Akt Protein Kinase Enhances Human Telomerase Activity through Phosphorylation of Telomerase Reverse Transcriptase Subunit*

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With the amino acid sequences of all reported Akt kinase physiological substrates, the possible Akt kinase substrate specificity has been suggested. The serine/threonine residue to be phosphorylated in these proteins is placed within stretches of amino acids with homology, and the arginine residues on the −5 and −3 positions and a hydrophobic amino acid on the +2 position are conserved relative to those of serine/threonine residues (XXXRRXXS/TXX). We noticed two putative Akt kinase phosphorylation sites (220GARRGGSSAS229) and (817AVRIRGKSYV826) in human telomerase reverse transcriptase (hTERT) subunit. To demonstrate that hTERT is an Akt kinase substrate protein, we performed the nonradioactive protein kinase assay with the fluorescein hTERT peptide (817AVRIRGKSYV826). We observed the phosphorylation of hTERT peptide by the human melanoma cell lysate or the activated recombinant Akt kinase proteins in vitro. With the treatment of the growth factor deprivation or okadaic acid, we also observed the up-regulation of both hTERT peptide phosphorylation and the telomerase activity. We noticed that Wortmannin down-regulates hTERT peptide phosphorylation and telomerase activity together. In addition, we observed the enhancement of telomerase activity with the pretreatment of Akt kinase in vitro. Thus, these observations suggest that Akt kinase enhances human telomerase activity through phosphorylation of hTERT subunit as one of its substrate proteins.

Akt protein kinase (a serine/threonine kinase, also called protein kinase B or Akt kinase) was identified at first as an oncogene because of its transformation ability of normal cells (1, 2). However, the role of Akt kinase was characterized as the anti-apoptosis protein, which protects the cell death induced by growth factor withdrawal and prompts the cell proliferation or survival (3–6). Moreover, with cDNA sequence homology search, the pleckstrin homology domain in Akt was identified (7). The recognition of pleckstrin homology domain, which binds to membrane with the phosphatidylinositol-3,4-diphosphate activation, provided a clue to explain the linkage between the activation of phosphatidylinositol 3-OH (PI3)1 kinase and Akt kinase activity. Furthermore, it was characterized that phosphatidylinositol-3,4-diphosphate localizes Akt kinase to membrane and alters its conformation, activating its kinase activity by phosphorylation of two different kinases, PI3-dependent protein kinase 1 phosphorylates Thr308 in the activation loop of the kinase domain of protein kinase B, and PI3-dependent protein kinase 2 phosphorylates Ser473 near the carboxyl terminus of protein kinase B (7–9). The release of Akt from membrane was also known to be a key regulatory step of Akt kinase. It was proposed that inositol triphosphate, presumably generated from phosphatidylinositol-4,5-diphosphate by phospholipase C-γ, could release pleckstrin homology domain-containing proteins from membrane (8–10). After releasing from membrane, the activated Akt kinase would become available to phosphorylate its downstream targets until inactivated by protein phosphatase 2A (PP2A). Therefore, this evidence indicated that Akt kinase is a key effector of PI3 kinase signal pathway, and the identification of Akt kinase authentic substrates provides clues to understand how PI3 kinase signal pathway contributes for the cell survival.

The first identified physiological substrate of Akt kinase was the glycogen synthase kinase 3 (GSK3) α and β and the heart isoenzyme 6-phosphofructo-2-kinase (10, 11). Phosphorylation of GSK3 by Akt kinase was reported to stimulate glycogen synthesis with the inactivation of GSK3 kinase and to affect other aspects of cellular function (11). It was proposed that GSK3 plays a role in the regulation of protein synthesis, modulation of transcription factors (AP-1 and cAMP response element-binding proteins), the cell fate determination (in Droshpila), and dorsal-ventral patterning (in Xenopus). Other researchers also suggested that Akt is upstream of the p70 ribosomal protein S6 kinase, but the connection is assumed to be indirect (11, 12). Recently, the inactivation of pro-apoptotic proteins (such as BAD protein or caspase-9) by Akt kinase phosphorylation was demonstrated (13–15). Besides BAD protein and caspase-9, H2B histone was also identified as an Akt kinase authentic substrate (16). From all reported Akt kinase substrate amino acid sequences, including BAD, PEK2, GSK3, H2B and caspase-9, the putative Akt kinase substrate consensus sequence was identified (10–16). The consensus sequence is conserved within the amino acid stretches; the arginine residues at positions −5 and −3 were positioned relative to those of serine/threonine residues to be phosphorylated by Akt kinase in these proteins (XXXRRXXS/TXX; the underline is a hydrophobic amino acid).

In our previous observation, we identified that human telomerase activity in SK-MEL 28 (a melanoma cell line) cells was enhanced in the serum-free condition without any activity change of tyrosine kinase (17). Even though the mechanism by human telomerase reverse transcriptase; DMEM, Dulbecco’s modified essential medium; FBS, fetal bovine serum; PKC, protein kinase C; GST, glutathione S-transferase.
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which telomerase is regulated in a cell is not yet established, several researchers reported that telomerase activity is effectively inhibited with the serine/threonine kinase inhibitors than the tyrosine kinase inhibitors (18, 19). Other researchers also observed that the incubation of cell nuclear telomerase extracts with PP2A, inactivating the active Akt kinase with its dephosphorylation activity, abolished the telomerase activity. Thus, they suggested that the telomerase activity is regulated by the serine/threonine phosphorylation (9, 19). Moreover, it was reported that PI3 kinase plays a role for the induction of telomerase activity in B cells (20). Therefore, we speculated that human telomerase is regulated by Akt kinase in PI3 kinase pathway for the cell proliferation or survival.

To demonstrate our speculation, we inspected human telomerase amino acid sequences with the putative Akt kinase substrate consensus sequence. Two putative Akt kinase phosphorylation sites (all serine residues) (220)GARRRGGSAS(229) and (817)AVRIRGKSYY(826) in human telomerase reverse transcriptase (hTERT, telomerase catalytic subunit), not in human telomerase associate protein subunit, have been identified (21, 22). We observed the phosphorylation of the fluorescein-labeled hTERT peptide (817)AVRIRGKSYY(826) by the human melanoma cell lysate or the activated Akt kinase in vitro. In addition, we observed the up-regulation of hTERT phosphorylation and the telomerase activity in a human melanoma cell line with the treatment of okadaic acid (a PP2A inhibitor) or the growth factor deprivation. With the treatment of wortmannin (a specific PI3-Akt kinase inhibitor), however, we noticed the down-regulation of hTERT phosphorylation and the telomerase activity. Moreover the enhancement of telomerase activity with the pretreatment of Akt kinase in vitro was also observed. Therefore, these observations strongly suggest that Akt kinase activates human telomerase through hTERT phosphorylation, as one of Akt kinase target proteins.

EXPERIMENTAL PROCEDURES

Cell Culture—SK-MEL 28 cell (a human melanoma cell line) was purchased from ATCC (Manassas, VA). Media and supplements were obtained from Life Technologies, Inc. The cell line was maintained in Dulbecco’s modified essential medium (DMEM) containing 10% heat-inactivated (30 min at 56 °C) fetal bovine serum (FBS), 10 units of potassium penicillin/ml, 100 μg of streptomycin/ml, 2 mM glutamine, and 20 mM sodium bicarbonate. The cells were incubated at 5% CO2, 95% humidity, and 37 °C chamber. The growth media was changed every 3 days.

Akt Protein Kinase Assay—Akt kinase assay was performed with the protocol provided by Promega (Madison, Wisconsin) PepTag nonradioactive protein kinase C (PKC) assay system, except for the substrate peptides. For Akt kinase substrates, the fluorescein (fluorescein isothiocyanate) was conjugated on the amino terminus of peptide H2B histone (39RKRISRKEEYS) (20) and hTERT (AVRIRGKSYY) (21) oligopeptides were purchased from Peptor Co. (Daejun, Korea). 5 μg of fluorescein in oligopeptide was incubated with 10 μl of differential treated cell lysates or the activated Akt kinase in 20 μl of protein kinase reaction mixture (20 mM HEPES, pH 7.2, 10 mM MgCl2, 10 mM MnCl2, 1 mM dithiothreitol, 0.2 mM EDTA, 20 μM ATP, 5 μM of phosphatidylserine, protein kinase activator) at 30 °C for 30 min. The reactions were stopped by heating to 95 °C for 10 min. The phosphorylated peptide was separated on 0.8% agarose gel at 100 V for 15 min. The phosphorylated products, which gained one more negative charge, were migrated to the anode. After the gel was photographed on a transilluminator, the phosphorylated peptides were cut out from the gel and radioactivity was measured with the spectrophotometer, following the protocol provided by the assay kit manufacturer.

Activation of the Recombinant GST-Akt Kinase Protein—Akt kinase-negative beads and PP2A were purchased from Upstate Biotechnology Inc. (Lake Placid, NY). Akt kinase was activated with the protocol provided by the manufacturer. SK-MEL 28 cells (107) grown in the serum-free condition for 24 h were lysed with radioimmune precipitation buffer lysate buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM sodium orthovanadate, 100 μg/ml phenylmethylsulfonyl fluoride, 1 μg of aprotinin). The cell lysate was preincubated with GST-agarose beads. Akt kinase-agarose beads (20 μg) were incubated with 50 μl of preincubated cell lysate and protein kinase assay buffer (20 mM HEPES, pH 7.2, 10 mM MgCl2, 10 mM MnCl2, 1 mM dithiothreitol, 0.2 mM EDTA, 20 μM ATP, 5 μg of phosphatidylserine, protein kinase activator) in a final volume 100 μl at 30 °C. The beads were precipitated and washed three times with the excess cell lysis buffer. The final pellet was used for the Akt kinase assay.

Telomerase Assay—SK-MEL 28 cells (1 × 106) were plated onto a 100-mm plate and grown in 10% FBS DMEM for 3 days. When the cell confluence became 70%, media were changed with 10% FBS DMEM or DMEM with out serum for the control experiment. For the other set of plates containing 70% confluent cells, media were changed with 10% FBS DMEM containing wortmannin or okadaic acid (Calbiochem-Novabiochem). After the plates were incubated for the telomerase assay, the cells were harvested, and the cell number was counted. The counted cells were centrifuged at 3000 × g for 10 min at 4 °C. For the each reaction, 2 × 104 cells were transferred into a fresh Eppendorf tube. The telomerase reaction mixture with the cell extract (corresponding to 5 × 103 cells or 2 μg of protein) was amplified by the telomeric repeat amplification protocol (17, 23). For the pretreatment experiment, we used the activated recombinant Akt kinase, the rat brain PKC (Promega), or PP2A. 20 μl of the reaction mixture (12 μl of SK-MEL 28 cell lysate, 10 ng of enzyme, and 4 μl of 5× assay buffer) was incubated at 30 °C for 30 min. 5 μl of the pretreatment reaction mixture was used for the telomerase assay. The assay procedure of telomerase followed the protocol provided by the telomerase polymerase chain reaction enzyme-linked immunosorbent assay manufacturer, Roche Molecular Biochemicals. The optical density of the samples was measured with a microtiter plate reader at 450 nm within 30 min after addition of the stop solution. Alternatively, 12% nondenatured acrylamide gel electrophoresis was performed with 20 μl of polymerase chain reaction product and transferred on nitrocellulose paper. The biotinylated telomerase product was probed with the streptavidin-peroxidase conjugate (Amersham Pharmacia Biotech) following the assay procedure of the manufacturer.

RESULTS

The Enhancement of Akt Kinase and Telomerase Activities in SK-MEL 28 Cells with the Treatment of Growth Factor Deprivation—We observed that the telomerase activity in SK-MEL 28 cells (human melanoma cell line) is 2-fold increased in the serum-free medium for 24 h of treatment (Fig. 1). Because many cellular protein activities are enhanced by phosphorylation, we speculated that the enhancement of telomerase activity in serum-free conditions is also because of its phosphorylation by certain protein kinase(s), resulting in the activation of telomerase. To determine which protein kinase is responsible for the enhancement of telomerase activity in the growth factor deprivation condition, we assayed the telomerase activity from the cells treated with several protein kinase inhibitors. We observed that bisindolylmaleimide (a serine/threonine kinase inhibitor) inhibited the telomerase activity effectively in a time- and dose-dependent manner, whereas genistein (a tyrosine kinase inhibitor) did not efficiently inhibit the telomerase activity (17, 18). Moreover, we observed that the tyrosine protein kinase activity is not enhanced with the treatment of growth factor deprivation (data not shown). Thus, we assumed that the increase of telomerase activity in the serum-free condition is because of the serine/threonine kinase(s). Recently, other researchers also suggested that activation of Akt kinase is one mechanism to explain how PI3 kinase can mediate survival of H19-7 cells during serum deprivation or differentiation (5). Therefore, based on this evidence, we speculated that the activation of Akt kinase in SK-MEL 28 cells with the growth factor deprivation enhances the telomerase activity. If Akt kinase is activated, we used the nonradioactive protein kinase assay method with the fluorescein H2B, histone peptide (39RKRISRKEEYS) (39), which is known as an Akt kinase substrate (13). As shown Fig. 1, Akt kinase activity was up-regulated with the treatment of growth factor deprivation in a time-dependent manner. This result led us next to examine whether hTERT subunit or human telom-
The Regulation of Human Telomerase Activity and hTERT Phosphorylation with Drugs Affecting Akt Kinase Activity—For the further demonstration that Akt kinase regulates the telomerase activity as its substrate, we used wortmannin and okadaic acid to modulate Akt kinase activity. It was reported that wortmannin is a phosphatidylinositol-Akt kinase pathway inhibitor, resulting in the specific Akt kinase inactivation (8, 9, 11). To address that wortmannin is a phosphatidylinositol-Akt kinase pathway inhibitor, we used the activated Akt kinase. GST-Akt recombinant kinase expressed in Escherichia coli was activated with human melanoma cell lysate. With the increase of assay time, the amount of phosphorylated hTERT peptide was increased (Fig. 2A). As shown in Fig. 2B, the increase of hTERT peptide phosphorylation was also observed with the increase of the activated Akt kinase concentration. For the control experiment, we used the inactive recombinant GST-Akt kinase protein, which has no kinase activity (Fig. 2, A and B). Furthermore, we observed that the other hTERT synthetic peptide (220 GARRGGAS 229) and GST-hTERT 870–883 recombinant protein, which contains the putative phosphorylation site (817AVRIRGKSYY826), were phosphorylated by the activated Akt kinase (data not shown). Thus, these results demonstrated that Akt kinase phosphorylates hTERT as one of its target proteins.

The Phosphorylation of hTERT Subunit by Akt Kinase—Inspecting the amino acid sequences of all known Akt kinase substrates (H2B, BAD, PFK2, GSK3, and caspase-9), the serine residue is found within stretches of amino acids with homology (Table I). The arginine residues at positions −2, −3, and −5 and the hydrophobic amino acid at the +2 position are conserved relative to the serine/threonine residues. It was reported that wortmannin is a phosphatidylinositol-Akt kinase pathway inhibitor, resulting in the specific Akt kinase inactivation (8, 9, 11). We assayed the telomerase activity of human melanoma cell line with wortmannin treatment (0, 50, 100 nM for 2 h treatment). As shown in Fig. 3A, wortmannin inhibited human telomerase activity in a dose-dependent manner. On the other hand, okadaic acid, a PP2A inhibitor, is known to activate Akt kinase with its phosphorylation (8, 9, 11). To address that the sequences (XXRXXSSSTXX) were not found in human telomerase-associated protein subunit that contains several PKC recognition sites (24).

With the fluorescein hTERT (817AVRIRGKSYY826) peptide, we observed that the hTERT peptide was phosphorylated by the human melanoma cell lysate, which contains Akt kinase activity (Fig. 1). To further determine that the serine residue (817AVRIRGKSYY826) in hTERT is phosphorylated by Akt kinase, we used the activated Akt kinase. GST-Akt recombinant kinase expressed in Escherichia coli was activated with human melanoma cell lysate. With the increase of assay time, the amount of phosphorylated hTERT peptide was increased (Fig. 2A). As shown in Fig. 2B, the increase of hTERT peptide phosphorylation was also observed with the increase of the activated Akt kinase concentration. For the control experiment, we used the inactive recombinant GST-Akt kinase protein, which has no kinase activity (Fig. 2, A and B). Furthermore, we observed that the other hTERT synthetic peptide (220 GARRGGAS 229) and GST-hTERT 870–883 recombinant protein, which contains the putative phosphorylation site (817AVRIRGKSYY826), were phosphorylated by the activated Akt kinase (data not shown). Thus, these results demonstrated that Akt kinase phosphorylates hTERT as one of its target proteins.

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Akt Kinase Phosphorylates hTERT

**FIG. 2.** Phosphorylation of hTERT peptide by Akt kinase. To demonstrate that Akt kinase phosphorylates the fluorescein hTERT (KLVFFAEVRHRGRKSYWY) peptide (see Table 1), a protein kinase assay was performed as described in Fig. 1. With the activated recombinant Akt kinase, we observed the increase of hTERT peptide phosphorylation, depending on the reaction time (A) or the enzyme concentration (B). For the control experiment, we also used the inactivated recombinant Akt kinase. The phosphorylated product (●) and unphosphorylated substrate (□) are marked. The ratio of product to substrate (P/S) was indicated below.

**FIG. 3.** Akt kinase regulates human telomerase activity with hTERT phosphorylation. To determine whether human telomerase activity is regulated by Akt kinase activity, telomerase activity in human melanoma cell line with the treatment of wortmannin or okadaic acid was observed. Akt kinase or telomerase activity assay was performed with the protocol as described in Fig. 1. With the treatment of Wortmannin, telomerase activity was down-regulated, whereas it was up-regulated with the treatment of okadaic acid in a dose-dependent manner (A). The values are the mean of three replicates. Each bar indicates the S.E. The positive control was 293 cell lysate, provided by the manufacturer. The negative control was heat-treated (65 °C, 10 min) SK-MEL 28 cell lysate.

**FIG. 4.** The activation of telomerase with Akt kinase. The telomerase activity of human melanoma cell lysate after the pretreatment with 10 ng of the activated Akt kinase, rat brain PKC, or PP2A was measured as described in Fig. 3. Human telomerase activity was enhanced with Akt kinase or PKC, whereas it was inhibited with PP2A. The positive control was 293 cell lysate, provided by the manufacturer. Negative control was heat-treated (65 °C, 10 min) SK-MEL 28 cell lysate.

The activation or inactivation of Akt kinase in the cell is one of the critical regulatory points to deliver either a survival or an apoptotic signal. Thus, the upstream signal transduction pathway in which Akt kinase is regulated in the cell becomes a research area interested intensively (8–11). To understand how Akt kinase function in the PI3 kinase pathway contributes to the cell proliferation/survival, the identification of substrate

**DISCUSSION**

The activation or inactivation of Akt kinase in the cell is one of the critical regulatory points to deliver either a survival or an apoptotic signal. Thus, the upstream signal transduction pathway in which Akt kinase is regulated in the cell becomes a research area interested intensively (8–11). To understand how Akt kinase function in the PI3 kinase pathway contributes to the cell proliferation/survival, the identification of substrate
protein that is phosphorylated by Akt kinase and the characterization of how Akt kinase phosphorylation modulates the protein function (either activation or inhibition) also seem to be important.

In this article, we demonstrated that hTERT is one of Akt kinase authentic substrate proteins with the nonradioactive Akt kinase assay method, and human telomerase activity is enhanced through hTERT phosphorylation by Akt kinase. With the growth factor deprivation, the up-regulation of human telomerase activity was observed (Fig. 1). However, any increase of tyrosine kinase activity was not observed in the growth factor-free condition, except Akt kinase. Thus, we speculated that Akt kinase, which was induced to resist apoptosis with the treatment of growth factor deprivation, also enhances human telomerase activity, and inspected human telomerase amino acid sequences with the putative Akt kinase substrate consensus sequence (XXRXRXS/TXX; the underline represents a hydrophobic amino acid). We noticed two putative Akt kinase phosphorylation sites (220GARRGGSAS229 and 817AVRIRGKSY826) in hTERT (21). To demonstrate whether hTERT is phosphorylated by Akt kinase, we used the fluorescein hTERT peptide (817AVRIRGKSY826) as a substrate for the human melanoma cell lysate or the activated Akt kinase protein. As shown in Figs. 1, 2, and 3, both the positive control substrate, H2B peptide (80RKRSRKESYS89) and hTERT peptide were phosphorylated by the activated Akt kinase and the cell lysate, respectively. In addition, we observed the down-regulation of the telomerase activity and hTERT phosphorylation with the treatment of wortmannin, a specific Akt kinase inhibitor from fructo-2-kinase, and GLUT4, which is highly conserved as Akt kinase substrate consensus sequences (XXRXRXS/TXX; the underline is a hydrophobic amino acid), we noticed several possible Akt kinase substrate proteins, including the apoptosis-related proteins, RIP-like kinase (30), and caspase-7 (31). Recently, it was reported that caspase-9 is inactivated by Akt kinase phosphorylation for the cell survival or proliferation (15). Thus, we speculate that RIP-like kinase and caspase-7 activities seem to be inactivated by Akt kinase phosphorylation, similar to the regulation mechanism of caspase-9. Moreover, we noticed the consensus sequence in the several GTPase proteins, including CDC42 and rac1 (32). These consensus sequences are also noticed in human IκB-related protein (33) and MEKK3 (34). Even though the relationships between Akt kinase and these proteins are presently unknown, Akt kinase phosphorylation sites of these proteins may contribute the signal cross-talk between two different signal pathways. Interestingly, GLUT4, a glucose transport protein, also contains the consensus Akt kinase phosphorylation site (29). We assume that because glucose is the basic energy source for the cell survival, Akt kinase is also adopted to involve the cell glucose metabolism with the phosphorylation of GSK3, 6-phosphofructo-2-kinase, and GLUT4.

In summary, the phosphorylation site of Akt kinase, which triggers a plethora of potential biological outcomes, seems to be highly conserved as Akt kinase substrate consensus sequences (XXRXRXS/TXX). Thus, the inspection of protein sequences with this consensus sequence may help to identify Akt kinase substrate protein. However, because some of its substrate proteins are activated or inactivated by Akt kinase phosphorylation for the cell proliferation/survival or the signal cross-talk, it is also necessary to determine whether Akt kinase phosphorylation activates or inactivates each Akt kinase substrate protein function. Thus, the identification of Akt kinase substrate pro-

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Recently, Akt kinase family proteins (Akt1, Akt2, and Akt3) have been characterized (26–28). Because of their amino acid sequence homology, each Akt kinase-specific function seems to be for the redundancy. Thus, we suppose that Akt2 and 3 are also the responsible kinase for the telomerase phosphorylation. However, it remains to be determined whether Akt2 and Akt3 also have the same substrate specificity. Interestingly, inspecting the amino acid sequences of Akt1, 2, and 3, we identify the consensus phosphorylation sites (TERPRPNTFY) that can explain why Akt kinase was autophosphorylated in other research data (13). Until now, two different Akt kinase and their phosphorylation sites on Akt kinase are characterized. They are PI3-dependent protein kinase 1, which phosphorylates Thr808 in the activation loop of the kinase domain of protein kinase B, and PI3-dependent protein kinase 2, which phosphorylates Ser473 near the carboxyl terminus. Thus, it is necessary to characterize how the autophosphorylation of Akt kinase on (TERPRPNTFY) regulates its function in vivo.

Several biological important proteins, including BAD protein, caspase-9, H2B histone, PEK2, and GSK3, have been identified as the substrates of Akt kinase (10–16). The arginine residues at positions −5 and −3 were conserved relative to those of serine residues to be phosphorylated in these proteins. With the replacement either −3 or −5 arginine with alanine in the Akt kinase substrate consensus sequences, the serine phosphorylation by Akt kinase was abolished completely (16).2 Thus, it seems to be that Akt kinase substrate specificity (XXRXRXS/TXX) is conserved well in the other proteins as Akt kinase substrate. Therefore, the inspection of these consensus amino acid sequences may help to determine whether these proteins are the possible Akt kinase substrate. With Akt kinase substrate consensus sequences (XXRXRXS/TXX; the underline is a hydrophobic amino acid), we noticed several possible Akt kinase substrate proteins, including the apoptosis-related proteins, RIP-like kinase (30), and caspase-7 (31). Recently, it was reported that caspase-9 is inactivated by Akt kinase phosphorylation for the cell survival or proliferation (15). Thus, we speculate that RIP-like kinase and caspase-7 activities seem to be inactivated by Akt kinase phosphorylation, similar to the regulation mechanism of caspase-9. Moreover, we noticed the consensus sequence in the several GTPase proteins, including CDC42 and rac1 (32). These consensus sequences are also noticed in human IκB-related protein (33) and MEKK3 (34). Even though the relationships between Akt kinase and these proteins are presently unknown, Akt kinase phosphorylation sites of these proteins may contribute the signal cross-talk between two different signal pathways. Interestingly, GLUT4, a glucose transport protein, also contains the consensus Akt kinase phosphorylation site (29). We assume that because glucose is the basic energy source for the cell survival, Akt kinase is also adopted to involve the cell glucose metabolism with the phosphorylation of GSK3, 6-phosphofructo-2-kinase, and GLUT4.

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2 S. S. Kang, T. Kwon, D. Y. Kwon, and S. I. Do, unpublished data.
tein and the characterization of its functional modification by Akt kinase phosphorylation may provide clues to understanding how Akt kinase functions contributes to protect the cell apoptosis or promote the cell proliferation collectively. In this article, we demonstrate that Akt kinase activates human telomerase activity, which plays an important role in keeping the telomere length for the cell proliferation through the phosphorylation of hTERT subunit, as one of Akt kinase target proteins.

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