Biochemical and Biophysical Research Communications 407 (2011) 86–91

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

PIDD4, a novel PIDD isoform without the LRR domain, can independently induce cell apoptosis in cytoplasm

Lin Huang [1], Dingding Han [1], Xianmei Yang, Bo Qin, Guoqing Ji, Long Yu *

State Key Laboratory of Genetic Engineering, Institute of Genetics, School of Life Sciences, Fudan University, Shanghai 200433, PR China

ARTICLE INFO

Article history:
Received 21 February 2011
Available online 28 February 2011

Keywords:
PIDD
Apoptosis
Splicing variant
p53

ABSTRACT

PIDD1 (P53-induced death domain) is a pro-apoptotic gene which can be induced by p53. So far, three alternative splicing products of human PIDD gene have been identified. Here we report a new splicing variant of this gene and named it PIDD4. The coding sequence of PIDD4 contains intron 3 and a 60 bp insert at the 5′ of exon 3. Each insertion has an in-frame stop codon, which makes PIDD4 get translated from exon 5 then. Therefore, PIDD4 protein lacks the 32 KD N-terminal peptide, missing the LRR domain found in the other three isoforms. In this study, we have shown that the expression of PIDD4 is also regulated by p53, and as PIDD2, it is not expressed in heart either. Moreover, PIDD4 is the only isoform which is expressed in skeletal muscle. This isoform mainly localizes in the cytoplasm, and produces a relatively higher proportion of PIDD-CC fragment. Overexpression of PIDD4 independently promotes apoptosis.

1. Introduction

Apoptosis is physiological important during the process of vertebrate development [1]. It can be identified with series of morphological changes, including plasma membrane blebbing, cell shrinkage, chromatin condensation and fragmentation. At present two different apoptosis pathways have been studied in depth: one is the death-receptor-dependent pathway, also called the extrinsic pathway; the other is mitochondria-dependent pathway, or the intrinsic pathway [2].

PIDD (p53-induced death domain) was first identified to be induced by p53 and was able to promote apoptosis [3]. So far, three alternative splicing isoforms of PIDD gene have been reported, including PIDD1 (isoform 1) [3], PIDD2 (isoform 2, also known as LRDD) [4] and PIDD3 (isoform 3) [5]. And they have different functions in the cell. PIDD1 has been shown to be the molecular switch between the cellular survival pathway and the apoptosis pathway under conditions as damage DNA [6]. PIDD1 undergoes autocatalytic cleavage to generate a 48 kDa (containing LRRs, PIDD1-N) and a 51 kDa (including death domain, PIDD1-C) fragments. The PIDD1-C further auto-cleavages itself to produce a short 37 kDa (PIDD1-CC) segment [7]. These three fragments activate different downstream signaling pathways respectively to regulate the cell cycle. PIDD1-C goes into the nucleus and complex with RIP1 (an adaptor protein, Receptor-Interacting Protein 1) and NEMO to activate NF-κB pathway [8], inhibiting apoptosis caused by DNA damage. On the other side, PIDD1-CC stays in the cytoplasm and associates with RAIDD (RIP-associated ICH-1/ECD3-homologous protein with a death domain) and pro-caspase 2 to participate in the mitochondrial apoptosis pathway [9]. Comparing to PIDD1, amino acids 580–590 (of PIDD1) are absent in PIDD2. Since the auto-cleavage site for PIDD-CC locates at amino acid 588, PIDD2 cannot generate PIDD-CC as PIDD1 does. On the contrary, PIDD2 protects cells and inhibits apoptosis induced by PIDD1 [5]. PIDD3 contains a deletion of 17 amino acids starting from amino acid 705, and it cannot induce apoptosis independently, only showing an augmentative pro-apoptotic effect when co-expressed with PIDD1 [5]. Moreover, recent report indicates that the three protein isoforms can all be stimulated by DNA damage to activate NF-κB pathway [5].

In this study, we have identified a new splicing isoform for human PIDD gene (PIDD4). Its encoding protein lacks the LRR domain, and can produce a higher proportion of PIDD-CC fragment. PIDD4 is regulated by p53 and expresses in skeletal muscle while the other three isoforms do not. Immunofluorescence experiments show that it localizes in the cytoplasm. Over-expression of PIDD4 independently promotes apoptosis and this has been confirmed by FACS experiments.

Abbreviations: PIDD, P53-induced death domain; LRR, leucine rich repeat; LRDD, leucine repeat death domain containing protein; RIP, Receptor-Interacting Protein.

* Corresponding author. Address: State Key Laboratory of Genetic Engineering, Institute of Genetics, School of Life Sciences, Fudan University, 220 Handan Road, Shanghai 200433, PR China. Fax: +86 21 65643404.
E-mail address: longyu@fudan.edu.cn (L. Yu).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.02.114

2. Materials and methods

2.1. Database searches and cloning of PIDD4

Online BLAST searches, EST mining and protein sequence retrieval were performed via the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/entrez). The full length ORF of human PIDD4 was cloned from testis cDNA of MTC (multiple tissue cDNA) panels (Clontech) with specific primers (forward B1: 5′-AATGCCCAGACTGTTCCTGACCTCA-3′; reverse B2: 5′-AGGTGGG GGCTCTGCCCATC-3′). The amplification was performed as follows: 1 cycle of 95 °C for 5 min, 35 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 2 min, followed by 1 cycle of 72 °C for 10 min. The product was then subcloned into the pMD18-T vector (Takara), pcDNA3.1 (Invitrogen) and pEGFP-C1 (Clontech). The 5′-UTR was also amplified from human testis cDNA library. Primers (forward A1: 5′-GCAG-GAGATGCTTCAGAGGATTCGG-3′; reverse A2: 5′-GAGGTCAGGAACA GTCTGGGCATT-3′) were used for PCR at the condition of 95 °C for 5 min, 35 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 2 min, followed by 1 cycle of 72 °C for 10 min. All the products were sequenced from both orientations.

2.2. RT-PCR analysis

Human MTC (multiple tissue cDNA) panels (Clontech) including bone marrow, stomach, bladder, lung, placenta, pancreas, heart, spleen, liver, thymus, testis, Intestine, uterus, ovary, brain, skeleton, muscle and prostate served as templates to study the distribution of human PIDD4 mRNA. Primer pairs designed in intron3 and exon6 respectively (forward: 5′-CAGGAGTGTCCCAGCGCCGTG-3′; reverse: 5′-CTTGAGGGGTCACAGGAAAGCTGTC-3′) were used for RT-PCR at the condition of 95 °C for 5 min, 35 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 2 min, followed by 1 cycle of 72 °C for 10 min. 5′-GAAGGTGAAGGTCGGAGTC-3′ and 5′-GAAGATGGT GATGGGATTTC-3′ were used to amplify GAPDH gene. The products were then separated by DNA electrophoresis in 2% (w/v) agarose gel.

2.3. Cell culture and transfection

AD293 cells and Saos-2 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Gibco-BRL) at 37 °C in 5% CO_2 atmosphere. 2×10^5 cells at 80% confluence in 6-well plate were transiently transfected using Lipofectamine reagent (Invitrogen) according to the manufacturer's protocol. After 4 h incubation, the transfection mixture was replaced with 2 ml of DMEM plus 10% FBS. Cells were cultivated for an additional 24 h before collection.

2.4. Western blot

The AD293 cells were harvested 24 h after transfection. Then protein samples separated by SDS–PAGE were electrotransferred onto nitrocellulose membrane. The membrane was blocked at room temperature for 1 h with TBS containing 5% (w/v) skim milk. The membrane was then incubated overnight at 4 °C with mouse anti-Myc monoclonal antibody (1:1000 dilutions, Sigma), washed three times with a mixture of TBS and 0.1% Tween 20 (Sigma) and incubated with HRP-conjugated goat anti-mouse antibody (1:5000 dilution, Santa Cruz) at room temperature for 1 h. The membrane was then washed again with TBS-T and developed with the ECL system (Santa Cruz).

2.5. Immunofluorescence assay

AD293 cells were plated on coverslips and transfected with Lipofectamine (Invitrogen). After 36 h, cells were washed twice with PBS (pH 7.4) and fixed in 4% paraformaldehyde for 10 min at room temperature. Then cells were resolved by 0.1% (v/v) Triton X-100 for 5 min, washed again and stained with Hoechst 33258 (5 lg/mL) for 10 min at room temperature in the dark. Images were viewed using fluorescence microscope (LEICA).

2.6. Flow cytometry analysis

After transfection for 36 h, cells were harvested and incubated with RNAase (100 lg/ml) and propidium iodide (50 g/mL) for 5 min at 37 °C. The degree of apoptosis was indicated by the percentage of cells in the sub-G1 fraction.

2.7. Quantitative real-time PCR

RNA was extracted with Trizol (Invitrogen) from cultured cells and reverse transcripted to cDNA with reverse transcription kit (Invitrogen) according to the manufacturer's protocol. Quantitative real-time PCR was performed using the SYBR green Supermix kit (Takara) with the iCycler detection system (Bio-RAD) at the condition of 95 °C for 5 min, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s and 72 °C for 20 s. Quantified transcripts of GAPDH gene was used as endogenous RNA controls. All experiments were performed thrice for each data point.

3. Results

3.1. Identification of a new human PIDD splicing variant

When searching human EST databases for sequences with homology to the conserved death domain motif, we obtained a series of human ESTs and assembled them into a contig containing 3364 nucleotides. This contig represents a new human PIDD splicing variant, and we named it PIDD4 (GenBank Accession No. AY295873). ESTs supporting this new isoform include DA582346, BE2999282, BG290727, CN427942.1 and BM023243. Comparing to PIDD1, PIDD4 coding region contains an extra of 60 nucleotides at the 5′ end of exon 3, as well as intron 3 (Fig. 1A). There are stop codons locating in both the 5′ end of exon 3 and intron 3. Therefore, the translation of PIDD4 starts from the ATG codon in exon 5 and results a protein product of 597 amino acids (the predicted molecular weight is 66.7 KD) (Fig. S1). From Fig. 1A and Fig. S1, we can also see that PIDD2 gets translated from exon 3. Since the inserts do not alter the ORF of the rest sequence, PIDD4 only lacks the N-terminal LRR domain which is identified in other three PIDD isoforms (Fig. 1B). We have amplified PIDD regions from exon 2–5 as well as from exon 5–16 from human testis cDNA library. And the sequencing result confirmed the existence of PIDD4 splicing variant (Fig. 1C and D and Fig. S1).

3.2. The tissue expression profile of PIDD4

Utilizing primers located in intron 3 and exon 4 respectively, RT-PCR was performed to detect the distribution of PIDD4 in 18 human tissues. The results showed that PIDD4 expresses in all the tested tissues except heart. PIDD4 has a high expression level in testis, ovary, colon, bladder and spleen, and its expression level is much lower in liver, kidney, bone marrow, prostate, stomach and skeletal muscle (Fig. 2A). When compared with other isoforms, PIDD4 does not express in heart as PIDD2, but uniquely expresses in skeletal muscle though the expression level is very low, (Fig. 2B). It has been shown that the transcription of PIDD1 can be regulated by p53 [4]. Since PIDD4 and PIDD1 share the same promoter, we further examined the mRNA level of PIDD4 after p53 over-expression with

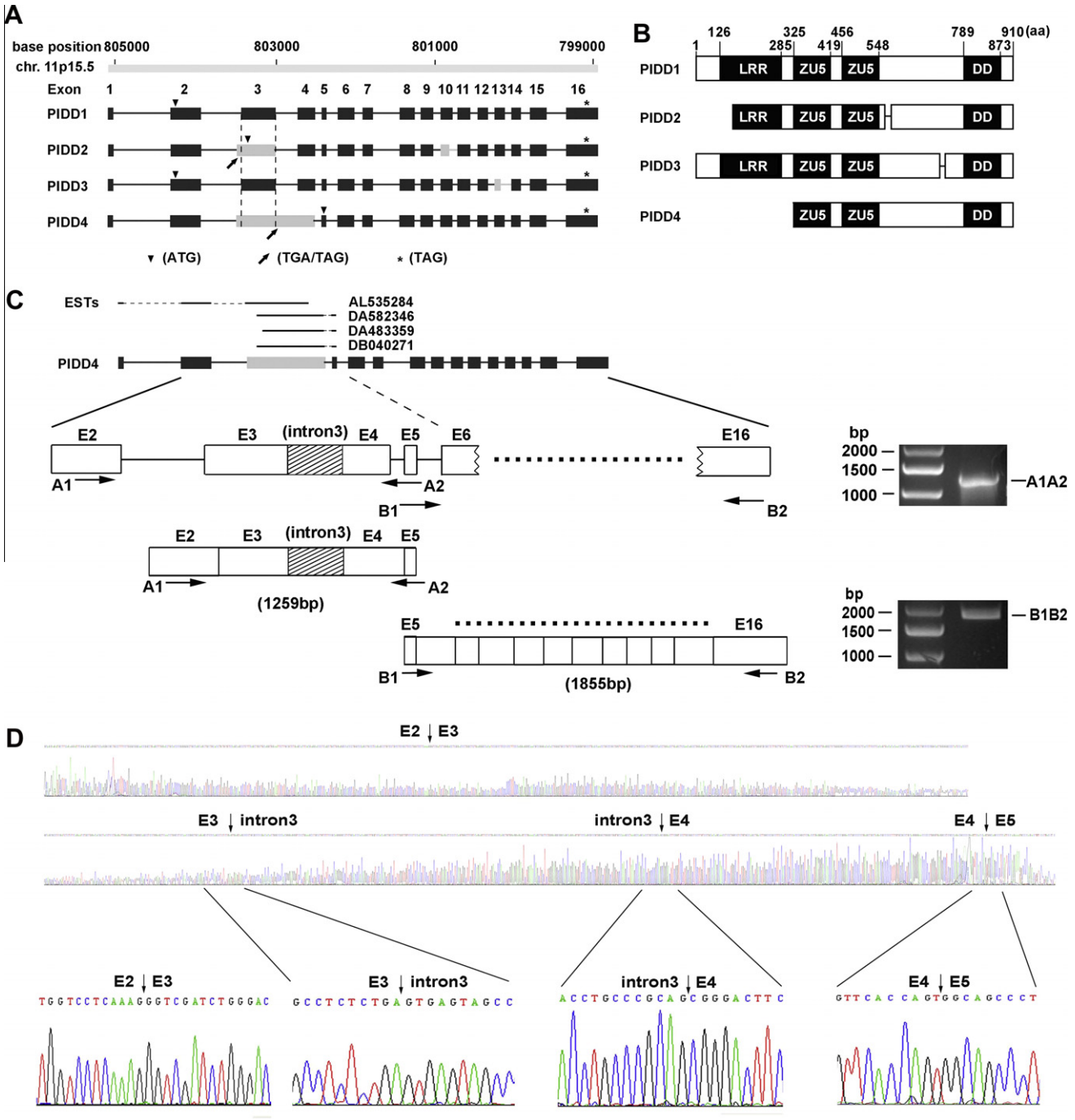

Fig. 1. Alternative splicing variants of human PIDD gene. (A) Schematic diagram of the alternative splicing variants of human PIDD gene. Introns and exons are indicated with lines and boxes, respectively. And the exons which could be alternatively spliced are gray colored. (B) Domain comparison of the four PIDD isoforms, the starting and ending amino acids for each domain have been shown in the figure. (C) Identification of PIDD4 transcript with RT-PCR. We listed the supporting ESTs here, and primers used for RT-PCR from human testis cDNA library are indicated as arrows. (D) Sequencing result of the 5′-UTR region of PIDD4 subtype.

real-time quantitative PCR. As expected, PIDD mRNA increased significantly after p53 induction (Fig. 2C), suggesting that PIDD4 expression may also be subjected to p53 regulation.

3.3. Subcellular localization and auto-cleavage of PIDD4

In order to study the subcellular localization of PIDD4, we cloned PIDD4 into pEGFP-C1 vector and transfected the construct into AD293 cells, and the empty vector was used as a negative control. 36 h after transfection, cells were fixed with 4% paraformaldehyde, and the nucleus was stained with Hoechst. We found that PIDD4 localized in the cytoplasm of AD293 cells (Fig. 3A).

Previous studies have shown that PIDD1 could undergo auto-cleavage, resulting in three fragments of PIDD1-N, PIDD1-C, and PIDD-CC. And PIDD1-C and PIDD1-CC activate NF-κB and caspase-2 pathways, respectively [7–9]. In order to investigate whether PIDD4 can also undergo auto-cleavage, we over-expressed PIDD4 in the Saos-2 cells. Utilizing the C-terminal myc tag of pcDNA3.1 and the N-terminal GFP tag of pEGFPC1 vectors, we

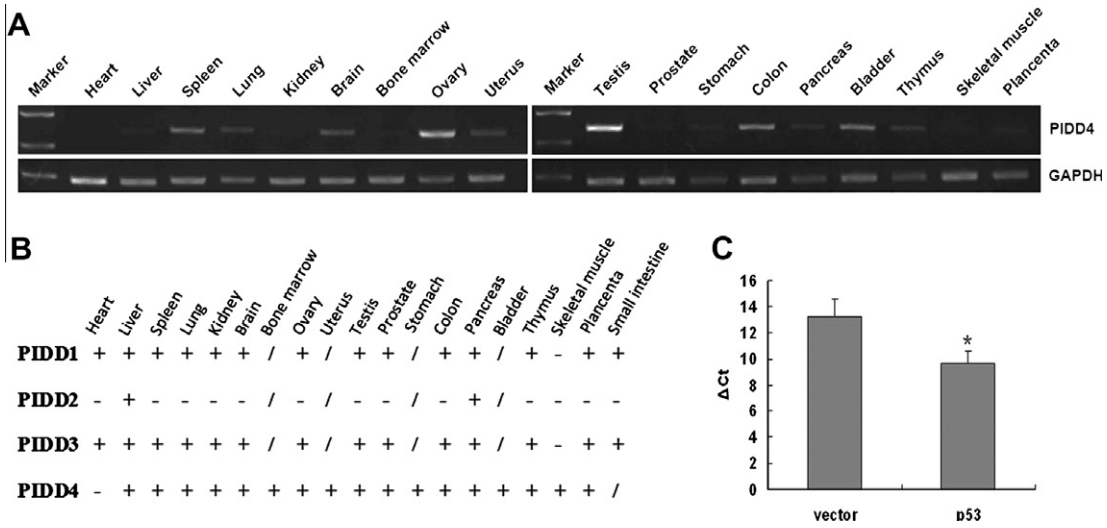

Fig. 2. Expression profile of PIDD4 in human tissues. (A) Distribution of PIDD4 in 18 human tissues. PIDD4 is expressed in all the tissues except heart. It is expressed in testis, ovary, colon, bladder and spleen at high levels, while in liver, kidney, bone marrow, prostate, stomach and skeletal muscle at trace levels. As a control, human GAPDH gene was also amplified in the same conditions. (B) Distribution comparison of the four PIDD isoforms in human tissues. (C) Expression of PIDD4 is regulated by p53. PIDD4 mRNA level was checked with real-time quantitative PCR after p53 over-expression. $^{*}p < 0.05$, *t*-test.

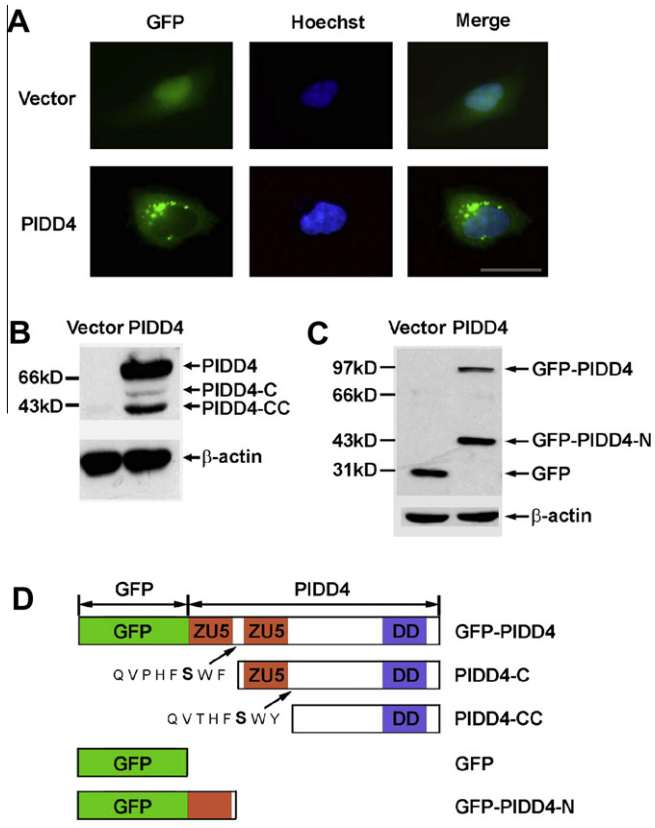

Fig. 3. Subcellular localization and auto-cleavage of PIDD4. (A) PIDD4 locates in the cytoplasm. AD293 cells were transfected with pEGFP-C1-PIDD4 construct (empty vector pEGFP-C1 was used as control). And 36 h after transfection, cells were fixed by 4% paraformaldehyde and stained with Hoechst for nuclei. The scale bar represents 10 μm. (B) Auto-cleavage of PIDD4 protein results into PIDD1-C and PIDD-CC. Saos-2 cells were transfected with pcDNA3.1-myc-PIDD4 (pcDNA3.1-myc vector as control). 24 h after transfection, cell lysates were detected with anti-Myc antibody by Western blot. β-actin is the intracellular control here. (C) Auto-cleavage of PIDD4 protein also produces PIDD1-N. This time, pEGFP-N1-PIDD4 construct was used. (D) Schematic representation of auto-cleavage of PIDD4. The amino acid sequences around the cleavage sites (Ser) are indicated. DD, death domain; ZU5, ZU5 domain.

checked the formation of auto-cleavage fragments, if any, of PIDD4 with Western blot. The results show that PIDD4 is about 67 kDa, consistent with previous prediction. And auto-cleavage of PIDD4 resulted in PIDD4-C (51 kDa), PIDD4-CC (37 kDa) and PIDD4-N (16 kDa) (Fig. 3B–D). Interestingly, the proportion of PIDD4-CC was significantly greater than that of PIDD4-C, which is opposite in the case of PIDD1 [7].

3.4. Over-expression of PIDD4 can promote apoptosis independently

In order to study the role of PIDD4 in apoptosis, Saos-2 cells were transfected with PIDD4 and analyzed by flow cytometry. Comparing to the control group, Saos-2 cells transfected with PIDD4 showed an obvious sub-G1 peak, indicating a significantly increased apoptotic cell population. The sub-G1 ratio of the Saos-2 cells transfected with pcDNA3.1-PIDD4 was 10.85%, while that of the control group was 1.34% (Fig. 4A). The difference is statistically significant.

We further verified the effect of PIDD4 on apoptosis with cell morphology. The apoptotic cells still can uptake Hoechst 33258 dye. As cell nucleus shrink during apoptosis, the nucleus of apoptotic cells showed condensed chromatin, which produce more intense blue fluorescence than normal cells [10]. Therefore, we transfected AD293 cells with pEGFPC1-PIDD4, and 36 h after transfection, cells were stained with Hoechst 33258. For cells transfected with empty vector, we found that their nucleus showed uniform light blue color. The nucleus of the pEGFPC1-PIDD4 positive cells showed bright blue fluorescence, and the cells also possess typical apoptotic morphological changes like nuclear condensation and apoptotic bodies. By characteristic morphological analysis, the apoptotic rate of the AD293 cells over-expressing PIDD4 (therefore GFP positive) was 36.0% while that of the control group was only 10.0%. And the difference is extremely significant in statistics (Fig. 4B). Our results indicated that PIDD4 can independently induce apoptosis.

4. Discussion

So far three PIDD isoforms have been reported. All three subtypes contain a C-terminal death domain and can affect apoptosis via activation of NF-κB pathway. Moreover, each isoform has its unique features. PIDD1 is a p53 dependent gene, and can induce

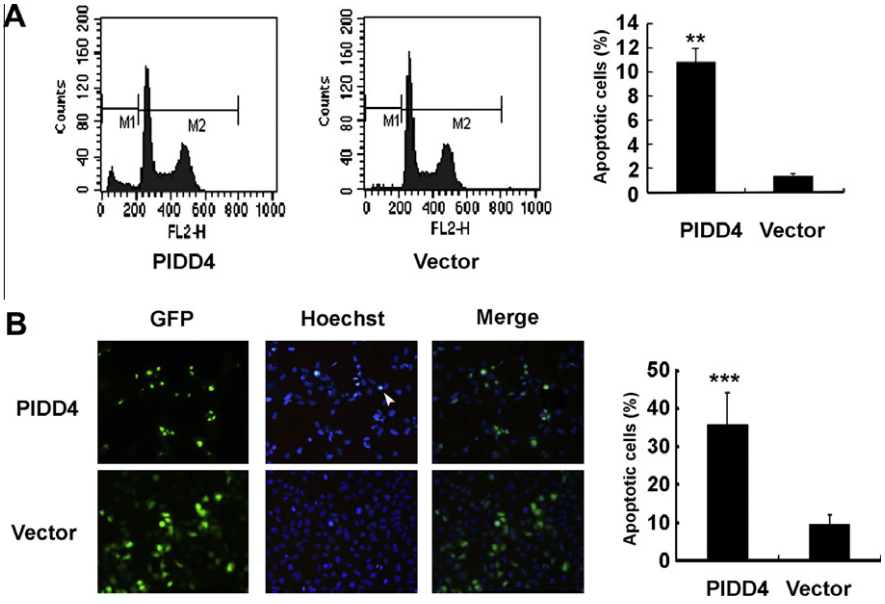

Fig. 4. Over-expression of PIDD4 promotes apoptosis. (A) Over-expression of PIDD4 increased sub-G1 phase cells. Saos-2 cells were transfected with PIDD4 construct or empty vector, and were subjected to flow cytometry analysis 36 h after transfection. Sub-G1 phase was labeled with M1. $^{**}p < 0.01$, *t*-test. (B) PIDD4 can induce nuclear condensation. AD293 cells were transfected with pEGFPC1-PIDD4. 36 h after transfection, cells were stained with Hoechst 33258. We then calculated the apoptic cells (with bright blue nuclei) on the proportion of GFP-positive cells. $^{***}p < 0.001$, *t*-test. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

apoptosis [9]. PIDD2 interacts with two other death domain-containing proteins FADD and MADD through their leucine-rich repeats (LRRs), and it can protect cells from apoptosis induced by PIDD1. On the contrary, co-expression of PIDD3 with PIDD1 increases the sensitivity of cells to genotoxic stress [5].

In this study, we first cloned and identified a new splicing isoform of human PIDD gene, PIDD4. Comparing to the most studied PIDD1 subtype, PIDD4 harbors an extra 60 bp insertion of 5′ end of exon 3, which contains a stop codon, making the translation initiate from the next start codon. And this is the same as for PIDD2. In addition, PIDD4 has another insertion of intron 3 (between exon 3 and 4), which also contains a stop codon. Eventually the translation of PIDD4 begins from the start ATG in exon 5. PIDD4 codes 597 amino acids and has a predicted molecular weight of 66.7 kDa. It contains two N-terminal ZU-5 domains (which present in ZO-1 and Unc5-like netrin receptors) and a C-terminal DD domain (death domain). Since there is no frameshift happening during alternative splicing, PIDD4 only lacks the N-terminal LRR domain when compared with PIDD1. And all other domains of these two isoforms are exactly the same. We have proven the existence of this series of insertion in PIDD4 isoform experimentally. The exon 2–5 as well as exon 5–16 had been amplified by PCR, and sequenced for confirmation.

To further explore the PIDD4 mRNA distribution, we used RT-PCR analysis of the expression of PIDD4 in 18 human tissues, and found that it was expressed in all 17 tissues except heart. PIDD4 has a high expression level in the testis, ovary, colon, bladder and spleen, while in liver, kidney, bone marrow, prostate, stomach and skeletal muscle, its expression was very low. This is different to the expression profiles reported for PIDD1 [3]. PIDD1 is detected in high abundance in kidney, but only barely in testis, and this is just the opposite of PIDD4. PIDD1 expresses in heart, while neither PIDD4 nor PIDD2 does so. These results suggest that PIDD4 and PIDD1 may play roles in a different degree in different tissues. In addition, we also found that PIDD4 uniquely expresses in skeletal muscle, though the expression level is very low. The transcription of PIDD1 is regulated by p53 [3]. Since PIDD4 and PIDD1 have the same promoter, we predict that PIDD4 is also regulated by p53. Indeed, our real-time quantitative PCR result showed that over-expression of p53 increased the mRNA level of PIDD4 significantly.

With EGFP-PIDD4 fusion protein, we found that PIDD4 mainly located in the cytoplasm of nuclear periphery and had a spotty distribution. The precise positioning of PIDD4 still needs further study. Previous report has shown that PIDD1, as a molecular switch, can undergo auto-cleavage to generate PIDD1-C and PIDD-CC, and these two fragments activate NF-κB and caspase-2 pathway, respectively [8,9]. Moreover, the amount of PIDD1-C fragment is higher than PIDD1-CC. We found PIDD4 can also selfcut into PIDD4-C and PIDD4-CC two fragments, but it is interesting that PIDD4-CC level is more than PIDD4-C, suggesting PIDD4 may have more significant pro-apoptotic capacity than PIDD1. Besides PIDD1-C and PIDD1-CC, auto-cleavage of PIDD1 generates another fragment containing the N-terminal LRR and ZU-5 domains, namely PIDD1-N [7]. In 2007, Tinel et al. constructed a PIDD1 mutation missing its N-terminal LRR domain, and this mutant contains similar domains to PIDD4. They found that the mutant can effectively activate NF-κB, thereby activating the survival pathway, and its activating ability was even more than that of the full-length PIDD1 [7]. PIDD2 is antagonistic to PIDD1 in apoptosis, and PIDD3 only strengthens the pro-apoptotic function of PIDD1 when in co-expression with it. Here, we showed that PIDD4 might be a new PIDD family member which can induce apoptosis independently, and its ability to promote apoptosis might be more than that of PIDD1.

In order to study the role of PIDD4 during apoptotic process, we analyzed cells over-expressing PIDD4 in the perspectives of cell cycle and morphology. Experimental results showed that PIDD4 induced the increase of Sub-G1 cells and apparent nucleus shrinkage, and both are characteristics of apoptosis. All these showed that PIDD4 can induce apoptosis independently, but the specific molecular mechanisms still need further study.

Acknowledgments

This work was supported by the National 973 Program of China (2004CB518605), the National 863 Project of China (2006AA020501), the National Key Sci-Tech Special Project of Chi-

na (2008ZX10002-020), the Project of the Shanghai Municipal Science and Technology Commission (03dz14086) and the National Natural Science Foundation of China (30024001, 30771188).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.02.114.

References

[1] D. Hanahan, R.A. Weinberg, The hallmarks of cancer, Cell 100 (2000) 57–70.
[2] S.P. Cregan, A. Fortin, J.G. MacLaurin, S.M. Callaghan, F. Cecconi, S.W. Yu, T.M. Dawson, V.L. Dawson, D.S. Park, G. Kroemer, R.S. Slack, Apoptosis-inducing factor is involved in the regulation of caspase-independent neuronal cell death, J. Cell Biol. 158 (2002) 507–517.
[3] Y. Lin, W. Ma, S. Benchimol, Pidd, a new death-domain-containing protein, is induced by p53 and promotes apoptosis, Nat. Genet. 26 (2000) 122–127.
[4] J.B. Telliez, K.M. Bean, L.L. Lin, LRDD A novel leucine rich repeat and death domain containing protein, Biochim. Biophys. Acta 1478 (2000) 280–288.
[5] S. Cuenin, A. Tinel, S. Janssens, J. Tschopp, P53-induced protein with a death domain (PIDD) isoforms differentially activate nuclear factor-kappaB and caspase-2 in response to genotoxic stress, Oncogene 27 (2008) 387–396.
[6] Z.H. Wu, A. Mabb, S. Miyamoto, PIDD: a switch hitter, Cell 123 (2005) 980–982.
[7] A. Tinel, S. Janssens, S. Lippens, S. Cuenin, E. Logette, B. Jaccard, M. Quadroni, J. Tschopp, Autoproteolysis of PIDD marks the bifurcation between pro-death caspase-2 and pro-survival NF-kappaB pathway, EMBO J. 26 (2007) 197–208.
[8] S. Janssens, A. Tinel, S. Lippens, J. Tschopp, PIDD mediates NF-kappaB activation in response to DNA damage, Cell 123 (2005) 1079–1092.
[9] C. Berube, L.M. Boucher, W. Ma, A. Wakeham, L. Salmena, R. Hakem, W.C. Yeh, T.W. Mak, S. Benchimol, Apoptosis caused by p53-induced protein with death domain (PIDD) depends on the death adapter protein RAIDD, Proc. Natl. Acad. Sci. USA 102 (2005) 14314–14320.
[10] A.H. Wyllie, R.G. Morris, A.L. Smith, D. Dunlop, Chromatin cleavage in apoptosis: association with condensed chromatin morphology and dependence on macromolecular synthesis, J. Pathol. 142 (1984) 67–77.