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Regulation of telomerase activity and anti-apoptotic function by protein–protein interaction and phosphorylation

Judith Haendeler, Jörg Hoffmann, Sandy Rahman, Andreas M. Zeiher, Stefanie Dimmeler*

Molecular Cardiology, Department of Internal Medicine IV, University of Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany

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Abstract The enzyme telomerase is necessary for the synthesis and maintenance of telomere length. The catalytic subunit, telomerase reverse transcriptase (TERT), is regulated by interaction with the 90 kDa heat shock protein (HSP90) and by Akt-dependent phosphorylation. Here, we demonstrate that HSP90 and Akt physically interact with TERT. Treatment of cells with novobiocin, which blocks C-terminal interaction of HSP90, disrupted HSP90 binding to Akt, induced Akt dephosphorylation and significantly reduced telomerase activity. The reduction of TERT activity by novobiocin was associated with an increase in apoptosis. Likewise, the induction of Akt dephosphorylation by protein phosphatase 2A (PP2A) reduced telomerase activity. HSP90 is known to prevent PP2A-mediated dephosphorylation of Akt. To investigate whether the effect of novobiocin is due to the reduction of Akt or TERT phosphorylation, we overexpressed a phospho-mimetic, active Akt (T308D/S473D). Akt(T308D/S473D) prevented novobiocin-induced reduction of telomerase activity and the stimulation of apoptosis. Moreover, overexpression of a dominant negative PP2A construct (PP2A(L199P)) as well as incubation with the PP2A inhibitor okadaic acid blocked the inhibition of telomerase activity by novobiocin. These data suggest that the association between HSP90, Akt and TERT in concert with the phosphorylation of TERT is necessary for maintaining telomerase activity and inhibition of apoptosis.

Key words: Akt; Apoptosis; HSP90; PP2A; Telomerase

1. Introduction

Telomeres are the physical ends of the chromosomes. They are composed of the repeated sequence TTAGGG and are responsible for maintaining chromosomal stability and integrity [1,2]. Telomeres can function as a mitotic clock, since telomeres are progressively shortened during each cell division. Telomere shortening forces human primary cells to stop dividing, when a critical minimum telomere length is reached [3,4]. At a critical point of 7 kb of telomere length cells come into the state of cellular senescence. However, recent data by Karlseder et al. suggested that overexpression of TRF2, a telomeric DNA binding protein, increased the rate of telomere shortening in primary cells without accelerating senescence [5].

The enzyme telomerase consists of the reverse transcriptase subunit (TERT), which contains the catalytic activity of the enzyme, and the associated RNA component, which serves as the template for synthesis of the telomeric sequence [6–9]. Recent publications have underscored the importance of the catalytic subunit TERT, since introduction of TERT into human cells extended both their life-span and their telomeres to lengths typical of young cells [10–12].

The regulation of TERT involves transcriptional and post-transcriptional mechanisms [13]. Post-transcriptional activation of the enzymatic activity of TERT by phosphorylation through the kinases PKC, ERK1/2 and Akt has been shown [13–16]. Especially for endothelial cells, we demonstrated that Akt is essential for TERT activation [17]. Moreover, Li et al. have shown that in the presence of protein phosphatase 2A (PP2A), but not of protein phosphatase 1 or protein phosphatase 2B, telomerase activity is markedly inhibited in vitro, suggesting that dephosphorylation locks telomerase into an inactive conformation [18]. Binding of TERT to proteins seems also to be required for TERT assembly and activity [19]. The heat shock proteins HSP90, HSP70 and p23 have been shown to be functionally associated with TERT [19,20]. HSP90 is an abundant and highly conserved protein involved in different cellular processes. In contrast to other heat shock proteins, most of the identified targets of HSP90 are signaling proteins [21]. HSP90 acts as a chaperone for unstable signal transducers and keeps them preserved for activation and for conformational changes [21]. Geldanamycin and novobiocin are two competitive inhibitors of HSP90 by binding to the N-terminus and to the C-terminus of HSP90, respectively, and thereby interfering with the association of HSP90 with different proteins. In particular, novobiocin has been shown to interfere with Hsc70, p23 and Raf1 binding to HSP90 [22]. In a recent study, Sato et al. identified the binding site for Akt to HSP90 at residues 327–340. By binding of Akt to HSP90, HSP90 protected Akt from PP2A-mediated dephosphorylation and maintained Akt in its phosphorylated and, thereby, activated form [23].

Therefore, we investigated whether human TERT (hTERT) associates with HSP90 and Akt and whether this association is important for telomerase activity and function. Here, we report that hTERT associates with HSP90 and Akt in human embryonic kidney cells as well as in endothelial cells. This association in concert with phosphorylation of hTERT is required for telomerase activity. PP2A reduced telomerase activity, suggesting a PP2A-sensitive phosphorylation of hTERT. Moreover, protein complex formation and phosphorylation of hTERT is also necessary to inhibit apoptosis and caspase-3

*Corresponding author. Fax: (49)-69-6301 7113.
E-mail address: dimmeler@em.uni-frankfurt.de (S. Dimmeler).

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activity, suggesting that the regulation of telomerase activity and its anti-apoptotic effect is post-transcriptionally modulated by phosphorylation and the interaction with binding proteins.

2. Materials and methods

2.1. Cell culture

293 cells were maintained in DMEM containing 10% fetal calf serum as described [24].

Human umbilical vein endothelial cells (HUVEC) were obtained from newborn cords and purchased from Clonetics (Cologne, Germany) at passage 0. HUVEC were cultured in endothelial basal medium supplemented with hydrocortisone (1 μg/ml), bovine brain extract (12 μg/ml), gentamicin (50 μg/ml), amphotericin B (50 ng/ml), epidermal growth factor (10 ng/ml), and 10% fetal calf serum. After detachment with trypsin, cells were grown for at least 18 h.

2.2. Transfection

The plasmid encoding hTERT wt was a gift from Dr. Weinberg [25] and was subcloned into pcDNA3.1 myc-his (Invitrogen). The phospho-mimetic, active Akt(T308D/S473D) and the non-phosphorylatable Akt(T308A/S473A) were cloned as previously described [26]. PP2A wt was cloned out of a cDNA library (fetal liver human) and subcloned into pcDNA4 his (Invitrogen). The leucine at position 199 was mutated to proline using site-directed mutagenesis, generating the dominant negative PP2A(L199P) construct. HUVEC were transfected with 3 μg plasmid and 25 μl Superfect as described previously with a transfection efficiency of 40% obtained by counting β-galactosidase-positive cells after transfection of lacZ in pcDNA3.1 myc-his (Invitrogen) [27].

293 cells were transfected with Lipofectamine/Plus according to the manufacturer's protocol (Gibco BRL) as previously described with a transfection efficiency of 95% obtained by counting β-galactosidase-positive cells after transfection of lacZ in pcDNA3.1 myc-his (Invitrogen) [24]. The final amount of transfected DNA for a 60 mm dish was 1.3 μg DNA.

2.3. Telomerase activity measurement

Telomerase activity was measured using a commercially available polymerase chain reaction (PCR)-based assay according to the manufacturer's protocol (Roche) as described previously [28]. TRAP assays were performed using biotin-labeled TS primers (provided with the assay). After PCR amplification, PCR products were resolved on a 12% non-denaturing polyacrylamide gel. After transfer to positively charged nylon membranes, the 6 bp telomerase-specific ladder was detected using streptavidin–horseradish peroxidase and the enhanced chemiluminescence system (Amersham). The linearity of the assay was assured by the positive controls provided by the company; as a negative control H_2O was used containing the biotinylated primers.

2.4. Immunoprecipitation and immunoblotting

Cells were lysed in RIPA buffer (50 mM Tris–HCl pH 8.0, 150 mM NaCl, 1% Nonidet P40, 0.5% deoxycholic acid, 0.1% sodium dodecyl sulfate (SDS)). 300–500 μg protein of cell lysate was immunoprecipitated with 5 μg HSP90 antibody (mouse, Transduction Laboratories), 5 μg myc antibody (sc-40, mouse, Santa Cruz) or 5 μg Akt antibody (clone 5G3, mouse, Cell Signaling) overnight at 4°C. After incubation with A/G Plus agarose for 2 h at 4°C, resulting beads were washed with lysis buffer four times. Agarose beads were subjected to SDS–polyacrylamide gel electrophoresis (PAGE) sample buffer and dissolved on a 10% SDS–PAGE.

Immunoblotting was performed with antibodies directed against HSP90 (overnight, 4°C, 1:500, Transduction Laboratories), phospho-Ser473-Akt (overnight, 4°C, 1:500, Cell Signaling), myc (2 h, room temperature, 1:250, Santa Cruz), tubulin (2 h, room temperature, 1:500, Neomarkers), and Akt (overnight, 4°C, 1:500, Cell Signaling). Antibodies were detected by the enhanced chemiluminescence system (Amersham).

2.5. Detection of apoptosis

Cells were washed with phosphate-buffered saline (PBS) and fixed in 4% formaldehyde. Cells were stained with 4′,6-diamidinophenylindole (DAPI; 0.2 μg/ml in 10 mM Tris–HCl, pH 7, 10 mM EDTA, 100 mM NaCl) for 30 min. Then, cells were washed with PBS and nuclear morphology was assessed by fluorescence microscopy. Alternatively, HUVEC were cotransfected with 2.25 μg plasmid and 0.75 μg lacZ and apoptosis was detected by counting the morphological changes of the transfected β-galactosidase-positive cells.

Alternatively, detection of cell death was performed by FACS analysis using annexin V-PE binding and 7-amino-actinomycin (7AAD) staining (Pharmingen). Annexin V is a Ca^{2+}-dependent phospholipid binding protein that has a high affinity to phosphatidylserine, which is exposed on the cell surface of apoptotic cells. In contrast, 7AAD is only cell-permeable when the cell membranes are damaged, indicative of necrotic cell death. Therefore, apoptotic cells were defined as annexin V-positive, 7AAD-negative cells. In brief, cells were trypsinized from the dish and pelleted. After washing twice with annexin binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4), cell pellets were resuspended in 50 μl of annexin binding buffer and incubated with 2.5 ng/ml annexin V-PE and 2.5 ng/ml 7AAD for 20 min. The reaction was terminated by adding 250 μl of annexin binding buffer and analyzed using FACS.

2.6. Caspase activity

For detection of caspase-3 activity, HUVEC were lysed in buffer (1% Triton X-100, 0.32 M sucrose, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 2 mM dithiothreitol, 10 mM Tris–HCl, pH 8) for 15 min, 4°C, followed by centrifugation (20 000 × g, 10 min). Caspase-3 activity was detected in resulting supernatants using the fluorogenic substrate 7-amino-4-coumarin-DEVD as described previously [27].

2.7. Statistics

Statistical analysis was performed with Student's t-test or ANOVA (SPSS Software).

3. Results

3.1. HSP90 and Akt associate with TERT

First, we wanted to address whether HSP90, Akt and TERT were associated with each other. Since we were not able to immunoprecipitate endogenous hTERT with commercially available antibodies, we transfected endothelial cells with myc-tagged hTERT wt and performed an immunoprecipitation directed against HSP90, Akt or the myc tag. We found that Akt and hTERT were in complex with HSP90 (Fig. 1A and data not shown). Similar results were also obtained in 293 cells, suggesting that the complex formation is not cell-specific (data not shown).

3.2. Association of HSP90 and Akt with hTERT is required for telomerase activity

To investigate the functional role of the association between HSP90, Akt and hTERT for the activity of telomerase, we used novobiocin, a coumarin-type antibiotic. Novobiocin is a competitive inhibitor for association of HSP90 to different proteins by binding to the C-terminus [22]. Recently, we showed that the phosphoinositide 3-kinase (PI3-kinase)/Akt pathway is required for telomerase activity in endothelial cells using the pharmacological inhibitor of PI3-kinase, Ly294002 [17]. Sato et al. showed that phosphorylation of Akt is dependent on maintaining HSP90 binding to Akt. Therefore, it is tempting to speculate that novobiocin may influence the association of hTERT with Akt and HSP90. Indeed, novobiocin reduced the association of Akt and HSP90 and hTERT (Fig. 1A,B). Next, we investigated the effect of novobiocin on Akt phosphorylation and activation. As shown in Fig. 1C, treatment of endothelial cells with novobiocin reduced phosphorylation of Akt in a concentration-dependent manner. To determine whether the association of Akt, HSP90 and hTERT

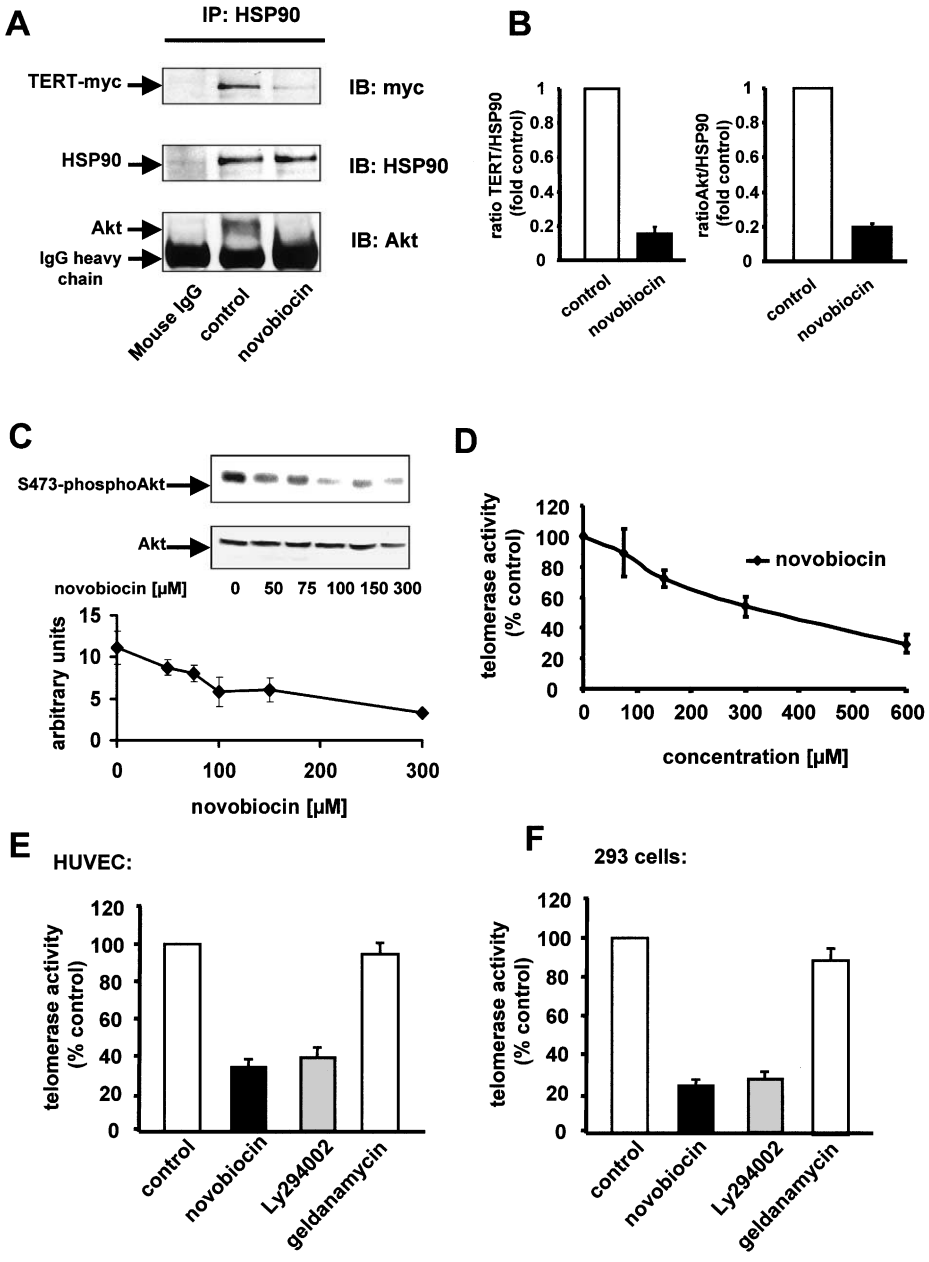

Fig. 1. A: Novobiocin disrupts the association of HSP90, hTERT wt and Akt. Endothelial cells were incubated with or without 300 μM novobiocin for 18 h and HSP90 was immunoprecipitated. The membranes were cut and the upper part was immunoblotted with anti-myc antibody (upper panel) and the lower part was immunoblotted with anti-Akt antibody (lower panel). Membranes were stripped and reprobed with an anti-HSP90 antibody (middle panel). Anti-mouse immunoglobulin was used to check the immunoprecipitation. B: Blots were quantified by scanning densitometry using the Scion Image program ($n = 6$). Data are means ± S.E.M. C: Novobiocin inhibits phosphorylation of Akt in a concentration-dependent manner. Endothelial cells were incubated with different novobiocin concentrations as indicated for 18 h. Immunoblotting was performed using an anti-S473 phospho-Akt antibody (upper panel). Membranes were stripped and reprobed with an anti-Akt antibody (middle panel). Blots were quantified by scanning densitometry using the Scion Image program ($n = 4$). Data are means ± S.E.M. (lower panel). D: Novobiocin inhibits telomerase activity. Endothelial cells were incubated for 18 h with different concentrations of novobiocin as indicated. After cell lysis, telomerase activity was measured in the resulting cell lysates ($n = 6$). Data are means ± S.E.M. E: Ly294002 and novobiocin inhibit telomerase activity to a similar extent. Endothelial cells were incubated with 10 μM Ly294002, 600 μM novobiocin or 10 μM geldanamycin for 18 h and telomerase activity was measured ($n = 6$). Data are means ± S.E.M. F: Ly294002 and novobiocin inhibit telomerase activity to a similar extent. 293 cells were incubated with 10 μM Ly294002, 600 μM novobiocin or 10 μM geldanamycin for 18 h and telomerase activity was measured ($n = 6$). Data are means ± S.E.M.

plays a role in maintaining telomerase activity, we incubated cells with novobiocin. Incubation with novobiocin reduced telomerase activity in a concentration-dependent manner with an IC_{50} of 233 μM (Fig. 1D). Taking into account that inhibition of the PI3-kinase pathway by Ly294002 reduced telomerase activity [17], we compared the effects of Ly294002 and novobiocin. As demonstrated in Fig. 1E, Ly294002 and novobiocin had comparable inhibitory effects on telomerase activity. In contrast, geldanamycin, another competitive inhibitor, which binds to the N-terminus of

HSP90 and thereby interferes with the association of HSP90 with different proteins [21], had no effect on telomerase activity in endothelial cells (Fig. 1E). Similar results for telomerase activity were obtained when 293 cells were treated with Ly294002, novobiocin or geldanamycin (Fig. 1F).

3.3. Effect of Akt and PP2A on telomerase activity

Having demonstrated that novobiocin reduced telomerase activity by inhibiting complex formation of hTERT with Akt and HSP90 and phosphorylation of Akt, we asked whether novobiocin-induced dephosphorylation and inactivation of Akt accounts for the inhibitory effect of novobiocin on telomerase activity. Therefore, we overexpressed a phospho-mimetic, active Akt construct, Akt(T308D/S473D), and a non-phosphorylatable Akt construct, Akt(T308A/S473A). It has previously been demonstrated that Akt(T308D/S473D) inhibited apoptosis in different cell types and that Akt(T308A/S473A) reduced telomerase activity [17,29]. As shown in Fig. 2A, overexpression of the active Akt(T308D/S473D) construct inhibited the novobiocin-induced reduction of telomerase activity. In contrast, the non-phosphorylatable Akt(T308A/S473A) further reduced novobiocin-induced reduction of telomerase activity in endothelial cells (Fig. 2A). These data indicate that phosphorylation of hTERT by Akt may be required for telomerase activity. Our data demonstrated that association of TERT/HSP90/Akt and phosphorylation of hTERT by Akt are important to maintain telomerase activity. However, it is not clear whether HSP90 has a direct effect on TERT or whether HSP90 keeps Akt in its active state and thereby maintains TERT phosphorylated. Because binding of HSP90 to Akt protected Akt from PP2A-mediated dephosphorylation in vitro [23], we cloned PP2A wt and the dominant negative PP2A(L199P) construct [30]. PP2A(L199P) has been shown to be catalytically impaired when transfected into yeast [30]. We found that under basal conditions overexpression of PP2A wt into 293 cells results in a significant reduction of telomerase activity compared to vector-transfected cells (vector-transfected cells: 0.875 ± 0.034 OD/mg protein, PP2A wt-transfected cells: 0.698 ± 0.041 OD/mg protein). In contrast the dominant negative construct PP2A(L199P) enhanced telomerase activity (Fig. 2B). To further delineate the effect of PP2A on the inhibitory effect of novobiocin on telomerase activity, we incubated cells overexpressing PP2A(L199P) with novobiocin. As shown in Fig. 2B, overexpression of PP2A(L199P) blocked the novobiocin effect, demonstrating that dephosphorylation by PP2A is important for maintaining telomerase activity. Of note, PP2A consists of several subunits and it has been suggested that reconstituting the holoenzyme in vivo could be difficult [30]. Therefore, we additionally treated 293 cells with okadaic acid to inhibit PP2A activation. As shown in Fig. 2B, okadaic acid completely inhibited novobiocin-induced reduction of telomerase activity.

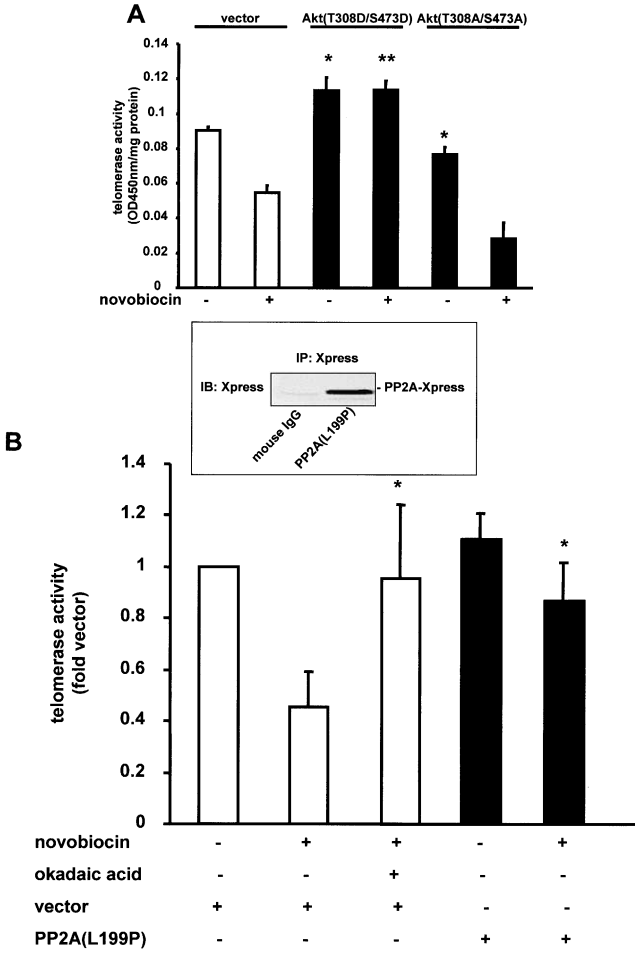

Fig. 2. A: Effects of Akt(T308D/S473D) and Akt(T308A/S473A) on novobiocin-induced reduction of telomerase activity in HUVEC. HUVEC were transfected with vector, Akt(T308D/S473D) or Akt (T308A/S473A) and incubated with 300 μM novobiocin for 18 h. After cell lysis, telomerase activity was measured in the resulting cell lysates ($n = 6$). Data are means ± S.E.M. $^{*}P < 0.05$ versus vector-transfected cells; $^{**}P < 0.05$ versus vector-transfected cells+novobiocin. B: Effect of PP2A(L199P) and okadaic acid on novobiocin-induced reduction of TERT activity. 293 cells were transfected with vector or PP2A(L199P). Cells were incubated with 300 μM novobiocin for 18 h in the presence or absence of 10 nM okadaic acid as indicated. After cell lysis, telomerase activity was measured in the resulting cell lysates ($n = 4$). Data are means ± S.E.M. $^{*}P < 0.05$ versus vector-transfected cells+novobiocin. The inset demonstrates overexpression of PP2A(L199P)-Xpress.

3.4. Novobiocin induced apoptosis and caspase activity in endothelial cells

Besides the well known function of TERT in counteracting telomere shortening, TERT has been suggested to exert anti-apoptotic functions [31,32]. To address the question whether complex formation of hTERT, HSP90 and Akt and phosphorylation are also necessary for the anti-apoptotic function of hTERT, we investigated whether novobiocin can induce apoptosis in endothelial cells and human embryonic kidney cells. Incubation with novobiocin for 18 h resulted in a concentration-dependent increase in the number of apoptotic cells as measured by FACS analysis and nuclear staining with DAPI (Fig. 3A and data not shown). The apoptosis induction by novobiocin was associated with an induction of caspase-3-like activity (Fig. 3B). Taking into account that overexpression of the active, phospho-mimetic Akt(T308D/S473D) construct restored telomerase activity after novobiocin treatment, we further investigated the effect of this construct on novobiocin-induced apoptosis. As demonstrated in Fig. 3C, overexpression of Akt(T308D/S473D) inhibited novobiocin-induced apoptosis, suggesting that the association between HSP90, Akt and hTERT in concert with the phosphorylation of TERT by Akt is necessary not only for maintaining telomerase activity, but also for inhibiting apoptosis. As expected,

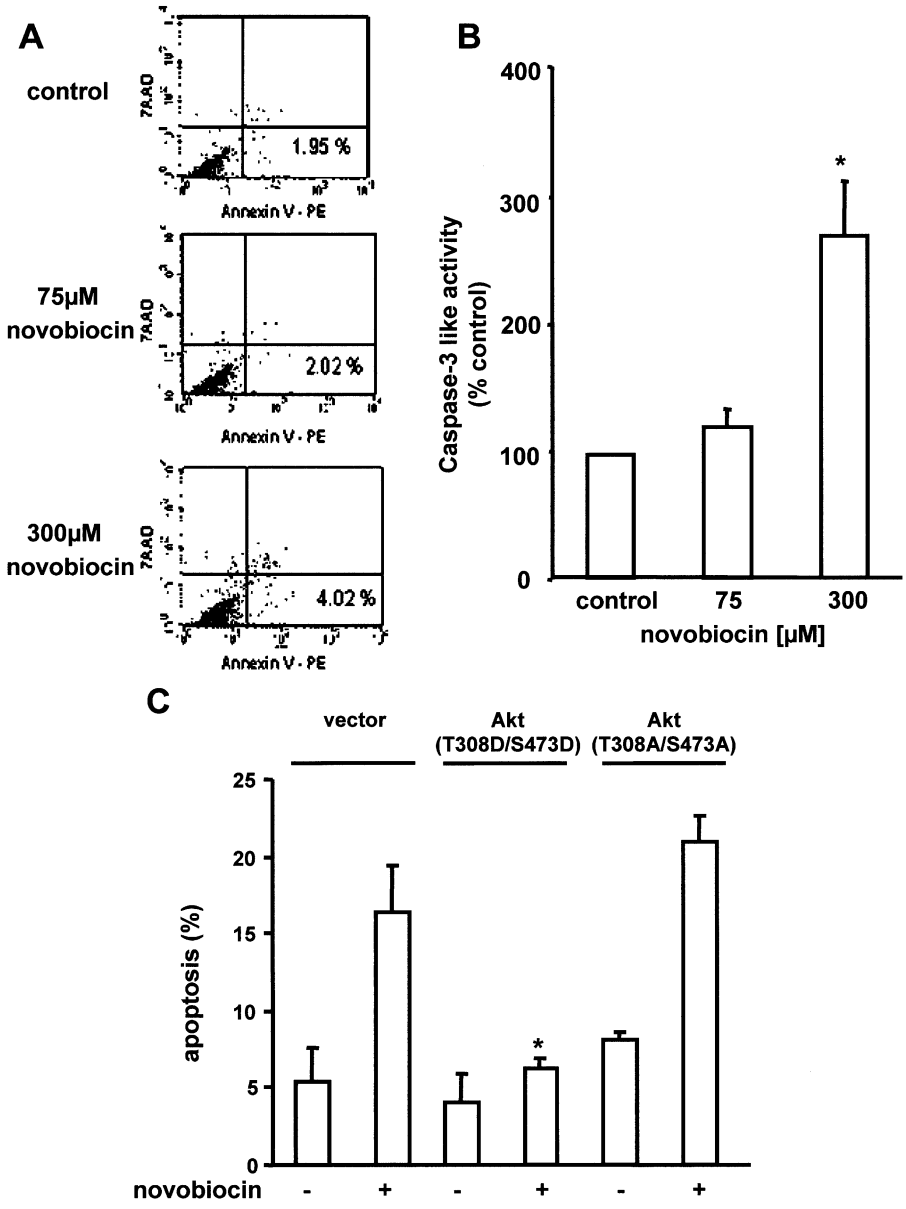

Fig. 3. A: Novobiocin induces apoptosis in endothelial cells. Endothelial cells were incubated with different novobiocin concentrations as indicated for 18 h. Apoptosis was measured by FACS analysis with annexin V-PE staining ($n=5$). A representative FACS analysis is shown. B: Novobiocin induces caspase-3-like activity in endothelial cells. Endothelial cells were incubated with different novobiocin concentrations as indicated for 18 h. Cells were lysed and caspase-3-like activity was measured as described in Section 2 ($n=3$). Data are means ± S.E.M. *$P<0.05$ versus cells treated with 75 μM novobiocin. C: Endothelial cells were cotransfected with vector and lacZ, Akt(T308D/S473D) and lacZ or Akt(T308A/S473A) and lacZ in a ratio of 3:1. After incubation with 300 μM novobiocin for 18 h, cells were stained with β-galactosidase and apoptosis was detected by counting the morphological changes of the transfected cells ($n=4$). Data are means ± S.E.M. *$P<0.01$ versus vector-transfected cells+novobiocin.

overexpression of the non-phosphorylatable construct Akt-(T308A/S473A) did not exert any inhibitory effect on apoptosis induction (Fig. 3C).

4. Discussion

The activity of the active subunit of the telomerase TERT is regulated by various post-transcriptional mechanisms including binding to heat shock proteins [13,19,20]. The present study demonstrates that TERT is associated not only with HSP90 but also with the protein kinase Akt. The binding of HSP90 to TERT was shown to be essential for the assembly of the telomerase complex [19]. Moreover, inhibition of HSP90 function in cells blocks assembly of active telomerase [19]. The underlying mechanism, however, is not fully elucidated. HSP90 is known to play a crucial role in regulation of various intracellular signaling pathways. Importantly, HSP90 prevents Akt inactivation by inhibiting dephosphorylation at Ser473 in vitro [23]. The preservation of Akt activity is mediated by interaction of Akt with HSP90 at residues 327–340, which is in close vicinity to the novobiocin binding site within HSP90. The present study now demonstrates that novobiocin leads to a dephosphorylation of Akt and inhibits Akt/HSP90 complex formation. In addition, novobiocin interfered with

the HSP90–TERT complex and potently inhibited telomerase activity. Interestingly, prevention of Akt dephosphorylation by overexpression of a phospho-mimetic Akt construct blocked novobiocin-induced inactivation of telomerase. These data would suggest that inactivation of telomerase induced by novobiocin is mainly caused by disruption of Akt–HSP90 interaction and consequent dephosphorylation and inactivation of Akt. Previous studies have shown that Akt can phosphorylate TERT and enhance its enzymatic activity in vitro [15,17].

Moreover, the data of the present study suggest that complex formation of hTERT with HSP90 and Akt in concert with hTERT phosphorylation is important for telomerase activity. Since telomerase activity is required to maintain telomere length at the end of the chromosomes in the nucleus, it is tempting to speculate that the HSP90–Akt–TERT complex may be formed in the nucleus. Preliminary data of our laboratory suggested that HSP90, Akt and TERT protein can be detected in nuclear fractions of cell lysates, underscoring the possibility of complex formation between HSP90, Akt and TERT in the nucleus (data not shown). The question remains how the complex is formed. It is tempting to speculate that after assembly of the TERT holoenzyme, which requires p23 and HSP90 [19], Akt may be recruited into the complex to phosphorylate and thereby fully activate TERT. However, further studies are required to address this question.

The dephosphorylation of Akt after disruption of the HSP90–Akt complex is mediated by PP2A in vitro [23]. Therefore, we investigated the contribution of PP2A for novobiocin-mediated TERT inactivation. Inhibition of PP2A by overexpression of a dominant negative construct prevented novobiocin-induced inactivation of TERT. In addition, PP2A wt reduced basal telomerase activity. These data are consistent with the concept that novobiocin exerts its inhibitory effects by allowing PP2A-mediated Akt inactivation. However, PP2A also markedly inhibited telomerase activity in vitro and this inhibition, also observed in human melanoma cell lysates, is mimicked by non-specific protein phosphatase alkaline phosphatase and prevented by the PP2A inhibitor okadaic acid [15,18]. Therefore, we cannot rule out that PP2A also exerts a direct effect on telomerase activity beside the inhibition of Akt-dependent phosphorylation. However, direct TERT inactivation by PP2A was only demonstrated in vitro [15,18], therefore further studies are necessary to address that point under in vivo conditions. At least, PP2A wt did not associate with hTERT wt (data not shown) and did not affect the enzymatic activity of TERT, when the phospho-mimetic Akt construct was co-transfected (data not shown).

In contrast to the study of Holt et al. [19], the inactivation of TERT was only detectable with novobiocin but not with geldanamycin, which specifically blocks N-terminal interaction of HSP90. One reason for this discrepancy could be the different experimental set-ups and possible cell type specificities. Whereas in our study endothelial cells and 293 cells were maintained under serum-containing conditions, the HT1080 cells used by Holt et al. were serum-starved for 14 days [19]. One might argue that in endothelial cells and 293 cells, HSP90 interacts via novobiocin-sensitive C-terminal interaction with Akt thereby promoting Akt-dependent phosphorylation of TERT. In contrast, direct interaction of TERT with HSP90 as described by Holt et al., which is sensitive to both geldanamycin and novobiocin, might not take place under our experimental conditions. This is in accordance with the finding that geldanamycin did not disturb TERT complex formation with HSP90 or Akt in our hands (data not shown).

Besides the well known function of TERT to elongate telomeres, TERT has also been shown to exert an anti-apoptotic function [31,32]. Thus, inhibition of TERT activity by overexpression of a dominant negative mutant or antisense oligonucleotides induced apoptosis [31–33]. Likewise, in our study inhibition of telomerase activity by novobiocin was associated with increased apoptosis of endothelial cells. Importantly, novobiocin-induced apoptosis was only prevented by overexpression of a phospho-mimetic Akt construct. These data are in accordance with the measurement of telomerase activity and support the conclusion that TERT phosphorylation renders the enzyme resistant against the inhibitory effect of novobiocin.

Taken together, our data suggest that telomerase activity and its anti-apoptotic function are regulated by association of TERT with Akt and HSP90 and consequently phosphorylation of TERT. The present study confirms previous reports that HSP90 is essential to maintain Akt in its phosphorylated and, thus, active state by preventing dephosphorylation by PP2A [23]. Thus, maintaining the association of HSP90, Akt and TERT may importantly contribute to the life-span of endothelial cells and to the integrity of the endothelial cell monolayer by protecting against apoptosis.

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