Telomerase Is Controlled by Protein Kinase Cα in Human Breast Cancer Cells*

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He Li, Linlin Zhao, Zhiyong Yang, John W. Funder, and Jun-Ping Liu‡

From the Molecular Signaling Laboratory, Baker Medical Research Institute, Commercial Road, Prahran, Victoria 3181, Australia

Telomerase, a specialized RNA-directed DNA polymerase that extends telomeres of eukaryotic chromosomes, is repressed in human somatic tissues and becomes activated during tumor progression in most human cancers. To date, little is known about how telomerase is activated and controlled in cancer, although activation is thought to be involved in cancer cell immortalization. Here, we report that human telomerase-associated protein 1 (hTEP1) and the telomerase catalytic subunit (human telomerase reverse transcriptase (hTERT)) are phosphoproteins and that their phosphorylation is a prerequisite for the activation of telomerase in intact human breast cancer cells. Identified by hTEP1 peptide affinity chromatography, protein kinase Cα mediates the phosphorylation of hTERT and induces a marked increase in telomerase activity. Thus, phosphorylation of hTEP1 and hTERT by protein kinase Cα represents an essential step in the generation of a functional telomerase complex in the initiation and maintenance of telomerase activity in human cancer.

In most eukaryotes, telomerase is responsible for the synthesis and maintenance of telomeric DNA that together with proteins forms a protective cap known as telomeres for eukaryotic linear chromosomes (1–10). The integrity of telomeres is essential for genome stability and chromosome paring and separation during cell division (11, 12). Damage of telomeres, if not repaired, causes cell cycle arrest, chromosome fusion, or degradation. Telomeres shorten, however, as a function of each cell division in normal human somatic cells, where telomerase activity is below detectable levels, contributing to the mechanisms underlying cell senescence. In most human cancers, telomeres stop shortening because of the de novo synthesis of telomeric DNA by activated telomerase. Telomerase may thus play a pivotal role in regulating the structure and function of telomeres during human cell immortalization and tumor progression.

Telomerase is a ribonucleoprotein reverse transcriptase. Telomerase RNA substrate from a variety of different organisms has been characterized (13–16). The RNA component contains the template for telomeric DNA addition and is essential for telomerase activity. Telomerase protein subunits have also been isolated from the ciliates Tetrahymena thermophila and Euploites aediculatus (17, 18) and from yeast and mammals (8, 9, 19, 20). In Tetrahymena, two telomerase protein subunits have been purified with the p80 protein binding to the RNA component and p95 binding to single-stranded telomeric DNA (17). Based on amino acid similarity to Tetrahymena p80, mammalian telomerase-associated protein 1 (TEP1) has been identified, which is an ~300-kDa polypeptide containing the Tetrahymena telomerase homology domain in the N-terminal region, a nucleotide-binding domain in the middle region, and multiple WD-40 repeats in the C-terminal portion (19, 20). Binding to telomerase RNA (19), TEP1 probably serves not only as a structural protein, but also as a regulatory subunit in mediating telomerase interaction with other molecules. The catalytic subunit of telomerase was initially purified from the ciliate E. aediculatus, called p123, which is homologous to the Est2p protein essential for telomere maintenance and telomerase activity in Saccharomyces cerevisiae (18, 21, 22). The human telomerase catalytic subunit or telomerase reverse transcriptase (hTERT) has recently been identified to be an ~130-kDa protein that is concomitantly expressed with the activation of telomerase during cellular immortalization and tumor progression (8, 9, 23, 24).

Although de novo expression of hTERT is essential for telomerase activity (8, 9), little is known about how telomerase is activated and maintained at the enzyme levels in human cancers. One possible mechanism is that the tertiary and quaternary structures of the large telomerase complex are modulated by protein phosphorylation in such a way that the enzyme is activated. This hypothesis is based on our previous finding that protein phosphatase 2A (PP2A) specifically inhibits telomerase activity in the nuclear lysates of human breast cancer cells (25). To establish if telomerase is directly regulated by protein phosphorylation and which protein kinase(s) are involved in the process, we have made use of affinity chromatography with a synthetic peptide corresponding to the N-terminal sequence of hTEP1 and produced polyclonal antibodies against hTEP1 and hTERT to isolate telomerase and its potentially responsible protein kinase(s) involved in the regulation of telomerase activity. Our data show that protein kinase Cα (PKCα) is selectively eluted from the hTEP1 peptide affinity column and mediates phosphorylation of hTEP1 and hTERT in intact human breast cancer cells. Phosphorylation of partially purified telomerase results in a marked increase in telomerase activity in vitro. Thus, activation of telomerase and subsequent maintenance of its activity may require a prerequisite phosphorylation of telomerase proteins by PKCα in human breast cancer cells.

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‡To whom correspondence and reprint requests should be addressed: Baker Medical Research Inst., P. O. Box 6492, St. Kilda Rd. Central, Melbourne, Victoria 8008, Australia. Tel.: 61-3-95224333; Fax: 61-3-95211362; E-mail: jun-ping.liu@baker.edu.au.

†The abbreviations used are: TEP1, telomerase-associated protein 1; hTEP1, human telomerase-associated protein 1; hTERT, human telomerase reverse transcriptase; PP2A, protein phosphatase 2A; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; PAGE, polyacrylamide gel electrophoresis.

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MATERIALS AND METHODS

Chemicals, Peptides, and Antibodies—PKC isoforms, the catalytic subunit of protein kinase A, PP2A, and phospholipase Cβ were from BIOMOL Research Laboratories Inc. (Plymouth Meeting, PA). Alkaline phosphatase was from New England Biolabs Inc. (Beverly, MA). DNA-dependent protein kinase was from Promega (Madison, WI). Cdc2 protein kinase was from Upstate Biotechnology, Inc. (Lake Placid, New York). ATP, Taq DNA polymerase, eDNA, and T4 gene 32 protein were from Boehringer Mannheim Australia Pty. Ltd. (New South Wales, Australia). [γ-32P]ATP and [α-32P]ATP were from Amerham Australia Pty. Ltd. (New South Wales, Australia). Gel electrophoresis reagents were from Bio-Rad. All other chemicals were from Sigma Chemical.

The telomerase peptides were synthesized by Chiron Technologies Pty. Ltd. (Victoria, Australia). Several peptides were made to produce telomerase peptide affinity column and hTERT-specific antibodies, and the peptides WGVTEEETRRNRQLEVC and DSEPTPHLKTRQRR were finally selected for making an affinity purification column used in this study and producing anti-hTERT antibodies, respectively. For anti-hTERT antibody, the peptide CAREKPQGSVAEEEDTD was used. Anti-hTERT and anti-hTERT antibodies were raised in rabbits by multiple immunization with the synthetic peptides. The antibodies were then affinity-purified against the respective antigenic peptides as described previously (26). Anti-PKC isoform antibodies and anti-Cdc2 kinase antibody were from Santa Cruz Biotechnology, Inc. (Santa Cruz CA). Anti-casein kinase IIα antibody and anti-mitogen-activated protein kinase antibody were from Transduction Laboratories (Lexington, KY). Anti-PP2Aβ antibody was from Upstate Biotechnology, Inc. The immunoreactive proteins were detected with ECL (Amerham Australia Pty. Ltd.).

Peptide Affinity Purification of Telomerase and Immunoblotting—
The hTEP1 peptide affinity column was prepared for purification of telomerase potential regulatory protein kinase and phosphatase by a method described previously (27). In the peptide affinity chromatography, 10 mg of total proteins of the nuclear telomerase extracts from human breast cancer PMC42 cells were loaded onto the column (bed volume of 10 ml) at a flow rate of ~0.5 ml/min. Following extensive washing (50 ml), the column was eluted with KCl (0–300 mM) and then a glycin buffer (0.1 mM, pH 2.4). The collected fractions were subjected to SDS-PAGE followed by immunoblotting using specific antibodies and the TRAP telomerase activity assay (3).

Protein Phosphorylation and Dephosphorylation in Vitro and in intact Cells—Equal amounts of proteins in the isolated telomerase fractions were subjected to incubation in phosphorylation buffer containing ATP (~40 μM, containing 3 μCi of [γ-32P]ATP) and Mg2+ (~1 mM), and the reaction was allowed to proceed at 30 °C for 10 min before being terminated by the addition of SDS sample buffer (28). For protein backphosphorylation, the proteins were subjected to dephosphorylation by incubation of the samples in the presence of an appropriate concentration of protein phosphatase as indicated at 30 °C for 5 min prior to phosphorylation (25, 28). After the dephosphorylation was terminated by inclusion of NaF (10 mM) and cooling at 0 °C, the samples were then subjected to protein phosphorylation with the adjustment of the buffer to phosphorylation conditions by adding 10–100× stock solutions (25, 28). For PKC-mediated protein phosphorylation, free Ca2+ (~200 μM), phosphatidylserine (40 μg/ml), PMA (10 μM), and purified recombinant PKC (1 μM) were included, and for detecting endogenous PKC activity, incubation was carried out in the presence or absence of the PKC inhibitors bisindolylmaleimide I (10 μM) and staurosporine (100 nM). The phosphorylated proteins were subsequently resolved by SDS-PAGE followed by autoradiography. For telomerase protein phosphorylation in intact human breast cancer cells, the cells were incubated with or without the PKC activator PMA for 3 h, and during the last 2 h, 32P was included in the phosphate-free medium to label the intracellular ATP pool and thus phosphoproteins. It was noted that PMA treatment did not induce significant change in the levels of 32P labeling in these cells. After being extensively washed, the cells were lysed, and the total lysates were subjected to immunoprecipitation. The immunoprecipitates were subjected to SDS-PAGE followed by autoradiography or to further incubations with PP2A plus or minus purified PKC from rat brain (28).

Immunoprecipitation and Telomerase Activity Assay—To determine direct phosphorylation and dephosphorylation of telomerase proteins in intact human breast cancer cells, hTERT and hTEP1 were immunoprecipitated from total cell lysates or partially purified telomerase fractions using specific antibodies against hTERT and hTEP1 peptides and a preimmune IgG as a control, essentially as described elsewhere (28).

To verify efficient precipitation of telomerase, telomerase activity was assessed in the immunoprecipitation by incubating the supernatant, wash fraction, and immunoprecipitates in a telomerase assay, with the immunoprecipitates being treated or not with PP2A. The measurement of telomerase activity was performed with the TRAP assay as described (3). For measuring telomerase activity in the immunoprecipitates, the telomerase reaction was carried out by incubating the immunoprecipitates at 30 °C for 20 min with shaking in a buffer described previously (25), which was then followed by centrifugation to remove the immuno-nprecipitated beads or phenol/chloroform extraction to obtain the de novo synthesized telomeres for polymerase chain reaction amplification. To analyze the effects of protein kinases and phosphatases on telomerase activity, partially purified telomerase was incubated with PP2A or alkaline phosphatase, and after termination of the reaction with NaF (10 mM), the samples were subjected to the telomerase activity assay or to further incubation of protein phosphorylation at 30 °C for
FIG. 2. PKCα mediates telomerase protein phosphorylation in vitro and in intact cells. A, immunoprecipitation of telomerase activity with anti-hTEP1 antibody from human breast cancer cells and role of protein phosphorylation. Total cell lysates of PMC42 cells were subjected to immunoprecipitation with preimmune IgG (lanes 1–3) or anti-hTEP1 antibody (lanes 4–8). The supernatants (S), wash fractions (W), and immunoprecipitates (IP) were then analyzed for telomerase activity. Some immunoprecipitates were additionally subjected to further incubation with (lane 8) or without (lane 7) PP2A (20 units) at 30 °C for 5 min, followed by termination of the reaction with NaF before the telomerase activity assay. B, hTEP1 is phosphorylated by PKCα and dephosphorylated by PP2A. Human breast cancer PMC42 cells were treated with the PKC activator PMA (200 nM; lanes 3–5) or diluent (lanes 1 and 2) for 2 h, and the intracellular ATP pool was labeled with 32P by inclusion of the radioisotope (0.5 mCi/1 × 10⁶ cells) in a phosphate-free medium. After extensive washing, the cells were lysed for immunoprecipitation with control IgG (lane 1) or anti-hTEP1 antibody (lanes 2–4). The immunoprecipitates were then subjected to incubation with (lanes 4 and 5) or without (lanes 1–3) PP2A (20 units) at 30 °C for 5 min, followed by termination of the reaction with NaF. After the dephosphorylation was terminated, the sample in lane 5 was subjected to further incubation with PKCα in phosphorylation buffer at 30 °C for 10 min, which was then terminated by adding SDS sample buffer and freezing the sample in liquid nitrogen. All samples were then subjected to SDS-PAGE followed by autoradiography (upper panel) and immunoblotting (lower panel). C, immunoblotting and co-immunoprecipitation of hTERT and hTEP1. Left panel, immunoblot detection of hTERT from different amounts of total nuclear lysates. The amounts of proteins loaded in each lane are indicated (lanes 1–3). Right panel, immunoblot detection of hTERT from immunoprecipitates obtained with the antibody against hTERT (lanes 4 and 5) or hTEP1 (lane 6), showing co-immunoprecipitation of hTERT and hTEP1. D, phosphorylation of hTERT in intact cells. Shown is an autoradiograph showing phosphorylation of proteins obtained by immunoprecipitation with control IgG (lanes 1 and 4) or the antibody against hTERT (lanes 2, 3, 5, and 6). Cells were labeled with 32P for 2 h in the presence (lanes 4–6) or absence (lanes 1–3) of the protein kinase C activator PMA (200 nM), and the total cell lysates were then subjected to immunoprecipitation. The immunoprecipitates were treated with (lanes 3 and 6) or without (lanes 1, 2, 4, and 5) purified PP2A (5 units) for 5 min at 30 °C before being subjected to SDS-PAGE followed by autoradiography (upper panel) and immunoblotting (lower panel).
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RESULTS AND DISCUSSION

Inappropriate telomerase activity is associated with a variety of human cancers (3). Our previous study showed that the activity of telomerase can be switched off at the enzyme level by PP2A-mediated protein dephosphorylation in the nuclei of human breast cancer cells (25). To further explore the mechanisms of regulation of telomerase activity by protein phosphorylation, telomerase was partially purified by hTEP1 peptide affinity chromatography, and protein kinase(s) potentially interacting with the hTEP1 peptide were analyzed by immunoblotting. As shown in Fig. 1A, although some telomerase passed straight through the peptide affinity column, after extensive washing (five times the column bed volume), a significant amount of telomerase activity could be eluted from the column in particular fractions, with the major peak corresponding to the KCl concentrations of ~120–150 mM. In contrast, a small amount of telomerase activity was eluted in these fractions from a column packed with a scrambled peptide (data not shown). These findings suggest that a functional telomerase complex is selectively retained in the hTEP1 peptide affinity chromatography column, probably through complex interactions involving multiple contacts between the telomerase complex and the hTEP1 peptide. This speculation is supported by the findings that a substantial amount of immunoreactive hTEP1 was coeluted with telomerase activity from the column (data not shown) and that immunoprecipitated hTEP1 formed multiple oligomers under particular conditions (data not shown). To identify protein kinase(s) putatively involved in regulating telomerase, column fractions were probed with various antibodies against different protein kinases. Interestingly, although no evidence for the presence of Cdk2 kinase, casein kinase IIα, mitogen-activated protein kinase, PKCβ1, PKCα, or PKCγ was found (data not shown), significant levels of immunoreactive PKCα were shown by Western blot analysis in some particular fractions from the low pH glycine elution (Fig. 1B). As a control for specificity, no PKCα was similarly eluted from the column in which beads were packed with a scrambled peptide (Fig. 1C). These data suggest that PKCα interacts with the hTEP1 peptide with a reasonably high affinity and a possible involvement of the kinase in modulating the structure and function of telomerase. Since the structure of hTEP1 is similar to that of Rack1, a receptor for activated PKC (29), in having a WD-40 repeat domain, it is tempting to speculate that the WD-40 repeats in hTEP1 are also involved in mediating PKC binding.

To explore whether telomerase proteins are phosphorylated by PKC, hTEP1 was immunoprecipitated from cultured human breast cancer cells prelabeled with 32P. Significant levels of telomerase activity were observed in the immunoprecipitates, and the activity was completely inhibited by PP2A (Fig. 2A), consistent with previous findings that hTEP1 co-immunoprecipitates with telomerase activity (19, 20) and suggesting that phosphorylation of telomerase protein component(s) is required for enzyme activity. Immunoprecipitated hTEP1 showed a major protein with a molecular mass of ~300 kDa (data not shown). Autoradiography of immunoprecipitated hTEP1, however, showed that hTEP1 is a phosphoprotein in intact cells (Fig. 2B). Incubation of cells with the PKC activator PMA increased the phosphorylation of hTEP1 (Fig. 2B), consistent with PKC mediating the phosphorylation of hTEP1 in human breast cancer cells. To determine if hTEP1 is a direct substrate of PKC and PP2A in vitro, the immunoprecipitates were then subjected to sequential dephosphorylation and rephosphorylation. Endogenously phosphorylated proteins showed near-complete dephosphorylation in the presence of purified PP2A and, following dephosphorylation, were subsequently rephosphorylated in the presence of purified PKCa (Fig. 2B). Omission of PKCa or inclusion of the specific PKC inhibitor bisindolylmaleimide I led to no rephosphorylation (data not shown). These data suggest that hTEP1 is a phosphoprotein directly controlled by PKC in vitro and, presumably, similarly in intact human breast cancer cells. Since the rephosphorylation by PKCa was not in full, it is possible that other protein kinase(s) are potentially involved as well.

A major phosphoprotein doublet with a molecular mass between 125 and 130 kDa was also phosphorylated by PKCa and dephosphorylated by PP2A in the hTEP1 immunoprecipitates (Fig. 2B). Since telomerase activity co-immunoprecipitated with hTEP1 from these cells (Fig. 2A) and since the protein doublet was of the appropriate size, it is possible that the phosphoprotein doublet is the catalytic subunit of telomerase (hTERT) co-immunoprecipitated with hTEP1. To determine if hTEP1 and hTERT co-immunoprecipitate, crude nuclear lysates were subjected to immunoprecipitation with antibody to either hTERT or hTEP1 (Fig. 2C). Incubation of cells with the PKC activator PMA increased the phosphorylation of hTEP1 (Fig. 2B), consistent with PKC mediating the phosphorylation of hTEP1 in human breast cancer cells. The relatively small changes in partial quantification of hTERT phosphorylation in control cells and in cells treated with PMA with or without PP2A dephosphorylation in vitro. Results are the means ± S.D. of three similar experiments. Asterisks indicate p < 0.05 in comparison with PP2A-un-treated groups (Student’s t test). ir-hTEP1, immunoreactive hTEP1.
the dephosphorylation of immunoprecipitated hTERT by PP2A could be due to the binding of anti-hTERT antibody or an involvement of other factors. The identities of the phosphoproteins at the top of the gel and with a molecular mass of ~43 kDa are under further investigation, although the phosphoproteins at the top of the gel could be co-immunoprecipitated hTEP1. These data also suggest that the phosphorylation and dephosphorylation of both hTERT and hTEP1 may thereby regulate the function of the telomerase complex in human breast cancer cells.

To determine roles for PKCa-mediated phosphorylation of telomerase proteins, the activity of partially purified telomerase proteins, the activity of partially purified nuclear telomerase by PKCa-mediated protein phosphorylation. Partially purified telomerase with relatively low activity from the nuclei of PMC42 cells (lanes 1 and 2) was incubated with PP2A (2 units; lane 3), a phosphorylation mixture (containing 10 μM PMA, 40 μg/ml phosphatidylserine (PS), and 40 μM ATP) (lane 4), or the phosphorylation mixture plus different concentrations of PKCa as indicated (lanes 5–10) for 10 min at 30 °C. Telomerase activity was then analyzed using the TRAP assay. Results are from one of four similar experiments. B, inhibition of partially purified telomerase from the peptide affinity chromatography column by alkaline phosphatase (ALP) and PP2A. Partially purified telomerase in fraction 5 from the peptide affinity chromatography column was subjected to alkaline phosphatase or PP2A dephosphorylation for 5 min at 30 °C, followed directly by NaF termination and then by telomerase activity analysis. The concentrations of the phosphatases used are indicated. One unit is equivalent to the release of 1 pmol of phosphate/min from 15 μmol of phosphorylase at 30 °C. B stands for buffers only (lane 1). C, PKCa reactivation of PP2A-inhibited telomerase. Partially purified telomerase in fraction 5 from the peptide affinity chromatography column was incubated with (lanes 2–7) or without (lane 1) PP2A (20 units) at 30 °C for 5 min. After the reaction was terminated, the samples in lanes 3–7 were further incubated with different concentrations of purified PKCa in a phosphorylation environment containing PMA (10 μM), phosphatidyserine (40 μg/ml), and ATP (40 μM) at 30 °C for 10 min, which was then followed by the telomerase activity assay (25). Lane 8 shows the telomerase activity as in lane 1 treated with RNase A as a control. D, reactivation of PP2A-inhibited telomerase from the nuclear extracts of PMC42 cells by different PKC isoforms. Total nuclear extracts (lanes 3 and 4) were treated with PP2A (lanes 5–18), and after termination with NaF, the samples in lanes 7–18 were further incubated with increasing concentrations (1, 10, and 100 nM) of different PKC isoforms, as indicated, before being subjected to the telomerase activity assay (25). B means buffers only (lanes 1 and 2). Results are from one of two to three similar experiments. Ctrl, control.
alse was analyzed following differential incubations with different protein kinases, including PKC isoforms, DNA-dependent protein kinase, Cdc2 kinase, and the catalytic subunit of protein kinase A. Although DNA-dependent protein kinase, Cdc2 kinase, and the catalytic subunit of protein kinase A had no effect (data not shown), incubation of the partially purified telomerase having relatively low activity with PKCo resulted in marked increases in telomerase activity. A dramatic increase in telomerase activity was observed at a PKCo concentration as low as \(10^{-13} \text{M}\) (Fig. 3A, lane 4 versus lane 5), and the increases were in a dose-dependent fashion with maximal effects at a concentration of \(10^{-11} \text{M}\) (Fig. 3A). This stimulatory effect was not seen for PKC activators alone (Fig. 2A, PS) or for PKCo in the absence of its activators (data not shown), suggesting that the phosphorylation activity of PKCo is required for the activation of telomerase.

In addition to stimulating the basal activity of partially purified telomerase, PKCo also completely reversed the inhibition of telomerase activity by PP2A. PP2A inhibited the activities of immunoprecipitated (Fig. 2A) and partially purified (Fig. 3B) telomerase, a concentration-dependent inhibition also found with the nonspecific alkaline phosphatase (Fig. 3B). The telomerase inhibited by PP2A could, however, be completely reactivated by PKCo over a range of concentrations (Fig. 3C), with a maximal effect at \(10^{-10} \text{M}\). Thus, the reversible phosphorylation and dephosphorylation of hTERT and hTEP1 as seen above were paralleled by the reversible increase and decrease in telomerase activity induced by PKCo and PP2A, respectively.

The finding that telomerase can be reversibly stimulated by PKCo and inhibited by PP2A strongly argues that the telomerase ribonucleoprotein complex exists in two structural conformations (phosphorylated and dephosphorylated) that are associated with high or low telomerase activities, structurally and functionally distinct forms of telomerase that can be switched to and fro by PKCo and PP2A. Increases in telomerase activity are paralleled by increased expression of the telomerase catalytic subunit hTERT (8, 9), in addition to the PKCo-mediated phosphorylation as shown here. It is thus likely that, during the process of telomerase activation in human breast cancer cells, the formation of a functionally active enzyme complex with newly produced hTERT and other telomerase components requires PKCo-induced phosphorylation of both hTERT and hTEP1.

Although the phosphorylation sites on hTERT and hTEP1 have yet to be determined, the interactions between PKCo and telomerase proteins appear to be mediated by direct binding of the kinase to the telomerase complex through regions other than the C1 and C2 domains of PKCo, as demonstrated by testing other PKCo isoforms for their effects on telomerase activity. Both the calcium-independent PKCo and PKCe and the atypical diacylglycerol-independent PKCo stimulated telomerase activity (Fig. 3D). This suggests that, although all PKCo isoforms possess the potential to regulate telomerase activity, only PKCo is associated with partially purified telomerase in the nuclei of human breast cancer cells, evidence for a specific regulatory role for PKCo in telomerase activity in these cells. Consistent with the observation for PKCo, all PKCo isoforms at concentrations \(>10^{-8} \text{M}\) caused an inhibition of telomerase activity (Fig. 3D), suggesting a complex interaction between telomerase and PKC. Since PKCo, PKCe, and PKCo possess no C2 domain and PKCo contains a half-C1 domain (30, 31), these data also suggest that intact C1 and C2 domains are not essential for regulating telomerase activity. Thus, interactions between PKC and the telomerase proteins appear to involve regions other than the C1 and C2 domains of PKC. Since previous studies have shown that PKCo inhibitors inhibit telomerase activity during T cell activation (32) and in cultured nasopharyngeal cancer cells (33) and that the PP2A inhibitor okadaic acid stimulates telomerase activity in cultured human breast cancer cells (25), it is possible that telomerase activity is dynamically maintained by a balance of the activities between PKCo and PP2A through phosphorylation and dephosphorylation of the telomerase proteins hTERT and hTEP1.

Taken together, these data establish a pathway whereby telomerase is activated in human cancer cells and implicate PKCo as playing a pivotal role in controlling telomerase activity in cultured human breast cancer cells. Given that expression of PKCo is markedly increased in the nuclei of human breast cancer cells, with a 30-fold increase in PKCo expression and a 10-fold increase in kinase activity (34–36), the connection between PKCo and telomerase suggests a possible mechanism whereby PKCo may be involved in tumor development (37). In addition, the reversible regulation of telomerase by PKCo and PP2A is also consistent with the hypothesis that a balance between activation of PKC (36, 37) and inhibition of PP2A (38) plays an important part in tumorigenesis. Finally, these findings provide a testable model for studying roles of telomerase in intact cancer cells and a potential therapeutic window for altering telomerase activity in anticancer therapy.

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