinsong Liu*

Department of Pathology, Division of Pathology and Laboratory Medicine, Unit 85, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030-4095, USA

Received 30 January 2003

Abstract

Human telomerase reverse transcriptase (hTERT), the catalytic subunit of human telomerase, is responsible for the synthesis and maintenance of the telomeric repeats at the distal ends of human chromosomes. Telomerase expression is repressed in normal human cells and is activated in immortal cells and during tumorigenesis, but the mechanism by which telomerase expression is regulated is not fully understood. Previous studies have shown that c-Myc stimulates hTERT transcription through the binding sites located on the hTERT promoter. In this study, we sought to determine whether BRCA1 inhibits hTERT transcription through its direct interaction with c-Myc. In ovarian cancer cells, c-Myc increased hTERT expression by threefold and BRCA1 completely abrogated this activity. A mutation in the c-Myc-binding site (E-box) of the hTERT promoter resulted in the loss of activation by c-Myc and in the loss of inhibition by BRCA1. Deletion of the c-Myc-binding domain in BRCA1 resulted in the loss of BRCA1's ability to inhibit transcription of the hTERT promoter. In addition, BRCA1 associates with c-Myc and inhibits the binding activity of c-Myc to the hTERT promoter. Our data indicate that BRCA1 is involved in regulating cellular immortalization through the modulation of c-Myc on the hTERT promoter.

Keywords: BRCA1; hTERT; c-Myc; Ovarian cancer

The ends of human chromosomes are composed of repetitive, noncoding DNA sequences (5′-TTAGG-3′) that are approximately 5–20 kb. These highly conserved and specialized structures provide protective mechanisms to stabilize chromosomes and protect chromosomes from random fusion events and degradation [1,2]. Telomerase is responsible for the synthesis and maintenance of the telomeric repeats [3]. Telomerase comprises several subunits, including an RNA subunit and a catalytic subunit, of the human telomerase reverse transcriptase (hTERT). The RNA subunit provides the template for the synthesis of telomeres [4]. The hTERT is responsible for the synthesis of telomeres using the RNA template [5,6]. With each cell division, the telomeres get shorter, and after dozens of these cell divisions, the telomeres decrease to a certain critical length and the cell dies [7,8]. By stable forced expression of hTERT, cells are able to bypass senescence and extend their lives [9]. Multiple studies have shown that telomerase activity is repressed in normal cells but activated in immortal cells and during tumorigenesis of breast and ovarian cancers [10–12]. These findings suggest that telomerase activation may play an important role in cellular immortalization and in early stages of tumor progression.

Several studies have shown that the c-Myc oncoprotein can activate hTERT transcription by directly binding to the E-box in the hTERT promoter and that it synchronistically interacts with nearby Sp1 [13–16]. In addition, two estrogen receptor-binding sites upstream of the hTERT promoter have been shown to mediate the hTERT response to estrogen stimulation [17]. Interestingly, *BRCA1*, a gene responsible for approximately half of all cases of hereditary breast cancer and for almost all cases of combined hereditary breast and ovarian cancers [18], has been shown to interact with c-Myc [19].

BRCA1 encodes a 1863-amino acid, 220-kDa nuclear phosphoprotein and acts in concert with DNA-repair enzymes to maintain the integrity of the genome. BRCA1 also interacts with hRad51, a human homologue of

* Corresponding author. Fax: 1-713-792-5529.
E-mail address: jliu@mdanderson.org (J. Liu).

0006-291X/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0006-291X(03)00318-8

bacterial RecA, a protein involved in homologous chromosome recombination and postradiation DNA repair [20]. A mutation in *p53* can accelerate breast cancer development in homozygous *BRCA1* (BRCA1$^{-/-}$) knockout mice [21], and mice heterozygous for *BRCA1*, *p53* double mutation (*BRCA1*$^{+/-}$/*p53*$^{+/-}$) have developed mammary gland tumors, after being exposed to low-frequency γ-radiation [22]. BRCA1-deficient cells display spontaneous chromosomal abnormalities, a defective G_2/M checkpoint, and centrosome amplification [23]. BRCA1 has been shown to mediate resistance to paclitaxel and ionizing irradiation in ovarian cancer [24].

Because c-Myc is amplified in many different types of human cancers, it is possible that BRCA1 forms a complex with c-Myc to regulate cellular growth, proliferation, and oncogenesis in different organs. However, the specific target gene for the BRCA1–c-Myc complex has not been identified. Because the hTERT promoter contains c-Myc-binding sites, we hypothesized that it may be a target for BRCA1. The purpose of this study was to determine whether BRCA1 can inhibit the transcription of hTERT through its direct interaction with c-Myc. Here, we provide direct evidence that BRCA1 may be involved in the regulation of cellular immortalization by regulating the transcription of hTERT through its interaction with c-Myc.

Materials and methods

Cell cultures. The ovarian cancer cell line SK-OV3, breast cancer cell line MCF-7, cervical cancer cell line HeLa, and the kidney cancer cell line 293T were purchased from the American Type Culture Collection (ATCC; Manassas, VA). The ovarian cancer cell line SNU-251 was a gift from Dr. Jae-Gahb Park (Korea Cell Line Bank, Seoul National University, Korea) and further characterized by Zhou et al. [24]. Briefly, the SNU-251 cell line is an endometrioid ovarian cell line that carries a single-base G-to-A transition in exon 23, resulting in a stop codon at amino acid 1815 of BRCA1 and a predicted loss of half of the second BRCA1 C-terminal (BRCT) repeat [25]. All cell lines were maintained in RPMI 1640 medium with 10% fetal bovine serum (FBS) in the presence of antibiotics. The 293T cells, HeLa cells, and MCF-7 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% FBS in the presence of antibiotics. All cells were cultured at 37 °C in a 5% CO_2 incubator.

Plasmid construction. Plasmid phTERT (−3328 ∼ +77 nt) containing the whole hTERT promoter (∼3.3 kb) was a gift from Dr. Satoru Kyo (Kanazawa University, Japan). The c-Myc expression plasmid was a gift from Dr. Robert Eisenman (Fred Hutchinson Cancer Research Center, Seattle, WA). The core promoter region (−169 ∼ +58 nt) of hTERT was obtained by polymerase chain reaction (PCR) amplification using the phTERT (−3328 ∼ +77 nt) plasmid as a template, cut by *Mlu*I and *Bgl*II, and subcloned into pGL3 basic vector to create the phTERT (−169 ∼ +58 nt) plasmid. The primers used to amplify the core promoter region were E-BOXI-W(F): 5′-GAACGCGTTCCCCACGTGGCGGAGGGAC-3′ and E-BOXII-W(R): 5′-GCAGATCTAGGGCTTCCCACGTGCGCAGC-3′. The plasmid phTERT (ΔMyc1, −169 ∼ +58 nt), which lost one of its c-Myc-binding sites, was generated by exchanging the three bases (CAC → TTT) that is underlines in E-box-m (F) primer and is located in the E-box of the core promoter from phTERT (−169 ∼ +58 nt). The primers used for E-box mutation were E-BOXI-M (F): 5′-GAACGCGTTCCCTTTGTGGCGGAGGGAC-3′ and BOXII-W(R): 5′-GCAGATCTAGGGCTTCCCACGTGCGCAGC-3′. The BRCA1 expression plasmid pcDNA3 (wtBRCA1) was constructed by excising BRCA1 cDNA with *Hin*dIII and *Eco*RV from a plasmid provided by Dr. Daniel A. Haber (Harvard Medical School, Boston, MA) and ligating the cDNA into pcDNA3 (Invitrogen, Carlsbad, CA). The mutant BRCA1 expression plasmid pcDNA3 (ΔMyc, BRCA1), which lacked a c-Myc-binding domain in BRCA1, was generated by replacing the cDNA BRCA1 fragment between the *Att*II and *Kpn*I sites in pcDNA3 (wtBRCA1) with a DNA fragment with deleted c-Myc binding domain from 644 to 1718 bp flanked by *Att*II and *Kpn*I in the primers used for PCR. This DNA fragment was obtained by PCR using the pcDNA3 (wtBRCA1) plasmid as the template and a pair of primers: BRCA1 (R): 5′-ACCAGGTACCAATGAAATACTGCT-3′ (2418–2441 bp) and BRCA1 (F): 5′-AGACGTCTCAGGGAACTAACCAAA CGGAGCAGAATGGTC-3′ (1719–1749 bp). The *Att*II and *Kpn*I restriction enzyme sites are indicated by underlining.

Transient transfection and luciferase assay. Various hTERT promoter constructs were cotransfected with the c-Myc expression plasmid in the presence or absence of the wild-type BRCA1 expression plasmid into different cancer cells. Luciferase (LUC) activity was measured 48 h after transfection. A total of 4 μg of plasmid was used in each transfection. Activity was normalized using β-galactosidase activity. LUC activity was assayed using the LUC reporter assay system (Promega, Madison, WI) and normalized as 100% in the presence of the c-Myc expression plasmid.

Immunoprecipitation and Western blot analysis. Cell extracts were prepared in 1× lysis buffer (50 mM Tris–HCl (pH 8.0), 120 mM NaCl, and 0.5% Nonidet P-40 (NP40)), 50 mM NaF, 1 mM sodium orthovanadate, 100 μg/ml polymethylsulfonyl fluoride (PMSF), 20 μg/ml aprotinin, and 10 μg/ml leupeptin. Protein was immunoprecipitated with anti-BRCA1 (ab1) antibody. Binding reactions were rotated overnight at 4 °C in the presence of protein extract, antibody, and protein G beads. The supernatant was removed and the protein G beads were then washed three or four times with the buffer containing 20 mM Tris–OAc (pH 7.9), 0.12 M KOAc, 6 mM $Mg(OAc)_2$, 0.1% NP40, 0.1 mM dithiothreitol (DTT), and 0.2 mg/ml bovine serum albumin (BSA). For Western blot analysis, samples were subjected to electrophoresis in 6% sodium dodecyl sulfate (SDS)–polyacrylamide gel and transferred to a nitrocellular membrane (Bio-Rad, Hercules, CA). The membrane was prehybridized with dried skim milk for 1 h and then blotted with a primary mouse anti-BRCA1 antibody (ab-1; Oncogene Research, Cambridge, MA) and a subsequent peroxidase-conjugated horse anti-mouse antibody (Amersham Pharmacia Biotech, Piscataway, NJ). The membrane was then washed with Tris-buffered saline (0.9% sodium chloride and 20 mM Tris–HCl (pH 7.4)) supplemented with 0.05% Tween 20 (TBST) three times for 10 min. An enhanced chemiluminescence kit was used to detect the signals (Amersham Pharmacia Biotech).

Nuclear cell extract preparation. Nuclear cell extract was prepared from 1×10^6 SK-OV3 cells. Cells were washed once with 1× phosphate-buffered saline (PBS) and twice with cold buffer A (10 mM Hepes (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, and 0.5 mM PMSF). After pelleted by centrifugation at 1000*g* for 5 min, cells were resuspended with two volumes of cold buffer A containing 0.1% NP40 and then stored on ice for 5 min. Nuclei from these cells were separated by centrifugation at 14,000*g*, 4 °C for 15 min and then dissolved by adding two volumes of cold buffer C (20 mM Hepes (pH 7.9), 1.5 mM $MgCl_2$, 0.5 mM DTT, 0.5 mM PMSF, 420 mM NaCl, 0.2 mM EDTA, and 25% glycerol) at 4 °C for 15 min. The nuclear cell extract in supernatant was collected by centrifugation at 14,000*g*, 4 °C for 10 min, and was ready to use or could be stored at −80 °C for later use.

Gel mobility shift assay. Nuclear cell extracts from SK-OV3 cells (10 μg) were incubated with a DNA binding buffer (50 mM Hepes (pH 7.9), 100 mM KCl, 2 mM EDTA, 2 mM DTT, 4% BSA, 6% glycerol, and 50 μg/ml poly(dC-dG)), in the presence or absence of an unlabeled competitor oligonucleotide (20-fold excess) or antibody (0.1–1 μg) on ice for 20 min. A 5000 cpm ^{32}p-labeled probe containing a wild-type

c-Myc-binding site in the hTERT promoter, 5′-GCGCTCCCCACG TGGCGGAGGG-3′ (nt: −172 ~ −150), was added to the nuclear extract, and the nuclear extract was then incubated at room temperature for 20 min. The oligonucleotide containing the mutant c-Myc-binding site is identical to the wild-type sequence probe, except for the two base pair changes (underlined): 5′-GCGCTCCCCACGGAGC GGAGGG-3′ (nt: −172 ~ −150). The anti-c-Myc antibody (N262) and normal serum immunoglobulin (Ig) G were obtained from Santa Cruz Biotechnology. (Santa Cruz, CA), and the anti-BRCA1 antibody (ab-1) was obtained from Oncogene Research (Cambridge, MA). Reaction mixtures were loaded on a 4% nondenatured polyacrylamide gel and then dried prior to the autoradiography.

Results

BRCA1 inhibits c-Myc-mediated transactivation of hTERT in SNU-251 and SK-OV3 cells

To examine the effect of BRCA1 on the transcriptional activity of the hTERT promoter, various hTERT promoters were subcloned into a pGL3 basic vector containing the LUC reporter gene. A schematic of the hTERT-LUC chimeric constructs is provided in Fig. 1A. The plasmid phTERT (−169 ~ +58 nt) was cotransfected with c-Myc expression plasmid into SK-OV3 cells (the wild-type BRCA1 cell line) or SNU-251 cells (the BRCA1-deficient cell line). The SNU-251 and SK-OV3 cells in which the plasmid phTERT (−169 ~ +58 nt) was cotransfected with the c-Myc expression plasmid showed an approximately threefold increase in the level of hTERT transcription, as indicated by the LUC activity (Fig. 1B, lane 2), compared with cells without the c-Myc expression plasmid (Fig. 1B, lane 1). Cotransfection with the wild-type BRCA1 expression plasmid inhibited the activation to almost the basal level in the SNU-251 cells, and to a lesser extent in the SK-OV3 cells (Fig. 1B, lane 4). This repression was dose dependent (Fig. 1B, lanes 3 and 4).

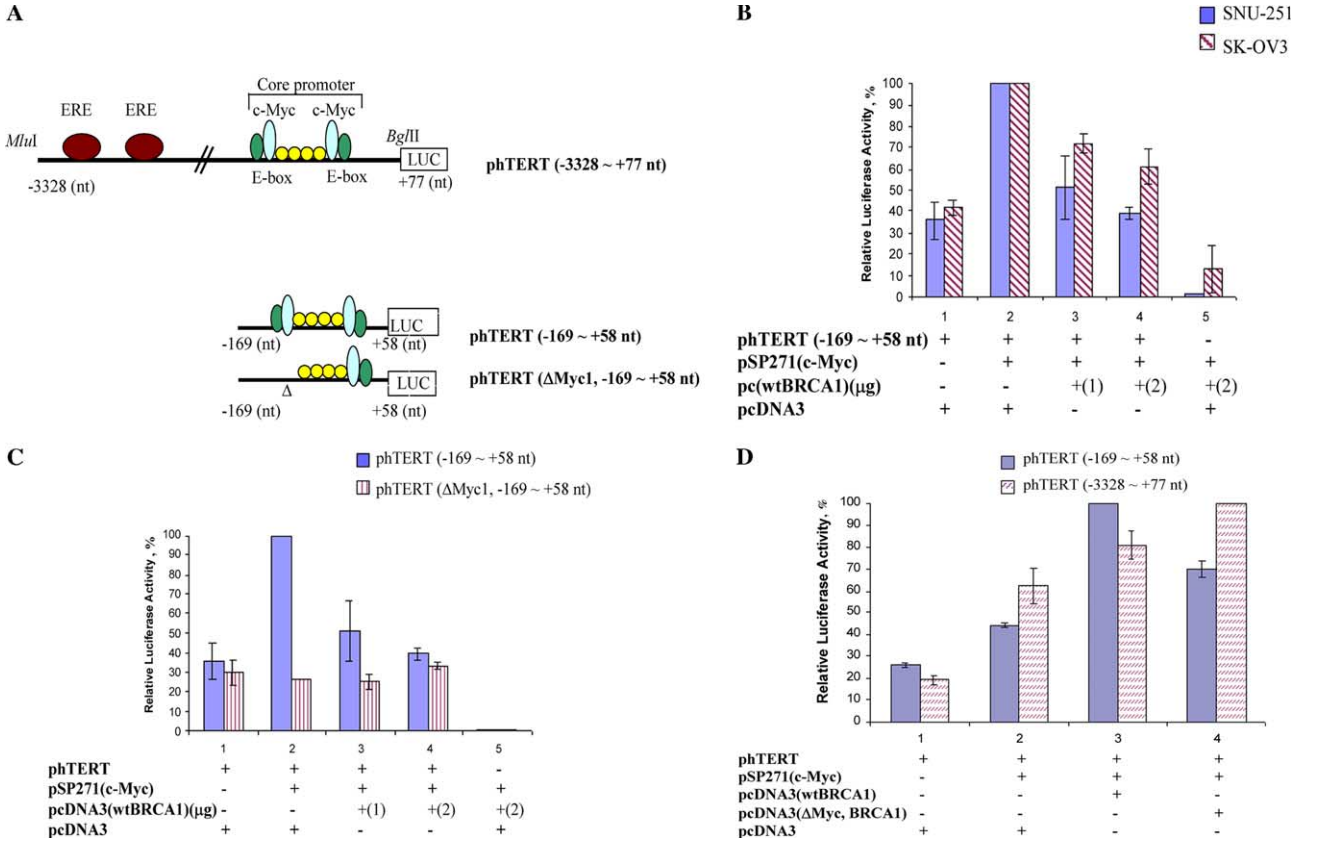

Fig. 1. (A) Schematic of hTERT-luciferase chimeric constructs. LUC, luciferase; ERE, estrogen response element; E-box, (5′-CACGTG-3′) sequence; nt, nucleotide. Numbers refer to the number of bases upstream (−) or downstream (+) of the ATG initiation codon of the hTERT gene. (B) The effect of c-Myc and BRCA1 on the level of hTERT transcription. Plasmid phTERT (−169 ~ +58 nt) was cotransfected with the c-Myc expression plasmid, in the presence or absence of the wild-type BRCA1 expression plasmid into SNU-251 or SK-OV3 cells. The name of each plasmid is indicated on the left lower position of the figure. The error bars represent the standard deviation. Where error bars are not seen, standard errors were too small to show on the graph. Each experiment was performed three times. The presence or absence of each plasmid used in each transfection is indicated by + or −. (C) The effect of the c-Myc-binding site in the hTERT promoter on the BRCA1-mediated inhibition in SNU-251 ovarian cancer cells. phTERT, hTERT-LUC plasmid. (D) The effect of c-Myc and BRCA1 on the hTERT promoter in 293T cells.

c-Myc-binding sites in the hTERT promoter are required for BRCA1-mediated inhibition

To examine the effect of BRCA1 on the transcriptional regulation of the hTERT promoter through the c-Myc DNA binding site, we performed LUC assays using an hTERT-LUC chimeric construct containing a mutation in the c-Myc-binding site of the hTERT promoter phTERT (ΔMyc1, $-169 \sim +58$ nt), together with its wild-type counterpart, phTERT ($-169 \sim +58$ nt), after they were transfected into SNU-251 cells. As shown in Fig. 1C, cells in which phTERT ($-169 \sim +58$ nt) was cotransfected with the c-Myc expression plasmid showed an approximately threefold stimulation of transcriptional activity (Fig. 1C, lane 2, indicated by the solid bar), compared with cells without the c-Myc expression plasmid (Fig. 1C, lane 1). A mutation in the c-Myc-binding site in plasmid phTERT (ΔMyc1, $-169 \sim +58$ nt) resulted in a loss of transactivation by c-Myc (Fig. 1C, lane 2, indicated by the dash bar). BRCA1 did not further decrease the basal level of transcription from the hTERT-LUC constructs (Fig. 1C, lanes 3 and 4), suggesting that BRCA1 inhibits the level of transcription through this c-Myc-binding site on the hTERT promoter. The 293T cells were similarly transfected and c-Myc stimulated the transcription from the hTERT promoter in the 293T cells, as it did in the SNU-251 and SK-OV3 cells (Fig. 1D, lane 2). However, cotransfection of the 293T cells with BRCA1 mildly stimulated the transcriptional activity of the hTERT promoter instead of repressing it as described above (Fig. 1D, lane 3). No repression of hTERT transcriptional activity by BRCA1 was detected in HeLa cells and MCF-7 cells (data not shown). On the basis of these results, we concluded that repression of transcriptional activity of the catalytic subunit of hTERT by BRCA1 is ovarian cancer-cell specific. Such repression was more dramatic in the SNU-251 ovarian cancer cell lines carrying the homozygous BRCA1 mutation.

Mutant BRCA1 protein without the c-Myc-binding domain lost its ability to inhibit the transcription of hTERT promoter in SNU-251 cells

An earlier study [19] showed that BRCA1 interacted with c-Myc through a specific binding domain in the BRCA1 protein. Whether this specific binding domain plays an important role in inhibiting the c-Myc-mediated transactivation of the hTERT promoter is not known. To examine the role of the c-Myc-binding domain of BRCA1 in regulating the hTERT promoter, we constructed a mutant BRCA1 expression plasmid, pcDNA3 (ΔMyc, BRCA1), which contained the mutant BRCA1 without the c-Myc-binding domain (Fig. 2A). LUC assays were performed using the mutant BRCA1 and wild-type BRCA1 expression plasmids after they were cotransfected with either the hTERT core promoter ($-169 \sim +58$ nt) construct or the hTERT whole promoter ($-3330 \sim +58$ nt) construct in the presence of c-Myc expression plasmid into SNU-251 cells. We found no repression of LUC activity from the hTERT core promoter ($-169 \sim +58$ nt) (Fig. 2B, lane 4) and only a mild inhibition from the hTERT whole promoter ($-3330 \sim +58$ nt) (Fig. 2B, lane 4) cotransfected with the c-Myc expression plasmid and the mutant BRCA1 expression plasmid, compared with the wild-type BRCA1 plasmid (Fig. 2B, lane 3). These results demonstrated that the specific c-Myc-binding domain in BRCA1 is required to inhibit the transcription activation of the hTERT promoter.

BRCA1 associates with c-Myc and inhibits DNA-binding activity of c-Myc to the hTERT promoter

To determine whether BRCA1 associates with c-Myc, we coimmunoprecipitated c-Myc with BRCA1 by using anti-BRCA1 (ab1) antibody or preimmune control serum. Both BRCA1 and c-Myc proteins were detected in the immunoprecipitation complex, demonstrating that BRCA1 forms a complex with c-Myc in the ovarian cancer cell line SK-OV3 (Fig. 3A). No detectable level of

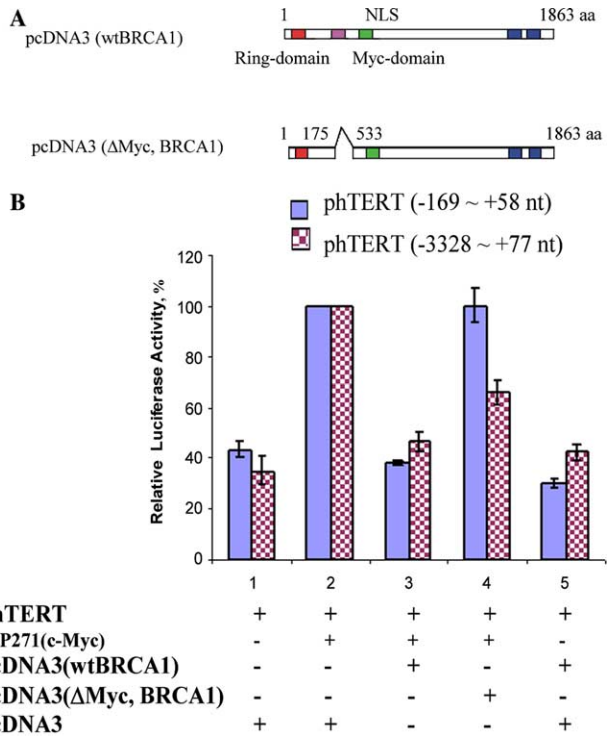

Fig. 2. (A) Schematic of mutant and wild-type BRCA1 constructs. aa, amino acid; NLS, nuclear localization signal; Myc-domain, c-Myc-binding domain in BRCA1. (B) The effect of the c-Myc-binding domain in BRCA1 on the level of transcriptional activity of hTERT promoter in SNU-251 cells. Each experiment was done three times. The error bars represent the standard deviations. Where error bars are not seen, standard errors were too small to show on the graph. phTERT, hTERT-LUC plasmid.

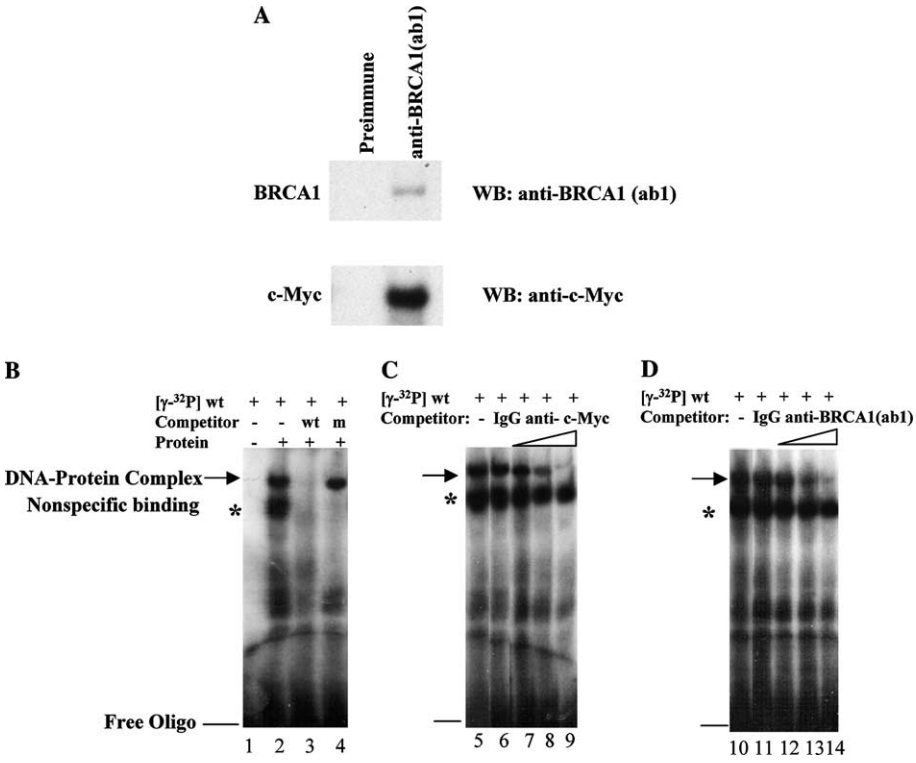

Fig. 3. Association of BRCA1 and c-Myc. (A) Coimmunoprecipitation of BRCA1 and c-Myc. BRCA1 and BRCA1-associated proteins in SK-OV3 cells were pulled down using anti-BRCA1 (ab1) antibody. The pull-down protein was detected by Western blotting (WB). Preimmune serum was included as a negative control. Preimmune, preimmune serum; anti-BRCA1 (ab1), anti-BRCA1 (ab1) anbibody; anti-c-Myc, anti-c-Myc antibody. (B) Detection of the c-Myc–BRCA1–DNA complex. A gel mobility shift assay was used to show that the c-Myc protein was able to form a specific DNA–c-Myc complex with the hTERT promoter. wt, wild-type c-Myc-binding oligonucleotide; m, mutant c-Myc-binding oligonucleotide. The specific c-Myc–DNA complex is indicated by the arrow; the nonspecific binding, by the asterisk. All lanes contained a [γ-^{32}P]-labeled oligonucleotide with a wild-type c-Myc-binding site. Lane 1, without nuclear extract; lane 2, with nuclear extract; lane 3, with nuclear extract and unlabeled wild-type oligonucleotides containing the c-Myc-binding site; and lane 4, with nuclear extract and an unlabeled oligonucleotide with a mutated c-Myc-binding site. (C) Inhibition of the binding of the c-Myc protein to DNA by anti-c-Myc antibody. An increased concentration of the anti-c-Myc antibody progressively decreased the binding of c-Myc to the hTERT promoter. IgG, preimmune serum. (D) Inhibition of the binding of c-Myc to DNA by an increased concentration of anti-BRCA1.

c-Myc was associated with the negative control. To further examine whether BRCA1 interacts with c-Myc on the hTERT promoter in vivo, we examined BRCA1 and c-Myc interaction using a gel mobility shift assay (GMSA). The oligonucleotides containing a c-Myc-binding site derived from the hTERT promoter were labeled with [γ-^{32}P] and incubated with nuclear extracts. The resulting DNA–protein complexes were detected in non-denaturing polyacrylamide gels. As shown in Fig. 3B, incubation of the oligonucleotides containing a c-Myc-binding site derived from the hTERT promoter yielded two bands (lane 2). The top band could be competed by an oligonucleotide containing a wild-type c-Myc-binding site (Fig. 3B, lane 3) but not by an oligonucleotide containing mutations in this binding site (Fig. 3B, lane 4). In contrast, the lower band was competed either by an oligonucleotide containing a wild-type or by a mutant c-Myc-binding site, suggesting that the top band represents the specific DNA–c-Myc complex and the lower band represents the nonspecific one (Fig. 3B, lanes 3 and 4). The specific DNA–c-Myc complex was further demonstrated (Fig. 3C) when incubation of an increased concentration of the anti-c-Myc antibody decreased the binding activity of the top band but not the lower band (lanes 7–9), similar to what was previously reported [14]. The DNA-binding activity of c-Myc was diminished when these DNA–c-Myc-binding complexes were incubated in the presence of the anti-BRCA1 antibody (Fig. 3D, lanes 12–14) but not in the presence of normal serum IgG (Fig. 3D, lane 11). The top band decreased markedly compared with the lower band, suggesting that the anti-BRCA1 antibody binds to the c-Myc–BRCA1 complex and decreases its binding to the c-Myc-binding sites. These results suggest that BRCA1 inhibits the transcriptional activity of hTERT through direct interaction with c-Myc in the core promoter of hTERT.

Discussion

The results presented demonstrate that the transcription of hTERT promoter is negatively regulated by

BRCA1. Although the function of BRCA1 has been studied extensively since its cloning in 1994 [26], our results demonstrate for the first time a direct link between BRCA1 and hTERT and suggest that BRCA1 regulates cellular immortalization through hTERT, which is a critical early step in carcinogenesis. On the basis of these findings, we propose that the negative regulation of hTERT by BRCA1 might be involved in the suppression of ovarian cancer development. A constitutive high level of the BRCA1 protein binds to the c-Myc protein, turns off transcription of hTERT, and prevents incidental cellular immortalization. In women with the heterozygous BRCA1 mutation or homozygous BRCA1 mutation after its wild-type allele of BRCA1 is lost, such an inhibitory effect by BRCA1 is attenuated or lost as a result of a decreased level or absence of the functional BRCA1 protein, which leads to a sustained high level of hTERT mRNA transcription and increased frequency of cellular immortalization. These immortalized cells are susceptible to additional genetic mutations and eventually grow into clinically detectable tumors. Li et al. [27] recently presented evidence that BRCA1 inhibits hTERT expression in breast cancer cells. In their study, BRCA1 alone appeared insufficient to inhibit the transcriptional activity of hTERT and required a novel cofactor called Nmi. In our study, however, this additional factor was not required for the inhibition of transcription by hTERT in ovarian cancer cells. In conclusion, our results demonstrate that hTERT promoter is a target gene for BRCA1 inhibition and that loss of such inhibition may lead to immortalization and to subsequent development of ovarian cancers.

Acknowledgments

This work was supported in part by institutional start-up funds, an institutional research grant, and a career development award from the M.D. Anderson Cancer Center Specialized Program Of Research Excellence *(SPORE) in Ovarian Cancer* (to J.L.). C.Z. is the recipient of a postdoctoral fellowship from the US Army Breast Cancer Research Program, Department of Defense, Grant No. DAMD17-99-1-9264 and the M.D. Anderson Cancer Center *SPORE in Ovarian Cancer*. We thank Dr. Daniel Haber for providing the BRCA1 cDNA, Dr. Satoru Kyo for the hTERT promoter, and Dr. Robert Eisenman for the pSP271(c-Myc) expression plasmid.

References

[1] E.H. Blackburn, Structure and function of telomeres, Nature 350 (1991) 569–573.

[2] R.K. Moyzis, J.M. Buckingham, L.S. Cram, M. Dani, L.L. Deaven, M.D. Jones, J. Meyne, R.L. Ratliff, J.R. Wu, A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes, Proc. Natl. Acad. Sci. USA 85 (1988) 6622–6626.

[3] C.W. Greider, E.H. Blackburn, Identification of a specific telomere terminal transferase activity in Tetrahymena extracts, Cell 43 (1985) 405–413.

[4] J. Feng, W.D. Funk, S.S. Wang, S.L. Weinrich, A.A. Avilion, C.P. Chiu, R.R. Adams, E. Chang, R.C. Allsopp, J. Yu, The RNA component of human telomerase, Science 269 (1995) 1236–1241.

[5] T.M. Nakamura, G.B. Morin, K.B. Chapman, S.L. Weinrich, W.H. Andrews, J. Lingner, C.B. Harley, T.R. Cech, Telomerase catalytic subunit homologs from fission yeast and human [see comments], Science 277 (1997) 955–959.

[6] M. Meyerson, C.M. Counter, E.N. Eaton, L.W. Ellisen, P. Steiner, S.D. Caddle, L. Ziaugra, R.L. Beijersbergen, M.J. Davidoff, Q. Liu, S. Bacchetti, D.A. Haber, R.A. Weinberg, hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization, Cell 90 (1997) 785–795.

[7] C.B. Harley, A.B. Futcher, C.W. Greider, Telomeres shorten during ageing of human fibroblasts, Nature 345 (1990) 458–460.

[8] C.M. Counter, A.A. Avilion, C.E. LeFeuvre, N.G. Stewart, C.W. Greider, C.B. Harley, S. Bacchetti, Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity, Embo J. 11 (1992) 1921–1929.

[9] A.G. Bodnar, M. Ouellette, M. Frolkis, S.E. Holt, C.-P. Chiu, G.B. Morin, C.B. Harley, J.W. Shay, S. Lichtsteiner, W.E. Wright, Extension of life-span by introduction of telomerase into normal human cells, Science 279 (1998) 349–352.

[10] N.W. Kim, M.A. Piatyszek, K.R. Prowse, C.B. Harley, M.D. West, P.L. Ho, G.M. Coviello, W.E. Wright, S.L. Weinrich, J.W. Shay, Specific association of human telomerase activity with immortal cells and cancer [see comments], Science 266 (1994) 2011–2015.

[11] C.M. Counter, H.W. Hirte, S. Bacchetti, C.B. Harley, Telomerase activity in human ovarian carcinoma, Proc. Natl. Acad. Sci. USA 91 (1994) 2900–2904.

[12] J.W. Shay, S. Bacchetti, A survey of telomerase activity in human cancer, Eur. J. Cancer 33 (1997) 787–791.

[13] M. Takakura, S. Kyo, T. Kanaya, H. Hirano, J. Takeda, M. Yutsudo, M. Inoue, 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 (1999) 551–557.

[14] S. Kyo, M. Takakura, T. Taira, T. Kanaya, H. Itoh, M. Yutsudo, H. Ariga, M. Inoue, Sp1 cooperates with c-Myc to activate transcription of the human telomerase reverse transcriptase gene (hTERT), Nucleic Acids Res. 28 (2000) 669–677.

[15] K.J. Wu, C. Grandori, M. Amacker, N. Simon-Vermot, A. Polack, J. Lingner, R. Dalla-Favera, Direct activation of TERT transcription by c-MYC, Nat. Genet. 21 (1999) 220–224.

[16] R.A. Greenberg, R.C. O'Hagan, H. Deng, Q. Xiao, S.R. Hann, R.R. Adams, S. Lichtsteiner, L. Chin, G.B. Morin, R.A. DePinho, Telomerase reverse transcriptase gene is a direct target of c-Myc but is not functionally equivalent in cellular transformation, Oncogene 18 (1999) 1219–1226.

[17] S. Kyo, M. Takakura, T. Kanaya, W. Zhuo, K. Fujimoto, Y. Nishio, A. Orimo, M. Inoue, Estrogen activates telomerase, Cancer Res. 59 (1999) 5917–5921.

[18] S.A. Gayther, P.D. Pharoah, B.A. Ponder, The genetics of inherited breast cancer, J. Mammary Gland Biol. Neoplasia 3 (1998) 365–376.

[19] Q. Wang, H. Zhang, K. Kajino, M.I. Greene, BRCA1 binds c-Myc and inhibits its transcriptional and transforming activity in cells, Oncogene 17 (1998) 1939–1948.

[20] R. Scully, J. Chen, A. Plug, Y. Xiao, D. Weaver, J. Feunteun, T. Ashley, D.M. Livingston, Association of BRCA1 with Rad51 in mitotic and meiotic cells, Cell 88 (1997) 265–275.

[21] X. Xu, K.U. Wagner, D. Larson, Z. Weaver, C. Li, T. Ried, L. Hennighausen, A. Wynshaw-Boris, C.X. Deng, Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation [see comments], Nat. Genet. 22 (1999) 37–43.

[22] V.L. Cressman, D.C. Backlund, E.M. Hicks, L.C. Gowen, V. Godfrey, B.H. Koller, Mammary tumor formation in p53- and BRCA1-deficient mice, Cell Growth Differ. 10 (1999) 1–10.

[23] X. Xu, Z. Weaver, S.P. Linke, C. Li, J. Gotay, X.W. Wang, C.C. Harris, T. Ried, C.X. Deng, Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells, Mol. Cell 3 (1999) 389–395.

[24] C. Zhou, J.L. Smith, J. Liu, Role of BRCA1 in cellular resistance to paclitaxel and ionizing radiation in an ovarian cancer cell line carrying a defective BRCA1, Oncogene (in press).

[25] Y. Yuan, W.H. Kim, H.S. Han, J.H. Lee, H.S. Park, J.K. Chung, S.B. Kang, J.G. Park, Establishment and characterization of human ovarian carcinoma cell lines, Gynecol. Oncol. 66 (1997) 378–387.

[26] Y. Miki, J. Swensen, D. Shattuck-Eidens, P.A. Futreal, K. Harshman, S. Tavtigian, Q. Liu, C. Cochran, L.M. Bennett, W. Ding, et al., A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1, Science 266 (1994) 66–71.

[27] H. Li, T.-H. Lee, H. Avraham, A novel tricomplex of BRCA1, Nmi and c-Myc inhibits c-Myc induced hTERT promoter activity in breast cancer, J. Biol. Chem. 277 (2002) 20965–20973.