Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) induces cancer cell senescence by interacting with telomerase RNA component

Cancer Cell Senescence Induced by GAPDH. To investigate a potential role of GAPDH in telomere maintenance in breast cancer cells, we expressed recombinant GAPDH in MCF7 cells. Whereas we noted a reduced cell density in the MCF7 cell cultures stably expressing GFP–GAPDH consistent with previous findings of GAPDH-induced cancer cell death (3, 4, 7, 25, 28, 29), we found that there were significant changes in the morphology of residues and human telomerase RNA (hTERC) is composed of 451 nucleotides (15–18). Reconstitution of telomerase activity in telomerase-negative cells can be achieved by expression of hTERC, demonstrating that hTERC is the rate-limiting component of the enzyme complex (19, 20).

GAPDH has long been recognized as an important enzyme in catalyzing ATP production through the anaerobic glycolysis of a monosaccharide (generally glucose) to pyruvate in the cytosol (21, 22). Intriguingly as a gene encoding a single 38-kDa protein without alternate splicing, GAPDH displays multiple functions that are independent of its role in energy generation (reviewed in refs. 5, 22–24). A large body of evidence indicates that GAPDH responds to various stress insults by translocation to the nucleus (3–7, 25). In the nucleus, GAPDH is the key component of a gene transcriptome for cell division (6). Binding to nucleic acids, GAPDH is implicated in mediating RNA nuclear export (22), protecting mRNA from degradation through interaction with AU-rich elements (AREs) (26) and the repair of DNA (27). Moreover, studies have shown that GAPDH binds to telomeres directly (8, 9). These findings suggest that while catalyzing energy production, GAPDH also regulates chromosome stability and genome integrity by multiple mechanisms, including the maintenance of telomere homeostasis to facilitate longevity of cell proliferation (24). However, the mechanisms by which GAPDH is involved in telomere homeostasis are not known, and whether or not the regulation of telomere length by GAPDH is dependent on telomerase requires further investigation. The present study provides clear evidence demonstrating that GAPDH binds TERC, that this binding is controlled by glyceraldehyde-3-phosphate (G3P) and 5-nitrosothioglutathione (GSNO), and that GAPDH regulates telomerase activity and cancer cell proliferation.

Results and Discussion

Cancer Cell Senescence Induced by GAPDH. To investigate a potential role of GAPDH in telomere maintenance in breast cancer cells, we expressed recombinant GAPDH in MCF7 cells. Whereas we noted a reduced cell density in the MCF7 cell cultures stably expressing GFP–GAPDH consistent with previous findings of GAPDH-induced cancer cell death (3, 4, 7, 25, 28, 29), we found that there were significant changes in the morphology of
MCF7 cells expressing GFP-GAPDH. Cells expressing exogenous GAPDH exhibited an enlarged and flattened morphology characteristic of typical stress-induced premature senescence cells (Fig. 1). To characterize the incidence of this cell-senescent-like phenotype, cells expressing either GFP–GAPDH fusion or GFP alone were seeded at $10^5$ cells per 35-mm dish and allowed to proliferate and form colonies for 2 wk (Fig. 1A). In cells expressing GFP–GAPDH, there were $\sim 46\%$ of cell-senescent-like colonies demonstrating an enlarged flattened cell morphology, compared with 11% in GFP-alone controls ($P < 0.05$) (Fig. 1B). Colonies without senescence-like morphology were fewer in stable cell cultures of GFP–GAPDH, compared with GFP-alone control cells (54 versus 89%, respectively, $P < 0.05$) (Fig. 1B). Staining of senescence-like cells for the proliferation marker Ki67 showed a clear absence of Ki67 consistent with cellular senescence (Fig. 1C). Further staining of cells expressing elevated GAPDH for senescence-associated $\beta$-galactosidase (SA-$\beta$-Gal) activity confirmed the cellular senescence phenotype (Fig. 1D, Left). Approximately 91% of cells with enlarged flattened morphology showed SA-$\beta$-Gal positivity vs. less than 10% in the controls (Fig. 1D, Right). These data uniquely demonstrate that increased gene expression of GAPDH triggers breast cancer cell senescence.

**GAPDH Inhibition of Telomerase Activity.** To further demonstrate breast cancer cell senescence, we measured telomerase activity and telomere length, as the reduction of both are among the key molecular events underpinning the permanent cell cycle arrest of cancer cell senescence (30, 31). In cultured cells expressing GFP–GAPDH, there was a significant inhibition of telomerase activity and shortening of telomere length, compared with that in cell cultures expressing GFP alone (Fig. 2A and C). To determine whether elevated GAPDH gene expression might potentially alter the gene expression of hTERT, the catalytic subunit and rate-limiting component of telomerase (32, 33), we measured hTERT gene expression by real-time PCR and found that there were no significant changes in the hTERT gene expression levels (Fig. 2D). Because GAPDH has been shown to bind telomeric DNA directly (8, 9), we next determined whether purified human GAPDH has any effect on telomerase activity using breast cancer cell telomerase extracts. Interestingly, purified erythrocyte GAPDH (E-GAPDH) inhibited telomerase activity in a concentration-dependent manner in vitro (Fig. 2E and F). The findings that GAPDH inhibits telomerase activity in vitro and in vivo suggest the possibility that a posttranslational mechanism is responsible for the inhibition of telomerase through a complex interaction between GAPDH and telomerase.
Role of the Rossmann Fold in GAPDH Binding to hTERC. Because GAPDH inhibits telomerase directly in vitro and in vivo (Fig. 2), and influences the stability, location, and function of a variety of RNA species through direct interactions (reviewed in ref. 24), we examined whether an interaction between GAPDH and the telomerase RNA moiety hTERC exists. As a positive control for GAPDH RNA binding, an AU-rich RNA molecule (4x AUUUA) was used, as has been previously demonstrated to interact with GAPDH (26). As shown in Fig. 3, A and B, purified human GAPDH bound full-length hTERC (1–451) in a concentration-dependent manner. The hTERC binding appeared to be specific as unlabeled TERC, but not tRNA or rNTPs, competitively inhibited the binding of radioisotope-labeled 4x AUUUA in a dose-dependent manner (Fig. 3B). Both 5’ and 3’ halves of hTERC bound GAPDH in a concentration-dependent manner (Fig. 3C), suggesting that GAPDH may bind hTERC on opposite sites. To determine the structural specificity of GAPDH binding, we constructed recombinant GST–GAPDH fragments and found that the binding site of hTERC was within the N-terminal region of recombinant GST–GAPDH 1–151 but not in the C-terminal region of GST–GAPDH 148–335 (Fig. 3D).

The Rossmann fold in the N-terminal region of GAPDH mediates the binding of the dinucleotide NAD⁺ (34, 35). To determine the role of the Rossmann fold in GAPDH binding to hTERC, we carried out single amino acid substitutions in GAPDH by site-directed mutagenesis. As shown in Fig. 3E, a single amino acid mutation at D35, Y45, or S51 in GST–GAPDH dramatically reduced the binding of GST–GAPDH to hTERC 1–451, suggesting that the integrity of the Rossmann fold on GAPDH is required in mediating hTERC binding. To determine whether the Rossmann fold potentially binds telomeric DNA, we tested the binding capacity of radioisotope-labeled telomeric DNA to various GST–GAPDH fragments using an electrophoretic mobility shift assay (EMSA). Consistently, telomeric DNA oligonucleotides bound GST–GAPDH wild type and GST–GAPDH 1–151, but with a reduced level to GST–GAPDH 1–125. The introduction of a single amino acid mutation at D35, Y45, or S51 in GST–GAPDH 1–113, 1–71, 1–55, 1–45, or the C-terminal fragment 148–335 (Fig. S1). To further demonstrate a Rossmann fold-mediated binding of GAPDH to telomeric DNA, we used single amino acid mutations in the Rossmann fold of GST–GAPDH 1–151 and examined the interaction of these proteins with telomeric DNA oligonucleotides. As shown in Fig. 3F, whereas GAPDH full length and GAPDH 1–151 bound the telomeric oligonucleotides significantly, mutations at Y42, Y45, Y49, or S51 abolished this binding and mutations at the T99 or A125 reduced the binding. Thus, we have uniquely identified that the GAPDH Rossmann fold contains residues critically required for GAPDH to bind hTERC (Fig. 3G). Given the highly conserved structure of GAPDH (36) and telomeric RNA (17, 18) across different vertebrate species, it is possible that GAPDH–hTERC interaction is evolutionarily conserved. Our data also demonstrate that the GAPDH Rossmann fold can accommodate either hTERC or telomeric DNA.

To investigate a potential involvement of hTERT in hTERC interaction with GAPDH, we performed a pull-down assay using recombinant GST–hTERT fragments. GST–hTERT 423–538 that contains the hTERC binding site, and GST–hTERT 423–538 as controls, were incubated with breast cancer cell lysates, and after extensive washing, proteins bound to the GST–hTERT proteins were eluted with increasing concentrations of salt and concentrated for analysis by SDS/PAGE. A major protein was recovered at the molecular size of ~38 kDa from the GST–hTERT 423–658 eluents but not that of GST 423–538 (Fig. S2A). Mass spectrometry experiments showed that 12 peptides derived from the purified 38-kDa protein covered 51–59% of GAPDH (Table S1), identifying the 38-kDa binding protein to be dyskerin (34, 35). The Rossmann fold in hTERT that contains the hTERC binding site, and GST–hTERT 423–538 as controls, were incubated with breast cancer cell lysates, and after extensive washing, proteins bound to the GST–hTERT proteins were eluted with increasing concentrations of salt and concentrated for analysis by SDS/PAGE. A major protein was recovered at the molecular size of ~38 kDa from the GST–hTERT 423–658 eluents but not that of GST 423–538 (Fig. S2A). Mass spectrometry experiments showed that 12 peptides derived from the purified 38-kDa protein covered 51–59% of GAPDH (Table S1), identifying the 38-kDa binding protein to be dyskerin (34, 35). The Rossmann fold in hTERT that contains the hTERC binding site, and GST–hTERT 423–538 as controls, were incubated with breast cancer cell lysates, and after extensive washing, proteins bound to the GST–hTERT proteins were eluted with increasing concentrations of salt and concentrated for analysis by SDS/PAGE. A major protein was recovered at the molecular size of ~38 kDa from the GST–hTERT 423–658 eluents but not that of GST 423–538 (Fig. S2A).
Role of the C-Terminal Region of GAPDH in Telomerase Inhibition. With the combined evidence that the binding between the Rossmann fold and hTERC is not directly coupled to telomerase inhibition and that the C-terminal fragment of GAPDH retains telomerase inhibitory activity (Fig. 4 A–C), we next focused on an α-helical loop structure on the GAPDH C-terminal region that has been found to be important in a protein–protein interaction (37). Mutation of the GAPDH lysine residues 259 and 260 showed that GAPDH lysine 259 plays a critical role in GAPDH inhibition of telomerase activity (Fig. 4 D–F), consistent with the finding that this residue is important for the interaction of GAPDH with CRM1 (37). Expression of GAPDH K259N resulted in no change in telomere length or telomerase activity (Fig. 4 E and F), compared with those induced by GAPDH wild type or K260A. The reversal of GAPDH inhibition of telomerase activity and shortening of telomeres in breast cancer cells by mutating a single amino acid residue on the GALPD C-terminal region further confirms the specific structure–function relationship of GAPDH in regulating telomere homeostasis, and suggests a complex molecular interaction between telomerase and GAPDH.

Reversible Regulation of GAPDH Binding to hTERC. To investigate the functional significance of the interplay between hTERC and GAPDH in telomerase inhibition, we determined whether an excess amount of hTERC is capable of preventing GAPDH from interacting with the telomerase complex. Through the provision of in vitro transcribed hTERC on GAPDH inhibition of telomerase, we found that excess hTERC completely reversed the inhibitory effect of GAPDH on telomerase activity in a dose-dependent manner (Fig. 5A). This finding suggests that addition of exogenous hTERC blocks the GAPDH interaction with endogenous hTERC in the telomerase complex. Thus, hTERC binding may play a central role in positioning GAPDH with telomerase, enabling the GAPDH C-terminal domain to inhibit telomerase activity. To further examine the mechanisms regulating GAPDH interactions with telomerase machinery, we investigated the effect of the GAPDH enzymatic substrate G3P on the ability of GAPDH to inhibit telomerase activity using in vitro telomerase activity TRAP assays. Addition of G3P reversed the inhibition of telomerase activity in a dose-dependent manner (Fig. 5B), consistent with our findings that the C-terminal catalytic region of GAPDH mediates its telomerase inhibitory function (Fig. 4). Thus, these data demonstrate that G3P binding to GAPDH blocks GAPDH inhibition of telomerase activity.

Under stress conditions, cells generate nitric oxide (NO), which leads to the S-nitrosylation of GAPDH cysteine residues, abolishing catalytic function and inducing the proapoptotic role of GAPDH (35, 38, 39). To test the hypothesis that the NO donor GSNO might compromise GAPDH inhibition of telomerase activity, GAPDH was pretreated with increasing concentrations of GSNO and then incubated with telomerase extracts in vitro TRAP assays. Surprisingly, whereas control GAPDH inhibited telomerase activity by ∼68% of untreated controls, GAPDH that was treated with 1.6 mM GSNO inhibited telomerase activity by ∼35% of controls (Fig. 5C). These data indicate that GSNO modification impairs the ability of GAPDH to inhibit telomerase. Thus, whereas S-nitrosylation of GAPDH is required for GAPDH to enter the nucleus (4, 38–40), GAPDH inhibition of telomerase activity may require removal of S-nitrosylation from GAPDH, possibly by the recently demonstrated mechanism of trans-S-nitrosylation of GAPDH (38). Our findings suggest that GAPDH targeting of the telomerase complex is regulated by a complex modification of GAPDH including GSNO-induced modification of GAPDH as an additional layer of specificity control.
NAD⁺ Blocks GAPDH Binding but Does Not Inhibit Telomerase Activity. Consistent with a possibly shared binding site for NAD⁺ and telomere in the Rossmann fold, GAPDH association with the telomeric DNA was inhibited by NAD⁺ in a concentration-dependent manner, with an IC₅₀ and maximal inhibition being 13.1 and 50 μM, respectively (Fig. S5A and B). Incubation of telomerase extracts with GAPDH in the presence of increasing concentrations of GAPDH showed no significant effect of NAD⁺ on the basal and GAPDH-inhibited telomerase activity (Fig. S5C), demonstrating a distinguishable activity of GAPDH between telomeric DNA binding and telomerase inhibition. Because the concentration of NAD⁺ in cytoplasm or the nucleus of healthy cells is ≥70 μM, GAPDH binding in the nucleus may be inhibited under normal conditions as NAD⁺ binding blocks the telomeric DNA binding to the Rossmann fold. Interestingly, it has been demonstrated that cellular stress depletes NAD⁺ almost completely (41–43). Thus, the Rossmann fold on GAPDH may provide a cognate binding site for the telomeric DNA in a manner critically dependent on the elimination of NAD⁺ from the Rossmann fold under stress conditions. Cellular depletion of NAD⁺ induced by oxidative stress may thus serve as a regulatory switch to enable the GAPDH Rossmann fold to be accessed by the telomeric DNA, consistent with stress-induced GAPDH S-nitrosylation and nuclear translocation (3, 4).

Summary and Conclusion. Taken together, our findings demonstrate a unique phenomenon that GAPDH induces cancer cell senescence with significant inhibition of telomerase activity and shortening of telomere length. The molecular mechanisms of GAPDH inhibiting telomerase activity involve GAPDH N-terminal Rossmann fold binding to hTERT and C-terminal catalytic region inhibiting telomerase. We demonstrate that the interaction between GAPDH and telomerase is negatively regulated by the GAPDH substrate NAD⁺ and G3P, suggesting that the interaction between GAPDH and its substrate operates as an important check to prevent GAPDH switching from the metabolic pathway to telomere signaling. Furthermore, we demonstrate that GSNO also inhibits GAPDH suppressing telomerase, suggesting that NO regulates GAPDH structure and function by S-nitrosylation and indirect regulation of NAD⁺ production and binding to GAPDH. In conclusion, our results indicate that GAPDH interacts with the telomerase complex by directly binding the telomerase DNA moiety and subsequently inhibiting telomerase activity through its C-terminal region, ultimately resulting in telomere shortening and cell aging. Furthermore, this inhibition of telomerase activity is reversible and regulated by NAD⁺, G3P, and NO production.

Materials and Methods

Recombinant Fusion Proteins. Plasmid constructs expressing GST-fusion proteins were constructed by PCR cloning of desired GAPDH fragments onto pGEX-4T1 plasmid. The oligonucleotides used to produce the hTERT CDNA fragments were GGGGAAATTCGCCGGTGTCTGCTGCC and GGGGTGCACTTACGCAAGGGGTG for GST-hTERT 423–538 and GGGGAAATTCGCCGGTGTCTGCTGCC and GGGGAGGAGGAGTCCCCCA for GST-hTERT 423–658. Transformed BL21 DE3 Escherichia coli cells were grown overnight at 37 °C, induced with isopropyl-β-D-thiogalactopyranoside (IPTG) for 3–5 h and lysed in ice-cold GST lysis buffer. Glutathione sepharose 4B beads (GE Life Sciences) were incubated at 4 °C with lysates for 2–4 h followed by three to six washes in cold GST lysis buffer. Proteins were eluted by resuspending beads in 200–500 μL elution buffer (EB) with end-over-end rotation for 15 min at room temperature. Elution was repeated three times and pooled eluates loaded into Amicon buffer exchange columns (Millipore) to concentrate proteins. A total of 3 mL EB without glutathione was then added and centrifuged at 10,000 g for 15 min at 4 °C. Purified GST-hTERT proteins attached to the beads were mixed with breast cancer PMC42 cell lysates (30–40 mg). GST–hTERT-binding proteins were eluted from GST–hTERT proteins in an open column with increasing concentrations of salt followed by a glycine-HCl (0.1 M, pH 2.8) wash. Eluted binding proteins were concentrated by lyophilization and resold by SDS-PAGE and silver staining. Proteins were digested in the gel for mass spectrometry. The gene expression plasmid pGEXAT–GAPDH was a gift from Yan Luo (Institute of Molecular and Cell Biology, Singapore) (6).

Mammalian Cell Culture, Senescence Analysis, and Gene Transfection. The human breast cancer epithelial cell line MCF7 was cultured in DMEM (Invitrogen) supplemented with 4 mM glutamine, 3.7 mg/mL sodium bicarbonate, and 10% heat-inactivated FBS (vol/vol) (Gibco BRL) at 37 °C in 5% CO₂ humidified incubator. β-Galactosidase staining was performed for cell senescence by incubating cells with 2 mL of staining solution (1 mg/mL X-gal, 40 mM citric acid/Na phosphate buffer, pH 6.0, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 150 mM NaCl, and 150 mM MgCl₂). The stained plates were wrapped with parafilm to protect against pH changes and incubated overnight at 37 °C. The cells were rinsed and stored in PBS and analyzed by microscopy (Nikon). Cells were transfected with various amounts of plasmid DNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocols. Stable MCF7 cell lines were generated following transfection with specified pcDNA3-GFP–GAPDH plasmids (a kind gift from William E. Evans, St. Jude Children’s Research Hospital, Memphis, TN) by selection with 1 μM puromycin.
TN (37), and subsequent selection with 1 mg/mL G418 (Sigma-Aldrich). Single amino acid mutations of GAPDH were conducted by site-directed mutagenesis of the pcDNA3–GFP–GAPDH gene expression constructs.

Tolmerase Activity and Tolmer Longe Analysis. Tolmerase repletion amplification protocol assays and tolmer longe analysis were performed as previously described (44) with minor modifications (45, 46).

Immunofluorescence Microscopy and Western Blotting Analysis. Cells were seeded in chamber slides (Lab-Tek II: Nalgene Nunc International) at 60–80% confluence and transfected with gene expression plasmids. Analysis was conducted at indicated times, fixed with 4% paraformaldehyde in PBS for 15 min, permeabilized in 0.1% Triton X-100/PBS, blocked in 1% BSA. Cells were incubated with primary antibodies at 4 °C overnight and detected by Cy3-conjugated antimouse IgG secondary antibodies. Antibodies used were: mouse anti-GAPDH monoclonal antibody (clone 6C5, Abcam), rabbit GAPDH polyclonal antibody (sc-2035; Santa Cruz Biotechnology); β-actin and α-tubulin antibodies were from Chemicon. After washing with PBS-Tween, the membrane was incubated with a 1:20,000 dilution of peroxidase–β–galactosidase antibodies from Roche. After washing, the slides were developed using 0.1 mg/mL 4-chloro-1-naphthol in 0.1 M Tris–HCl (pH 9.5). The slides were counterstained with 1 μg/mL Hoechst 33,258 and mounted with ProLong Gold Antifade Reagent (Invitrogen). Images were acquired using a Leica TCS-SP5 confocal laser scanning microscope. All of the experiments were conducted at least twice, and results were expressed as the mean ± SEM.

In Vitro Transcription and RNA Binding Assays. Radiolabeled and unlabeled competitor RNA were produced using the MAXIScript T7 in vitro transcription kit (Ambion) according to manufacturer instructions. Before use in synthesis reactions, template DNA was excised from plasmids using restriction endonucleases and purified. For RNA binding assays 0.1 × 10^10–10 × 10^8 cpm RNA was incubated with 0.2–1.0 μg protein in the presence or absence of unlabeled competitor RNA for 30–60 min. Complexes were collected on 254 nm UV light for 10 min for UV cross-linking, followed by addition of 1 μL RNase mixture (Invitrogen) and incubation for 15 min at 37 °C. Samples were then subjected to SDS/PAGE on a 12% acrylamide TBE gel, dried between cellophane, and exposed to X-ray film overnight.

RNA Isolation and Gene Expression Analyses. Total RNA was isolated using the High Pure RNA Isolation kit (Roche Diagnostics). cDNA was reverse transcribed from 2 μg of RNA, using the ThermoScript RT-PCR kit (Invitrogen). Real-time PCR was conducted with SYBR Green Mastermix (Invitrogen) on an ABI Prism 7900 HT system using primers: hTERT, CATTTATCAAGCAGTTG- TGG and CGGACATCCCTGGCCTTCTT and β-actin, TCCCTGGAAGAGCTA- CGA and AGGAAGGAGCTTGAGAG.

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Supporting Information
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Fig. S1. Binding of different GAPDH fragments to telomeric DNA oligonucleotides. Upper, Coomassie blue staining of ∼1.0 μg indicated purified proteins. Lower, electrophoretic mobility shift assay. Purified proteins as indicated were individually incubated with radioisotope-labeled telomeric DNA oligonucleotide followed by electrophoresis and autoradiography. Data are representative of three similar experiments.
Fig. S2. hTERT sequence pulls down GAPDH and reverses GAPDH inhibition of telomerase activity. (A) Pull-down assay demonstrating a physical interactive complex of GAPDH and GST–hTERT fragment. GST–hTERT 423–538 and GST–hTERT 423–658 were incubated with breast cancer cell lysates, and after extensive washing, proteins bound to the GST–hTERT proteins were eluted with increasing concentrations of salt. A 38-kDa protein from the GST–hTERT 423–658 was specifically eluted. (B) Effect of transient expression of GFP–GAPDH, GFP–GAPDH plus dyskerin, and GFP–GAPDH plus hTERT in MCF7 cells on telomerase activity. Telomerase activity was measured by TRAP 48 h after transfection of cells with the construct combinations indicated.
Fig. S3. Effect of stable expression of GFP, GFP–GAPDH, GFP–GAPDH45G, GFP–GAPDH49G, GFP–GAPDH51G, and GFP–GAPDHK259N on telomerase activity of MCF7 cell cultures. Telomerase activity was measured by TRAP assay approximately 6 wk after transfection. Telomerase products and internal controls (ICs) are indicated. Data are representative of four similar experiments.
Fig. S4. Effects of GAPDH and GAPDH mutants on telomere length. (A) GFP control. (B) Wild-type GFP–GAPDH. (C) GFP–GAPDH Y45G. (D) GFP–GAPDH Y49G. (E) Graphical depiction of mean telomere signal frequency in MCF7 cell lines stably expressing indicated fusion proteins as determined by telomere Q-FISH ∼10 wk posttransfection. Data are means ± SD of three experiments.
Fig. S5. NAD⁺ blocked GAPDH binding telomeres but did not inhibit telomerase activity. (A) NAD⁺ inhibits binding of GAPDH to telomeric DNA. Erythrocyte GAPDH was incubated with radiolabeled [CCCTAA]₆ and NAD⁺. Lanes 1–9: 0, 50 nM, 0.5 μM, 5 μM, 50 μM, 0.5 mM, 1 mM, 2 mM, and 4 mM NAD⁺, respectively. (B) Scatter plot of densitometric values of bands corresponding to DNA bound GAPDH. The line of best fit, generated using SigmaPlot for Windows software version 10.0 (Systat), was used to estimate 50% inhibition of the GAPDH–DNA interaction by NAD⁺. (C) Effect of NAD⁺ on GAPDH-mediated inhibition of telomerase activity in vitro. Cancer cell lysates were incubated with (+) and without (−) erythrocyte GAPDH for 30 min during the substrate elongation step of the TRAP assay, in the presence of increasing amounts of NAD⁺ (0.0, 0.1, 1.0, 10.0, and 100.0 pmol, respectively). NAD⁺ was preincubated with GAPDH before addition of cell lysates.

Table S1. Identification of a 38-kDa protein to be GAPDH from GST–hTERT pull down

<table>
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<th>Experiment samples</th>
<th>Protein bound, kDa</th>
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