PDCDH10 Interacts With hTERT and Negatively Regulates Telomerase Activity

Li-Na Zhou, MD, Xing Hua, MD, Wu-Quan Deng, MD, Qi-Nan Wu, MD, Hao Mei, MSc, and Bing Chen, MD, PhD

Abstract: Telomerase catalyzes telomeric DNA synthesis, an essential process to maintain the length of telomere for continuous cell proliferation and genomic stability. Telomerase is activated in gametes, stem cells, and most tumor cells, and its activity is tightly controlled by a catalytic human telomerase reverse transcriptase (hTERT) subunit and a collection of associated proteins.

In the present work, normal human testis tissue was used for the first time to identify proteins involved in the telomerase regulation under normal physiological conditions.

Immunoprecipitation was performed using total protein lysates from the normal testis tissue and the proteins of interest were identified by microfluidic high-performance liquid chromatography and tandem mass spectrometry (HPLC-Chip-MS/MS). The regulatory role of PDCDH10 in telomerase activity was confirmed by a telomeric repeat amplification protocol (TRAP) assay, and the biological functions of it were characterized by in vitro proliferation, migration, and invasion assays.

A new in vivo hTERT interacting protein, protocadherin 10 (PCDH10), was identified. Overexpression of PCDH10 in pancreatic cancer cells impaired telomere elongation by inhibiting telomerase activity while having no obvious effect on hTERT expression at mRNA and protein levels. As a result of this critical function in telomerase regulation, PCDH10 was found to inhibit cell proliferation, migration, and invasion, suggesting a tumor suppressive role of this protein.

Our data suggested that PCDH10 played a critical role in cancer cell growth, negatively regulating telomerase activity, implicating a potential value in future therapeutic development against cancer.

PCDH10 Interacts With hTERT and Negatively Regulates Telomerase Activity


telomeres are repetitive guanine-rich sequences located at the ends of eukaryotic chromatin and they protect chromatin from deterioration and inappropriate recombination. Telomeres shorten after each round of cell division in normal human somatic cells, eventually limit cell replications, and cause replicative senescence. However, telomere shortening in immortalized cells is alleviated by telomerase, a special ribonucleoprotein enzyme that maintains telomere homeostasis by synthesizing and elongating telomeric repeats.

In normal physiology telomerase is only expressed in a limited number of cells, such as gametes, activated lymphocytes, and stem cells where natural replication is essential. However, over 90% of tumor cells express telomerase, making it a common phenotypic feature among different malignancies. The activation of telomerase has been found to be a pivotal step in carcinogenesis and its down-regulation is associated with the differentiation of tumor cells. Thus, the telomerase regulation mechanism is crucial for cancer cell survival.

Human telomerase reverse transcriptase (hTERT) is a catalytic component of the human telomerase complex and also the rate-limiting factor of telomerase activity. Its transcription is directly controlled by c-myc, SP1, p53, and Wilms tumor (WT)-1. In addition, telomerase activity can also be mediated at the post-translational level. Reversible phosphorylation of hTERT at serine/threonine or tyrosine residues, as a result of the activation of multiple kinases or phosphatasises, is important for its structure, localization, and catalytic activity. Identification of molecules and proteins involved in the telomerase complex is, therefore, a prerequisite to understanding the molecular mechanism underlying the delicately controlled elongation of telomeres under both physiological and pathological conditions.

Systematic proteomics is a powerful tool for screening protein–protein interactions, and its application in tumor models with overexpressed hTERT facilitates the identification of upstream regulators of telomerase. Using yeast 2-hybrid systems, the association of telomerase with HSP90, p23, Ku, and 14-3-3 signaling proteins was uncovered, improving our understanding of assembly of telomerase complex and its access to telomeric DNA ends. However, given that the human telomerase complex has an estimated mass of 1000 kDa, it is predicted that there are additional hTERT-associated proteins that remain to be identified.

While telomerase regulation in cancer cells has been well characterized, much less is known about the telomerase complex in normal biology. In healthy tissues, the telomerase is largely inactive due to the transcriptional repression of hTERT prior to birth, except for germinal tissues such as testis and...
ovary,14–16 lymph nodes,17 and some hyperplastic tissues.18,19 This dormant state of telomerase presents an ideal opportunity for the discovery of telomerase-suppressive factors, which may represent keys to future therapeutic development targeting telomerase activity.

In this study, we discovered a new hTERT-interacting protein, protocadherin 10 (PCDH10), in normal human testis tissues using immunoprecipitation followed by a microfluidic-based high-performance liquid chromatography and tandem mass spectrometry (HPLC-Chip-MS/MS), a powerful approach in the study of protein–protein interactions.20–22 The interaction of PCDH10 with hTERT was confirmed by reciprocal immunoprecipitation, and the inhibitory effects of this interaction on telomerase activity were characterized by a telomeric repeat amplification protocol (TRAP) assay. Moreover, the observations that overexpression of PCDH10 inhibits cancer cell proliferation, adhesion, migration, and invasion support a potential tumor suppressor role for PCDH10.

METHODS

Ethics Statement

Fresh normal testis tissues were collected from patients who received surgical treatment for prostate cancer at the Department of Urology, Southwest Hospital, The Third Military Medical University, China. All the patients were informed of the purpose and procedure of this study and written consent was obtained before surgery. The protocols used for this study were approved by the Research Ethics Committee of Southwest Hospital affiliated to The Third Military Medical University, Chongqing, China.

Immunopurification of hTERT-Associated Complex and HPLC-CHIP-MS-MS

A Pierce immunoprecipitation kit (Thermo Scientific, Waltham, MA) was used following the manufacturer’s instructions. Briefly, total protein was isolated with an immunoprecipitation lysis/wash buffer from fresh testis tissue diced to small pieces, in the presence of a complete protease inhibitor cocktail (Roche Diagnostics, Shanghai, China). Total protein concentration was determined by a bicinchoninic acid assay. The lysate was then precleared with the control agarose in a Pierce spin column and incubated overnight at 4°C with 20 μg of anti-hTERT polyclonal antibody (Abcam, Cambridge, UK) or an IgG control coupled to an AminoLink Plus Coupling resin. The resin was washed with the immunoprecipitation lysis/wash buffer before the immunoprecipitates were eluted with the elution buffer.

The immunoprecipitates were separated by 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), and the gel stained with 0.25% Cooamassie blue R-250 in 10% acetic acid/45% methanol for at least 3 hours with gentle shaking. After thoroughly destaining in 50% methanol and 10% ethanol, the gel was stored in distilled water at room temperature until HPLC-Chip-MS/MS analysis.

The bands of interest were excised from the SDS-PAGE gel and then were destained in ammonium bicarbonate/acetonitrile, reduced with dithiothreitol, alkylated with iodoacetamide, and dehydrated in acetonitrile. Automatic in-gel protein digestion was performed with a MassPrep station (Waters, Milford, MA) and 12.5 ng/μL trypsin in 50 mM ammonium bicarbonate for 5 hours at 37°C.

Digested peptides were separated using an Agilent 1200 series Nanoflow HPLC system (Agilent Technologies, Santa Clara, CA). The sample in solvent A (0.1% formic acid in water) was trapped in a Zorbax 300SB C18 enrichment column for 1.5 minutes and then was applied to an analytical column with a biphasic gradient of solvent B (90% acetonitrile, 10% water with 0.1% formic acid) ranging from 3% to 15% for 2 minutes, then from 15% to 50% for 70 minutes, with a fixed flow rate of 0.3 mL/min. Mass spectrometry was performed with an Agilent 1100 Series LC/MSD Trap MS with an Agilent orthogonal nanoelectrospray source. MS/MS analysis was controlled by a data-dependent setting. The nanoelectrospray source was operated at 2000 V with a PicoTip EMITTER (New Objective, Woburn, MA). Due to statistical fluctuations in the peptide precursor selection during MS/MS acquisition, LC-MS/MS assays were performed in triplicate for each sample to achieve appropriate proteome comparison.

The identification of peptides and proteins was automatically performed by Spectrum Mill software (Agilent) using the Swiss-Prot protein database (Geneva, Switzerland) and screened for tryptic peptides restricted to Homo sapiens.

Immunoprecipitation and Western Blotting

Total protein isolated from normal testis tissue was incubated with an anti-hTERT or anti-PCDH10 polyclonal antibody (Santa Cruz, Dallas, TX) coupled to an AminoLink Plus Coupling resin. Resin without antibody was used as a control. The resulting immunoprecipitates were separated on SDS(10%)-PAGE and transferred onto a polyvinylidene fluoride membrane. After blocking with 5% skim milk in phosphate-buffered saline (PBS), the membrane was incubated with the appropriate primary antibodies overnight at 4°C with gentle agitation, followed by 2-hour incubation with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Santa Cruz) at room temperature. The signal was visualized by using a SuperSignal West Dura Extended Duration Substrate (Thermo Scientific).

Cloning of the PCDH10 Gene

The full-length coding region of PCDH10 was amplified by reverse transcription-polymerase chain reaction (RT-PCR) from total RNA isolated from testis using TRIzol reagent (Thermo Scientific) according to the manufacturer’s protocol. RT-PCR was performed using a Real-Time PCR Master Mix (Toyobo, Japan). Specific primers for PCDH10 were designed according to the PCDH10 sequence registered in Genbank (NM_001098170) and synthesized by Shanghai Bioasia Biotech (Shanghai, China). The sequences of the forward and reverse PCR primers were 5'-GGGGTACCAT-GATTGTGCTATTATTGTTTGCCT-3' and 5'-GCTCTAGAC-TAGCATATCTTTTCCGTGTA-3', respectively. The PCR product was ligated into a pTA2 vector (Toyobo), which was transformed into DH5α competent Escherichia coli (American Type Culture Collection, Manassas, VA). The sequence of PCDH10 was confirmed by Sanger sequencing at Sangon Biotech (Shanghai, China).

Construction of an Adenovirus-Based PCDH10 Expressing Vector

An adenovirus-based PCDH10 expressing vector was constructed by DNA recombination using a pAdTrack-CMV and pAdEasyTMXl Adenoviral Vector System (Agilent Technologies). The pTA2-PCDH10 and pAdTrack-CMV shuttle plasmids were each digested by Kpn I and Xho I and ligated. The resulting recombinant shuttle plasmids were transformed into DH5α E. coli, selected, and linearized by Pme I. The pAdTrack-
CMV-PCDH10 obtained was then transferred into BJ5183 E. coli in which pAdEasy-PCDH10 was generated. Linearized by Pac I, the recombinant adenovirus plasmid, pAdEasy-PCDH10, was transfected into 293T cells using lipofectamine (Thermo Scientific) to generate and propagate the recombinant adenovirus, Ad-PCDH10. Virus titer was detected by using a tissue culture infective dose assay.

Cell Culture and Virus Transduction
The pancreatic cancer cell line, HS 776T (JENNIO Biological Technology, Guangzhou, China), was maintained in RPMI 1640 medium (Thermo Scientific) supplemented with 10% fetal bovine serum (FBS) in the presence of penicillin (100 units/mL) and streptomycin (100 units/mL) at 37°C in a humidified 5% CO2 atmosphere. For virus transduction, 1 x 10⁶ cells were seeded and cultured to 80% confluence in 75 cm² flasks, washed with PBS and antibiotic-free, serum-free medium. The virus containing medium (1 x 10⁹ plaque-forming units (PFU)/mL) was added to cover the HS 776 T cells and was incubated with the cells for 2 hours at 37°C before being examined microscopically for the expression of green fluorescent protein at different time points.

Real-Time PCR
Real-time PCR was performed using a SYBR Green Realtime PCR Master Mix (Toyobo) following the manufacturer’s instruction. Briefly, total RNA was extracted from cultured cells with TRIZol reagent and used as a template for complementary DNA synthesis driven by avian myeloblastosis virus (AMV) reverse transcriptase with oligo (dT) primers. Real-time PCR was performed with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a control. The primers used were designed based on the sequences registered in GenBank and synthesized by Shanghai Bioasia Biotech (Table 1). A melting curve was obtained to determine product purity. The threshold cycle (Ct) of the target mRNAs were normalized to that of GAPDH and used to calculate relative levels of the target transcripts.

Telomerase Assay
The telomerase activity was assessed by a telomeric repeat amplification protocol (TRAP) assay using a Telomerase Detection Kit (Millipore, Billerica, MA). Briefly, immunoprecipitation with an anti-PCDH10 antibody was performed using HIV HS 776T cells that were transduced by PCDH10 expressing viruses for 72 hours. TRAP lysis buffer were used as a negative control and cell extracts from telomerase-active 293 cells were used as a positive control. After 30 minutes of telomerase extraction, 30 minutes of primer elongation at 23°C, 3 minutes of telomerase inactivation at 94°C, depuration, and 30 cycles of amplification (94°C for 30 s, 50°C for 30 s, and 72°C for 90 s) were performed. From each RT-PCR sample, 25 μL aliquots were separated on a 12% nondenaturing polyacrylamide gel, which was then stained and visualized under ultraviolet (UV) light. A semiquantitative densitometric evaluation was conducted using ImageQuant 5.0 software (Molecular Dynamics, Sunnyvale, CA). All of these experiments were performed in triplicate.

Cell Proliferation Assay
Cell proliferation was assessed by monitoring cell viability after PCDH10 manipulation. Cells were seeded into 96-well plates (1 x 10⁴ cells/well) and transduced with the PCDH10-expressing or the empty control viruses. At various time points, 10 μL of CCK-8 solution was added to each well. After another 1 to 4 hours, absorbance at 450 nm was measured. The experiment was performed 3 times with 6 replicates for each sample.

Wound Healing Assay
A monolayer wound healing assay was performed to assess the impact of PCDH10 on cell mobility. HS 776T cells with stable expression of PCDH10 were grown in 35-mm dishes to form a monolayer, on which scratches were then carefully made using sterile tips. After 2 washes with fresh medium, the cells were cultured under normal conditions and imaged at 24 and 48 hours under a phase-contrast microscope. The experiments were repeated for 3 times.

Matrigel Invasion Assay
The effect of PCDH10 on cell invasion was assessed in a 24-well plate fitted with invasion chambers that were sealed with PET membrane (8-μm pore size) at the bottom and coated with Matrigel (Becton Dickinson, Franklin Lakes, NJ). HS 776T cells with and without stable PCDH10 expression were washed 3 times, and seeded into the top chamber at 4 x 10⁴ cells/well in 100-μL fresh medium containing 1% FBS, while in the bottom chamber 600-μL medium supplemented with 5 mg/mL fibronectin (Sigma) was added. The cells were incubated for 24 hours at 37°C, and those migrating through the Matrigel-coated membrane were stained with Gentian Violet and counted. The experiment was repeated 3 times.

Statistical Analysis
All values are expressed as the mean ± standard deviation (SD). Correlations between the expression of PCDH10, expression of hTERT, and telomerase activity in the cells examined were analyzed. Differences between experimental groups were assessed with a 2-tailed unpaired Student t test. Statistical analysis was performed using a SPSS 16.0 package (IBM, Armonk, NY) and P < 0.05 was considered to be statistically significant.

### Table 1. Primers Sequences of hTERT and hTERT-Interacting Protein Genes

| Target          | Forward Primer                      | Reverse Primer                      |
|-----------------|-------------------------------------|-------------------------------------|
| hTERT           | 5'-TATGCCCTGGTCATCCAGAAAGG-3'       | 5'-CAAGAAATCATCCACCAACACG-3'        |
| hTR             | 5'-CTGGGGGAGGTGTGGCCATT-3'          | 5'-CGAACGGGCCAGCAGCTGACAT-3'        |
| PCDH10          | 5'-GACATATGTTATTACCCACG-3'          | 5'-CAGGATCTTAAAAAAGTGAGGAGG-3'      |
| GAPDH           | 5'-GCTTCTACCCACCATGGGA-3'           | 5'-CGGCCATGCACCAGT TT-3'            |

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.
RESULTS

Identification of Novel hTERT-Binding Proteins

The hTERT-binding protein complex was immunoprecipitated from human testis tissue extracts using an hTERT antibody. The complex was separated by SDS-PAGE and was compared with human testis extracts that were incubated with an IgG control antibody. Two bands were detected in the former and not the latter (Bands 1 and 2, Fig. 1A) and they had molecular weights of ~120 and ~110 kDa. The 120-kDa band was confirmed to be hTERT in the Western blotting analysis (Fig. 1B) run in parallel using the same batch of immunoprecipitate samples as in Figure 1A. hTERT was not detected in the immunoprecipitate of IgG control, indicating the specificity of the hTERT antibody used in the experiment. Band 2 (~110 kDa) was unknown and subjected to HPLC-Chip-MS/MS analysis after reduction, alkylation, and trypsinization.

After HPLC-Chip-MS/MS, peptide mass fingerprinting was performed by searching against Swiss-Prot protein database. The protein, PCDH10, was consistently identified as one of the top 5 binding partners of hTERT in the 3 HPLC-Chip-MS/MS analyses performed (Fig. 1, C and D).

Confirmation of the Interactions Between PCDH10 and hTERT

To confirm the association of hTERT with the newly identified binding partner, PCDH10, reciprocal immunoprecipitation was performed. Total protein isolated from testis tissue was used for immunoprecipitation with anti-hTERT and anti-PCDH10, with isotype matched IgG as controls, and detected by Western blotting for PCDH10 and hTERT, respectively. The results are shown in Figure 2A and B. PCDH10 was detected in the immunoprecipitate of the anti-hTERT antibody, and hTERT was detected in the immunoprecipitate of the anti-PCDH10 antibody. These data demonstrate for the first time that hTERT associates with PCDH10 in vivo.

Construction of Recombinant Adenovirus Encoding Human PCDH10 Protein

The full-length coding region of PCDH10 was cloned from normal human testis tissue using RT-PCR (Fig. 3A). The amplified fragment was inserted into adenovirus shuttle vector pAdTrack-CMV. The obtained pAdTrack-CMV-PCDH10 and adenovirus genome-containing pAdEasy-1 plasmid underwent homologous recombination in E. coli BJS183 cells (Fig. 3B). Finally, the recombinant adenovirus Ad-PCDH10 was packed and propagated after pAdEasy-PCDH10 was transfected into 293T cells. The adenovirus of high purity was obtained after 5 days of propagation after pAdEasy-PCDH10 was transfected into E. coli BJ5183 cells (Fig. 3B).

Effect of PCDH10 Overexpression on Telomerase Functions

The interaction of PCDH10 with hTERT in vivo directed us toward examining its function in telomerase regulation. PCDH10 was overexpressed in HS 776T cells using the recombinant adenovirus, Ad-PCDH10, and cells treated with empty Ad vector and untransduced cells as controls. Western blotting and RT-PCR confirmed significantly higher levels of PCDH10 in cells transfected with Ad-PCDH10 adenovirus compared with the mock and vector controls (Fig. 4, A–D). Of note, the exogenously expressed PCDH10 had no effect on the expression of hTERT on both mRNA and protein levels and the abundance of telomerase RNA (hTR) (Fig. 4, A–D).

To assess the effect of PCDH10 on telomerase activity, immunoprecipitation was performed with an anti-PCDH10 antibody using cell extracts collected from cells overexpressing PCDH10 or the control cells. The immunoprecipitates were then used for TRAP assay. Unsurprisingly, more hTERT was pulled down with the anti-PCDH10 antibody in cells overexpressing PCDH10 (Fig. 4E, bottom panel). The TRAP assay results indicated a remarkable reduction in telomerase activity in the HS 776T cells with PCDH10 overexpression compared with the mock and vector controls (Fig. 4E and F). Taken together, these data demonstrate that PCDH10 was physically involved in the telomerase complex through its interactions with hTERT in vivo, and this association inhibits telomerase activity.

Effect of PCDH10 on Cell Proliferation, Migration, and Invasion

PCDH10 was overexpressed in HS 776T cells by transduction with adenovirus Ad-PCDH10 using the empty Ad vector and untransduced cells as controls. At 24, 48, 72, 96, 120, and 144 hours posttransduction, a CCK-8 assay was performed to assess cell viability. A gradual decrease in cell proliferation was observed at the 96, 120, and 140 hours time points for the cells that overexpressed PCDH10. Specifically, cell proliferation was decreased by 12.73% (P < 0.05), 19.87% (P < 0.01), and 30.14% (P < 0.001), respectively, compared with the untransduced control (Fig. 5A). Meanwhile, the cells treated with empty virus appeared unaffected. These data suggest that PCDH10 overexpression strongly inhibits tumor cell proliferation.

We next assessed the effect of PCDH10 overexpression on cell migration by a wound healing assay on the HS 776T cell monolayer. Cell motility was found to be unchanged for the HS 776T cells that were transduced with empty virus compared with untransduced cells (P > 0.05), whereas the cells overexpressing PCDH10 exhibited significantly slower motility across the scratch mark (Fig. 5B). The relative migration of the PCDH10 overexpressing cells was decreased by 30.07% after 24 hours (P < 0.001) and by 52.95% after 48 hours (P < 0.001), compared with the controls (Fig. 5C). These results suggest that PCDH10 inhibits tumor cell migration.

Furthermore, a matrigel invasion assay was also performed to examine the invasive potential of HS 776T cells with and without exogenous PCDH10 expression. As shown in Figure 5D, upregulation of PCDH10 significantly reduced the number of cells that migrated through the matrigel-coated membranes compared with the untransduced cells (P < 0.001), whereas the empty virus had no effect. In combination, these results suggest that PCDH10 overexpression potently inhibits the invasion of HS 776T cells.

DISCUSSION

The dormant state of telomerase in the majority of normal tissues suggests that hTERT-associated proteins may coordinately function as inhibitors of telomerase, and play a critical role in the restriction of cell replication to maintain tissue homeostasis. In the present investigation, we screened for hTERT interacting proteins in the normal testis by HPLC-Chip-MS/MS analysis of proteins immunoprecipitated with an hTERT antibody. This approach allowed us to more directly identify proteins interacting with hTERT at the physiological level, rather than using yeast hybrid systems which rely on in
FIGURE 1. Immunoprecipitation and HPLC-Clip-MS/MS analysis of hTERT-interacting proteins. A, Proteins interacting with hTERT were immunoprecipitated by an hTERT antibody from extracts collected from normal testis tissues. Immunoprecipitation reactions using IgG as a control were performed as a comparison. B, Specificity of the immunoprecipitation was confirmed by Western blotting. C and D, Peptide mass fingerprinting identified the PCDH10 protein.
vitro protein–protein interactions. To our knowledge, this study was the first to demonstrate that PCDH10 as an hTERT-interacting protein which negatively regulates telomerase activity and inhibits cancer cell proliferation, migration, and invasion.

The protein identified, PCDH10, belongs to the protocadherin subfamily of cadherins. This subfamily of proteins contain 6 extracellular cadherin domains, a transmembrane domain, and a cytoplasmic tail that differs them from the classical cadherins. As a newly discovered protein, the function of PCDH10 remains unclear. Accumulating evidence links it with signal transduction and growth control during development of the central nervous system. Importantly for this study, recent data indicated frequent transcriptional silencing and promoter methylation of PCDH10 in most carcinoma cell lines, including 82% of nasopharyngeal carcinoma, and 42% to 51% of other carcinomas, but not in normal tissues, although the molecular reason is unknown. In this study, we overexpressed PCDH10 in cancer cells where its expression is usually suppressed, and found significant decrease in telomerase activity (Fig. 4E). Our data provide the first experimental evidence that PCDH10 negatively regulates telomerase activity, explaining that, by silencing and methylating PCDH10 gene, cancer cells hijack telomerase activity to achieve telomere elongation and continuous proliferation capacity.

Marked inhibition of cell proliferation, migration, and matrigel invasion were observed with overexpression of PCDH10.
PCDH10 in a pancreatic cancer cell line (Fig. 5). These data correlate with an inhibitory role for PCDH10 in telomerase regulation, and are consistent with previous reports that PCDH10 may be a candidate tumor suppressor gene. Accordingly, inactivation of PCDH10 may permit tumor growth and expansion, and its ectopic expression in carcinoma cell lines dramatically inhibits tumor cell growth, migration, invasion, and colony formation. It is uncertain whether protocadherins such as PCDH10 are functionally redundant to classic cadherins. PCDH10 is unlikely a major regulator for cell–cell junction considering that mouse PCDH10 only weakly mediates cell aggregation. It may not interfere with the Wnt/β-catenin/Tcf signaling pathway like cadherins because protocadherins, including PCDH10, do not bind β-catenin. In this study, we identified PCDH10 as a negative factor of telomerase. RT-PCR and Western Blotting results exhibited that hTERT was expressed in the presence of PCDH10, suggesting that suppression of telomerase activity by PCDH10 is mediated through the interactions between PCDH10 and hTERT, rather than an inhibition of hTERT transcription (Fig. 4, A–D). Therefore, we speculate that interactions between PCDH10 and hTERT may directly inhibit telomerase activity. PCDH10 is a typical protocadherin that contains a cytoplasmic motif, CM-2, which is homologous to the laminin-type EGF-like domains. CM-2 is also similar to the C2HC-type zinc finger or zinc knuckle finger motif. Since some zinc fingers have been shown to mediate protein–protein interactions, the CM-2 motif in PCDH10 may mediate its interactions with other proteins. Previously, it has been shown that the introduction of hTERT into normal cells induces telomerase activity and confers a continuous replication capacity, whereas alterations in the C-terminus of hTERT disrupt its ability to maintain telomerase activity to immortalize a cell. Taken together, these observations suggest that the C-terminus of hTERT interacts with other essential factors for telomerase activity, and it will be interesting to investigate whether the C-terminus of hTERT interacts with the CM-2 domain of PCDH10.

FIGURE 5. Ectopic PCDH10 expression suppresses the proliferation, migration, and invasion of pancreatic carcinoma cells. HS 776T cells were transduced with an adenovirus expressing PCDH10 (PCDH10), whereas untransduced and empty adenovirus-transduced cells (Mock and Vector, respectively) served as controls. A, CCK-8 assay was used to monitor cell proliferation at various time points up to 140 h after transduction. The mean ± standard error values are shown (P < 0.01). B, C, Motility of HS 776T cells was assessed by wound migration assays. The migration of Mock, Vector, and PCDH10 cells across the wound is imaged in B, and relative migration is quantified in C (P < 0.01). D, The invasive capacity of Mock, Vector, and PCDH10 cells was assessed by matrigel invasion assays. The cells that migrated were stained with hematoxylin and eosin and imaged (D). E, The cells migrated were quantified and the data averaged from 3 independent experiments. P < 0.01.

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.
www.md-journal.com | 7
In the present study, we screened for hTERT interacting proteins in the normal testis tissue and identified PCDH10 as a novel hTERT interacting partner. Our data suggest that interactions between hTERT and PCDH10 contribute to the regulation of telomerase activity in the cell, since overexpression of PCDH10 significantly inhibited the telomerase activity, as well as the proliferation, migration, and invasion activity of a pancreatic cell line. To our knowledge, these findings provide the first-line evidence for an inhibitory role for PCDH10 in telomerase activity in normal tissue. Further studies are needed to improve our understanding of the regulatory pathways of telomerase which will eventually contribute to the development, prevention, diagnosis, and therapy of cancer.

REFERENCES

1. Blackburn EH. Structure and function of telomeres. Nature. 1991;350:569–573.

2. Greider CW, Blackburn EH. A telomeric sequence in the rna of tetrahymena telomerase required for telomere repeat synthesis. Nature. 1989;337:331–337.

3. Bodnar AG, Ouellette M, Frolkis M, et al. Extension of life-span by introduction of telomerase into normal human cells. Science. 1998;279:349–352.

4. Blackburn EH. Telomerase and cancer: Kirk A. Landon prize for basic cancer research lecture. Mol Cancer Res. 2005;3:477–482.

5. Artandi SE, DePinho RA. Telomeres and telomerase in cancer. Carcinogenesis. 2010;31:9–18.

6. Lingner J, Hughes TR, Shevchenko A, et al. Reverse transcriptase motifs in the catalytic subunit of telomerase. Science. 1997;276:561–567.

7. Takakura M, Kyo S, Kanaya T, et al. Cloning of human telomerase catalytic subunit (hTERT) gene promoter and identification of proximal core promoter sequences essential for transcriptional activation in immortalized and cancer cells. Cancer Res. 1999;59:551–557.

8. Cong YS, Wright WE, Shay JW. Human telomerase and its regulation. Microbiol Mol Biol Rev. 2002;66:407–425.

9. Holt SE, Aigner DL, Baur J, et al. Functional requirement of p23 and hsp90 in telomerase complexes. Genes Dev. 1999;13:817–820.

10. Seimiya H, Sawada H, Muramatsu Y, et al. Involvement of 14-3-3 motifs in the catalytic subunit of telomerase. EMBO J. 2000;19:2652–2661.

11. Stellwagen AE, Haimberger ZW, Veatch JR, et al. Ku interacts with telomerase rna to promote telomere addition at native and broken chromosome ends. Genes Dev. 2003;17:2384–2395.

12. Harrington L. Biochemical aspects of telomerase function. Cancer Lett. 2003;194:139–154.

13. Fujisawa M, Tanaka H, Tatsunami N, et al. Telomerase activity in the testis of infertile patients with selected causes. Hum Reprod. 1998;13:1476–1479.

14. Misiti S, Nanni S, Fontemaggi G, et al. Induction of htert expression and telomerase activity by estrogens in human ovary epithelium cells. Mol Cell Biol. 2000;20:3764–3771.

15. Kinugawa C, Murakami T, Okamura K, et al. Telomerase activity in normal ovaries and premature ovarian failure. Tohoku J Exp Med. 2000;190:231–238.

16. Baykal A, Thompson JA, Xu XC, et al. In situ human telomerase reverse transcriptase expression pattern in normal and neoplastic ovarian tissues. Oncol Rep. 2004;11:297–302.

17. Hiyama K, Hirai Y, Kyoizumi S, et al. Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. J Immunol. 1995;155:3711–3715.

18. Counter CM, Gupta J, Harley CB, et al. Telomerase activity in normal leukocytes and in hematologic malignancies. Blood. 1995;85:2315–2320.

19. Kim NW, Piatyszek MA, Prowse KR, et al. Specific association of human telomerase activity with immortal cells and cancer. Science. 1994;266:2011–2015.

20. Geetha T, Langlais P, Luo M, et al. Label-free proteomic identification of endogenous, insulin-stimulated interaction partners of insulin receptor substrate-1. J Am Soc Mass Spectrom. 2011;22:457–466.

21. Yu M, Wang J, Li W, et al. Proteomic screen defines the hepatocyte nuclear factor lalp4a-binding partners and identifies hmlb1 as a new cofactor of hnf1alpha. Nucleic Acids Res. 2008;36:1209–1219.

22. Hwang H, Bowen BP, Lefort N, et al. Proteomics analysis of human skeletal muscle reveals novel abnormalities in obesity and type 2 diabetes. Diabetes. 2010;59:33–42.

23. Frank M, Kemler R. Protocadherins. Curr Opin Cell Biol. 2002;14:557–562.

24. Wolverton T, Lalande M. Identification and characterization of three members of a novel subclass of protecadherins. Genomics. 2001;76:66–72.

25. Ying J, Li H, Seng TJ, et al. Functional epigenetics identifies a protocadherin pcdh10 as a candidate tumor suppressor for nasopharyngeal, esophageal and multiple other carcinomas with frequent methylation. Oncogene. 2006;25:1070–1080.

26. Yu B, Yang H, Zhang C, et al. High-resolution melting analysis of pcdh10 methylation levels in gastric, colorectal and pancreatic cancers. Neoplasma. 2010;57:247–252.

27. Narayan G, Scotto L, Neelakantan V, et al. Protocadherin pcdh10, involved in tumor progression, is a frequent and early target of promoter hypermethylation in cervical cancer. Genes Chromosomes Cancer. 2009;48:983–992.

28. Li Z, Chinn JC, Yang M, et al. Role of pcdh10 and its hypermethylation in human gastric cancer. Biochim Biophys Acta. 2012;1823:298–305.

29. Suzuki ST. Recent progress in protocadherin research. Exp Cell Res. 2000;261:13–18.

30. Counter CM, Hahn WC, Wei W, et al. Dissociation among in vitro telomerase activity, telomere maintenance, and cellular immortalization. Proc Natl Acad Sci U S A. 1998;95:14723–14728.