PDCD5 regulates cell proliferation, cell cycle progression and apoptosis

PENGHUI LI[1], HONGXIN FEI[2], LIHONG WANG[3], HUIYU XU[3], HAIYAN ZHANG[2] and LIHONG ZHENG[1]

Departments of [1]Biogenetics, [2]Histology and Embryology, and [3]Immunology, Qiqihar Medical University, Qiqihar, Heilongjiang 161006, P.R. China

Received February 13, 2017; Accepted August 3, 2017

DOI: 10.3892/ol.2017.7401

Abstract. Programmed cell death (PDCD)5 is cloned from human leukemia cell line TF-1. PDCD5 is one of the members of the programmed cell death protein family that is frequently involved in tumor growth and apoptosis. To investigate the molecular and cellular functions of PDCD5, the present study established a PDCD5 stably overexpressing A431 cell line and examined the role of PDCD5 in cell proliferation, cell cycle progression and apoptosis. The data demonstrated that overexpression of PDCD5 significantly inhibited cell proliferation, induced cell cycle arrest at G2/M phase and apoptosis in A431 cells. The expression profiles of certain key regulators of these cellular events were further investigated, including P53, B cell lymphoma (BCL)-2, BCL-2 associated X protein (BAX) and caspase (CASP)3. The data demonstrated that at the transcript and protein levels, P53, BAX and CASP3 were all upregulated in the PDCD5 stably overexpressing A431 cells whereas BCL-2 was downregulated, indicating that PDCD5 acts as an important upstream regulator of P53, BCL-2, BAX and CASP3. The data suggest that PDCD5 regulates cell proliferation, cell cycle progression and apoptosis in A431 cells. PDCD5 may be a novel tumor suppressor gene, and may be potentially used for cancer treatment in the future.

Introduction

Cell proliferation and apoptosis are key cellular events in the development of organisms (1). A number of biological processes, such as tissue development and homeostasis, require a balance between cell proliferation and apoptosis, dysregulation of which would result in different types of human diseases (1). In eukaryotes, the cell cycle consists of four stages: G1/G0, S, G2 and M. Each stage needs to be monitored by specific checkpoint to ensure that the genetic information of the cell is faithfully transmitted to the next generation. The G2/M checkpoint (DNA damage checkpoint) is an important cell cycle checkpoint to prevent the cell from entering the mitosis with DNA damage (2). In other words, cells will be arrested at G2/M phase when genomic DNA is damaged and needs to be repaired. All somatic cells proliferate via a mitotic process determined by successful progression of the cell cycle (3).

Programmed cell death 5 (PDCD5) is a one of the members of programmed cell death protein family. The gene *PDCD5*, alternatively named *TFAR19*, was originally cloned from human leukemia cell line TF-1 (4). This gene is localized on chromosome 19q12-q1311 and spans around 6 kb of genomic DNA that contains 5 introns and 6 exons. The open reading frame of *PDCD5* encodes a 125-aa protein that is highly conserved ranging from yeast to human (4). *PDCD5* is ubiquitously expressed in different tissues and involved in the regulation of apoptosis in different cell types (4-8). The apoptotic potential of PDCD5 may be partially resulted from its phosphorylation at serine 118 by CK2, which is required for the nuclear translocation of PDCD5 in response to genotoxic stress (9,10). Recently, it was shown that PDCD5 is also an important regulator of the non-apoptotic programmed cell death (PCD), designated 'paraptosis' (11). More recently, it was reported that PDCD5 also regulates autophagy to protect against cardiac remodeling (12). Dysregulation of *PDCD5* has been found to be involved in different type of tumors (13-22). The antitumor activity of PDCD5 has been also proposed (23-29) and low expression level of PDCD5 has been suggested to be a prognostic indicator for cancers (30). PDCD5 was also indicated to have the therapeutic potential in the treatment of rheumatoid arthritis and other autoimmune diseases because of its inflammatory effects (31,32). Knockout of *PDCD5* can also protect the brain from ischemic injury by inhibiting the PDCD5-VHL pathway (33).

PDCD5 is downregulated in the lung adenocarcinoma patients compared to the healthy controls, which indicates PDCD5 is a tumor suppressor gene associated with lung cancer (34). Single nucleotide polymorphism in the *PDCD5* gene locus was also found to be associated with non-small cell lung cancers (35). Recently, a few important interacting partners of PDCD5 have been discovered, including Tip60, CK2, CTT, p53, tumor suppressor protein pVHL and YY1-associated factor 2 (YAF-2) (9,36-41). In the genotoxic conditions, PDCD5 selectively mediates HDAC3 dissociation

Correspondence to: Professor Lihong Zheng, Department of Biogenetics, Qiqihar Medical University, Bukui North Street 333, Jianhua, Qiqihar, Heilongjiang 161006, P.R. China
E-mail: lihongzheng11@hotmail.com

Key words: cell proliferation, apoptosis, cell cycle progression, G2/M arrest, PDCD5

from p53, and induces HDAC3 degradation through the ubiquitin-dependent proteasomal pathway, which subsequently activates p53 as a result in response to the stress (42,43). The promoter activity of *PDCD5* is activated by the transcription factor NF-κB p65 (44) and the protein stability of *PDCD5* are positively regulated by YAF2 and OTUD5 (41,45), and negatively regulated by DNAJB1 (46).

In the present study, we investigate the roles of PDCD5 in cell proliferation, cell cycle progression and apoptosis by using a PDCD5 stably overexpressing A431 cell line. We further examine whether these changes of cellular processes caused by overexpression of PDCD5 are related to the P53 signaling pathway.

Materials and methods

Reagents and cell line. DMEM [10% fetal bovine serum (FBS), 2 mM glutamine, 1% penicillin/streptomycin]. The A431 cells were cultured at 37˚C incubator supplemented with 5% CO_2. dNTP (10 mM) and One Step SYBR® PrimeScript™ RT-PCR kit were purchased from Takara Bio (Dalian, China); Primers were synthesized by GeneCreate Biological Engineering Co., Ltd. (Wuhan, China); TRIzol was purchased from Invitrogen (Carlsbad, CA, USA); MTT was purchased from Sigma (St. Louis, MO, USA; cat. no. m5655); FBS was purchased from Gibco; PI and Annexin V-FITC were purchased from Beyotime. Antibodies were purchased from Cusabio. The PDCD5 overexpressing A431 cell line was established by GeneCreate Biological Engineering Co., Ltd. (Wuhan, China). The cell line stably transfected empty vector was used a control.

MTT assay. Cells splitted into each well of 96-well plate with the cell density ~1000-10000 cells/well. 180 µl of diluted cells was added into each well. 5 different time points including 12, 24, 48, 72 and 96 h were set-up and each time point has 5 replicates for PDCD5 overexpressing and control cells. The cells were cultured in the 37˚C incubator supplemented with 5% CO_2. 20 µl of MTT (5 mg/ml, 0.5% MTT) was added to each well and the cells were cultured for additional 4 h. The culture media were carefully removed and 100 µl DMSO was added into each well. The plate was gently shaken on the shaker at low speed for 10 min to dissolve the crystal completely. The absorbance of each well was measured at OD490 nm by using the ELISA reader.

Flow cytometry. The cells were synchronized with serum withdrawal (media without serum) for 24 h and then replenished with 10% FBS containing DMEM media for additional 48 h. The cells were then trypsinized and transferred to the collection tube. Cells were pelleted by centrifuge at 1000 rpm for 5 min. The supernatant was removed and cells were resuspended with ice-cold PBS and then transferred into 1.5 ml tube, and repelleted by centrifugation. The cells were then fixed in 1 ml of 70% cold ethanol for >2 h. The cells were pelleted again by centrifugation at 1000 rpm for 5 min. The supernatant was carefully aspirated and the cells were suspended with 1 ml ice-cold PBS. The cells were then repelleted and the supernatant was carefully removed. 0.5 ml PI solution (PI 5 mg, RNase 2 mg, 1.0% Triton X-100 0.25 ml, saline 65 ml, sodium citrate 100 mg, ddH_2O was added to bring total volume to 100 ml and the pH value was adjusted to 7.2-7.6; Stored at 4˚C in brown bottle and keep away from light) was added into the cells and incubated at 37˚C for 30 min without light exposure. The cells can be stored at 4˚C or kept on ice. Flow experiments should proceed within 24 h after PI staining. Flow cytometer (Beckman Moflo XDP) was set at 488 nm (excitation wavelength) to detect red fluorescence and light scattering. DNA content and light scattering analyses were performed by using Modfit software.

Real-time quantitative PCR (qPCR). Total RNA was extracted by using TRIzol method (Invitrogen) according to the instructions of the manual. 1 ml TRIzol was added to the cell pellet (containing ~$1x10^7$ cells), mixed well and incubated at RT for 5 min. 0.2 ml chloroform was then added, votexed for 15 sec and incubated for 3 min. The lysates were centrifuged at 12,000 rpm, 4˚C for 10 min. The supernatant was removed and mixed well with 0.5 ml cold isopropanol, and kept on ice for 20~30 min. The mixture was then centrifuged at 12,000 rpm, 4˚C for 10 min to pellet the RNA. The supernatant was removed and the pellet was washed with 1 ml 75% ethanol. The RNA/ethanol mixture was centrifuged again at 7,500 g for 5 min, and the supernatant was discarded. The RNA was air-dried and dissolved in ddH_2O. The qPCR reaction was as follows: RNA (template): 2 µl; SYBR® PrimeScript Master Mix (2x), 12.5 µl; forward primer (20 µM): 0.5 µl; reverse primer (20 µM): 0.5 µl; ddH_2O: 11.5 µl; total volume, 25 µl. GAPDH or β-actin was used as an internal control. Primer sequences are presented below in Table I. qRT-PCR program was as follows: 45˚C, 15 min; 95˚C, 5 min; 95˚C, 20 sec; 60˚C, 20 sec; 72˚C, 30 sec; 40 cycles. The data were analyzed by using $2^{-\Delta\Delta Cq}$ method.

Western blot analysis. Total protein was extracted from about $1x10^7$ A431 cells. The lysate was equally mixed with 2X loading buffer and boiled at 100˚C for 5 min, and then subjected to 15% sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE). Proteins were then electrotransferred onto a PVDF (Bio-Rad) membrane, which was then blocked with 5% non-fat milk in PBST (1xPBS with 0.05% Tween-20) at RT for 1 h. The membrane was then incubated with each individual primary antibody, including PDCD5, P53, BAX, BCL-2, CASP3 and GAPDH, at 37˚C for 1 h. GAPDH was used as a loading control. The membrane was then washed with PBST for 5 min/3 times and proceeded to incubate with secondary antibody (goat anti-mouse or goat anti-rabbit) at 37˚C for 1 h. After washing with PBST for 5 min/3 times, membrane-bound antibodies were detected with ECL (enhanced chemiluminescence) kit.

Results

Establishment of PDCD5 stably overexpressing A431 cell line. A431 is a cell line derived from human epidermoid carcinoma and has been widely used in the studies on cell cycle progression and tumor-related signaling pathways. Therefore, we introduced this cell line to our studies. The cells were transfected with PDCD5 construct and its empty vector, respectively. To confirm whether the stable cell line was successfully

Table I. Primers used for quantitative RT-PCR.

Gene	Primer	Sequence	Tm (˚C)
PDCD5	Forward	5'-ACAGATGGCAAGATATGGACA-3'	60
	Reverse	5'-TCCTAGACTTGTTCCGTTAAG-3'	
P53	Forward	5'-CAGCCAAGTCTGTGACTTGCA-3'	60
	Reverse	5'-GTGTGGAATCAACCCACAGCT-3'	
BAX	Forward	5'-CCCTTTTGCTTCAGGGTTTCATCCA-3'	60
	Reverse	5'-CTTGAGACACTCGCTCAGCTTCTTG-3'	
BCL-2	Forward	5'-CTGCACCTGACGCCCTTCACC-3'	60
	Reverse	5'-CACATGACCCCACCGAACTCAAAGA-3'	
CASP3	Forward	5'-CATGGAAGCGAATCAATGGACT-3'	60
	Reverse	5'-CTGTACCAGACCGAGATGTCA-3'	

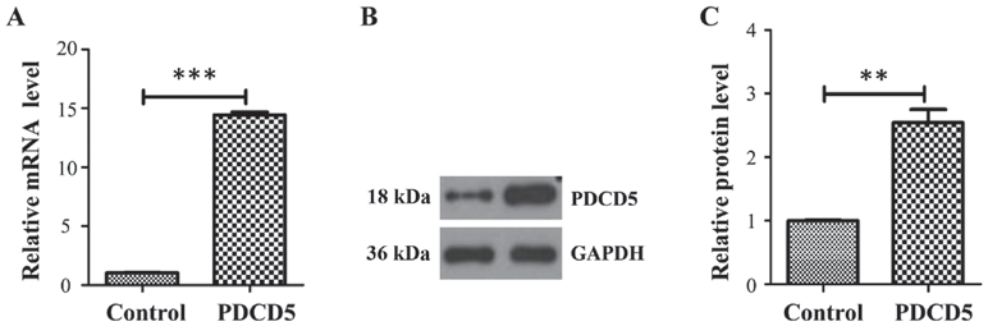

Figure 1. Verification of PDCD5 overexpression at the transcript and protein levels in the A431 overexpressing cells. (A) The transcript levels in the PDCD5 overexpressing A431 cells and its empty vector-transfected cells were analyzed by qRT-PCR. (B) The expression levels in the PDCD5 overexpressing A431 cells and its empty vector-transfected cells were detected by western blot. The protein level of PDCD5 is strikingly increased in the PDCD5 overexpressing A431 cells compared with the empty vector control. GAPDH was used as a loading control. (C) Quantification for the western blot data in (B) from there independent experiments. **P<0.01 and ***P<0.001. Control, empty vector-transfected A431 cells; PDCD5, PDCD5 overexpressing A431 cells.

established, we performed quantitative RT-PCR and western blot. At the transcript level, PDCD5 was increased ~15-fold in the PDCD5 stably overexpressing A431 cells compared with the empty vector control (Fig. 1A). At the protein level, PDCD5 was also strikingly increased in the PDCD5 overexpressing cells compared with the control (Fig. 1B and C).

Overexpression of PDCD5 inhibits A431 cell proliferation. To examine whether overexpression of PDCD5 affects cell proliferation, MTT assay was performed for the PDCD5 overexpressing cells and control cells at different time points, including 12, 24, 48, 72 and 96 h. The growth curves for these two cell lines were generated according to the OD values at these time points. The data indicated that at the time points of 72 and 96 h, cell proliferation in the PDCD5 stably overexpressing cells was significantly slower than that in the empty vector control cells (Fig. 2).

Overexpression of PDCD5 induces cell cycle arrest at G2/M phase in A431 cells. To further understand the inhibitory effect of PDCD5 on cell proliferation, we performed flow cytometry to investigate the distribution of specific phases of cell cycle in the PDCD5 stably overexpressing A431 cells and its empty vector control. The results showed that PDCD5 overexpressing cells were strikingly arrested at the G2/M phase of the cell cycle, compared with the control cells (Fig. 3A and B).

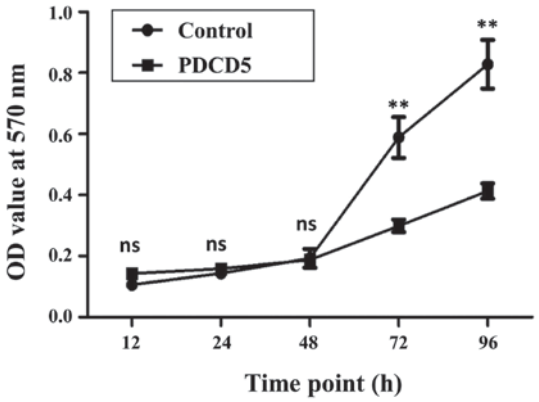

Figure 2. MTT assay was performed to monitor cell proliferation in PDCD5 overexpressing A431 cells and its control cells. The cells were splitted at the similar density for the PDCD5 overexpressing cells and the control cells. Cell growth was monitored at multiple time points, including 12, 24, 48, 72 and 96 h. From 72 h, the cell viability was significantly lower in the PDCD5 overexpressing cells compared with the control cells. **P<0.01. Control, empty vector-transfected A431 cells; PDCD5, PDCD5 overexpressing A431 cells.

Overexpression of PDCD5 induces apoptosis in A431 cells. Cell cycle arrest at G2/M is generally resulted from DNA damage. To investigate whether the PDCD5 overexpressing cells with DNA damage underwent apoptosis, we performed flow cytometric analysis as well. The data indicated that

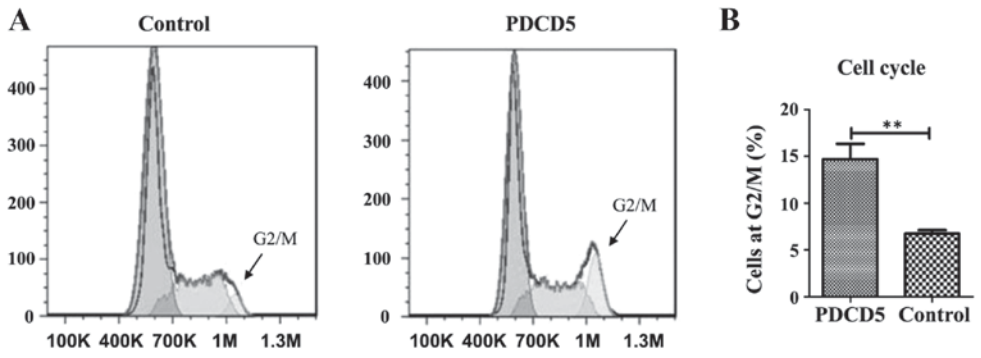

Figure 3. Overexpression of PDCD5 induces G2/M cell cycle arrest in A431 cells. After propidium iodide staining, flow cytometry was performed to determine the distribution of cell cycle phases, including G0/G1, S and G2/M, in the PDCD5 overexpressing cells and its control cells. (A) Cell cycle was strikingly arrested at the G2M phase in the PDCD5 overexpressing cells. Representative images from three independent experiments are shown. (B) Quantification of G2/M arrest in the PDCD5 overexpressing A431 cells and its control cells from three independent experiments. **P<0.01. Control, empty vector-transfected A431 cells; PDCD5, PDCD5 overexpressing A431 cells.

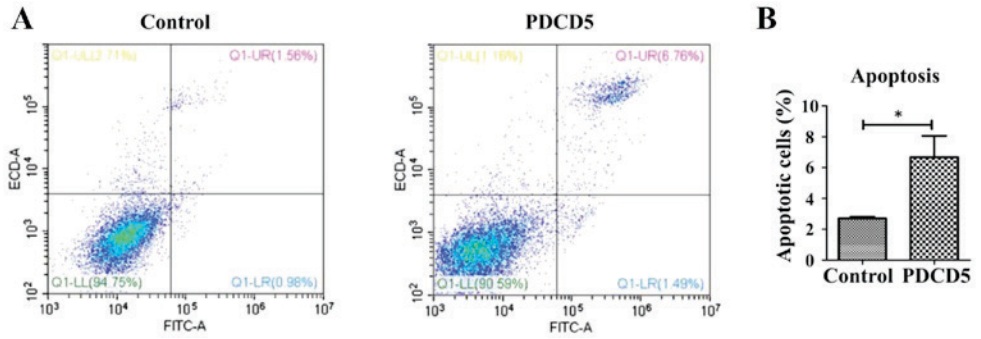

Figure 4. Overexpression of PDCD5 induces apoptosis in A431 cells. Flow cytometry was performed to determine apoptosis in the PDCD5 overexpressing cells and its control cells. (A) More apoptotic cells were clearly observed in the PDCD5 overexpressing cells compared with the control cells. Representative images from three independent experiments are shown. (B) Quantification for the percentage of apoptosis in the PDCD5 overexpressing A431 cells and its control cells from three independent experiments. *P<0.05. Control, empty vector-transfected A431 cells; PDCD5, PDCD5 overexpressing A431 cells.

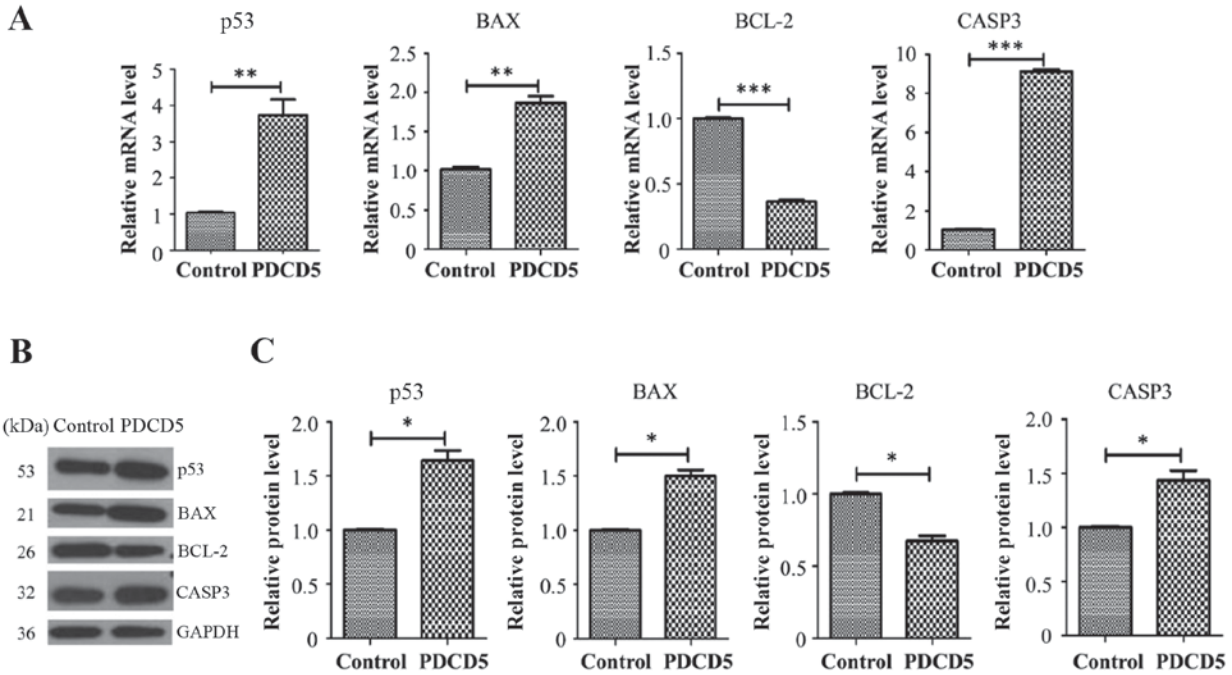

Figure 5. P53, BAX and CASP3 are upregulated while BCL-2 is downregulated in the PDCD5 overexpressing A431 cells. (A) The transcript levels of P53, BAX, BCL-2 and CASP3 in the PDCD5 overexpressing A431 cells and its control cells were analyzed by real-time RT-PCR. (B) The expression levels of P53, BAX, BCL-2 and CASP3 in the PDCD5 overexpressing A431 cells and its control cells were examined by western blot. GAPDH was used as a loading control. (C) Quantification for western blot data in (B) from there independent experiments. *P<0.05, **P<0.01 and ***P<0.001. Control, empty vector-transfected A431 cells; PDCD5, PDCD5 overexpressing A431 cells.

overexpression of PDCD5 strikingly induces apoptosis, compared with the control cells (Fig. 4A and B).

Overexpression of PDCD5 leads to dysregulation of P53, BAX, BCL-2 and CAPS3 in A431 cells. To further understand the molecular underpinnings that might be relevant to decreased proliferation, increased apoptosis and G2/M arrest in the PDCD5 overexpressing A431 cells, we examined the transcript and protein levels of tumor suppressor P53 and key molecules of apoptosis including BCL-2, BAX and CAPS3. Real-time RT-PCR results showed that *P53*, *BAX* and *CAPS3* were all upregulated in the PDCD5 stably overexpressing A431 cells while *BCL-2* is downregulated, compared with the empty vector control cells (Fig. 5A). Western blot by using specific antibodies against these proteins was then performed to further confirm RT-PCR results (Fig. 5B and C). The data showed that the pattern of protein dysregulations was basically consistent with that observed in the qRT-PCR data.

Discussion

Programmed cell death (PCD) is the death of a cell mediated by a serial of intracellular programs. There are three forms of PCD: Apoptosis, autophagy and programmed necrosis (47). It is well known that apoptosis is an orchestrated cellular process that can occur in physiological and pathological conditions (48). Cell proliferation is a cellular event that causes an increase of cell number. In human cancers, cell proliferation is out of control and apoptosis is suppressed (49). Cell proliferation is decreased when cell cycle arrest occurs. In the condition of DNA damage, cell cycle arrest will be initiated as an attempt to repair the damage, however, if the damage is too extensive to be repaired, the cell will undergo cell death in a way of apoptosis (50).

According to the NCBI database, there are currently 12 members in total in the PDCD protein family, namely PDCD1~PDCD12. Among them, PDCD8 and PDCD9 are officially known as AIFM1 and MRPS30, respectively. PDCD1, often known as PD-1, is the member that has been most extensively studied and shown to negatively regulate T cell responses, in collaboration with its two ligands, PD-L1 and PD-L2 (51-53). In addition to PDCD5, other programmed cell death proteins are also known to play important roles in apoptosis and/or cell cycle progression (54-57), and are also dysregulated in many types of human cancers (13,16,58-63). Opposite to what we observed for PDCD5, depletion of PDCD2 in human acute leukemia cells impairs their proliferation, induces cell cycle arrest and p53 activation while overexpression of PDCD2 facilitates cell growth in cancers (55,64). However, in gastric cancer cells, expression of PDCD2 seems to induce cell cycle arrest and apoptosis, which are also found to be p53-dependent (54). This suggests the connection between PDCD2 and cell cycle arrest might be tissue and cancer type-dependent.

In the present study, we used A431 cells as a cell model to investigate the role of PDCD5 in cell proliferation, cell cycle progression and apoptosis. As a human model epidermoid carcinoma cell line, A431 has been widely used in studies on the cell cycle and tumor related cell signaling pathways because epidermal growth factor receptor (EGFR) is known to be strikingly upregulated in these cells (65-68). In this study, we found that in the A431 cell, overexpression of PDCD5 inhibits cell proliferation, induces cell cycle arrest at G2/M phase and apoptosis. We next attempted to examine the molecular underpinnings of such dysregulations in these cellular events described above. Some key molecules involved in cell proliferation, cell cycle progression and apoptosis, including *P53*, *BAX, BCL-2* and *CASP-3,* were found to be dysregulated when PDCD5 was stably overexpressed in A431 cells.

P53 mutation has been found in over 50% of all human cancers (69). Loss of *p53* was recently found to induce cell proliferation via Ras-independent activation of the Raf/Mek/Erk signaling pathway (70). In line with these findings, overexpression of *p53* is known to inhibit cell proliferation (71,72). p53-dependent G1 and G2/M arrests of the cell cycle are important components of the cellular response to a variety of stresses, including DNA damage (73). In the PDCD5 overexpressing A431 cells, we observed upregulation of *P53,* reduced cell proliferation and G2/M arrest, which is consistent with previous reports. p53 activates Bax to mediate mitochondrial membrane permeabilization and apoptosis (74), and inhibits Bcl-2 in some conditions such as apoptotic response to DNA damage (75). Activation of p53 signaling pathway, including upregulation of p53, Bax, caspase-3 and downregulation of Bcl-2, was accompanied with G2/M cell cycle arrest in different cell types when treated with different drugs (76-78). These lines of evidences are in line with our observations in the PDCD5 stably overexpressing A431 cells.

It is noted that all our data in this study were generated by using a PDCD5 stably overexpressing cell model. In our future study, we might need to establish a PDCD5 knockdown cell model and knockout animal model to further confirm the roles of PDCD5 in cell proliferation, cell cycle progression and apoptosis. Moreover, the detailed molecular mechanism underlying the regulation of cell proliferation, cell cycle progression and apoptosis by PDCD5 also needs to be further investigated.

Acknowledgements

Thie present study was supported by the Project of Department of Education of Heilongjiang Province (grant no. 12531797).

References

1. Hipfner DR and Cohen SM: Connecting proliferation and apoptosis in development and disease. Nat Rev Mol Cell Biol 5: 805-815, 2004.
2. Löbrich M and Jeggo PA: The impact of a negligent G2/M checkpoint on genomic instability and cancer induction. Nat Rev Cancer 7: 861-869, 2007.
3. Alenzi FQ: Links between apoptosis, proliferation and the cell cycle. Br J Biomed Sci 61: 99-102, 2004.
4. Liu H, Wang Y, Zhang Y, Song Q, Di C, Chen G, Tang J and Ma D: TFAR19, a novel apoptosis-related gene cloned from human leukemia cell line TF-1, could enhance apoptosis of some tumor cells induced by growth factor withdrawal. Biochem Biophys Res Commun 254: 203-210, 1999.
5. Wang N, Lu HS, Guan ZP, Sun TZ, Chen YY, Ruan GR, Chen ZK, Jiang J and Bai CJ: Involvement of PDCD5 in the regulation of apoptosis in fibroblast-like synoviocytes of rheumatoid arthritis. Apoptosis 12: 1433-1441, 2007.
6. Chen LN, Wang Y, Ma DL and Chen YY: Short interfering RNA against the PDCD5 attenuates cell apoptosis and caspase-3 activity induced by Bax overexpression. Apoptosis 11: 101-111, 2006.

7. Yang YH, Zhao M, Li WM, Lu YY, Chen YY, Kang B and Lu YY: Expression of programmed cell death 5 gene involves in regulation of apoptosis in gastric tumor cells. Apoptosis 11: 993-1001, 2006.
8. Ruan GR, Zhao HS, Chang Y, Li JL, Qin YZ, Liu YR, Chen SS and Huang XJ: Adenovirus-mediated PDCD5 gene transfer sensitizes K562 cells to apoptosis induced by idarubicin in vitro and in vivo. Apoptosis 13: 641-648, 2008.
9. Salvi M, Xu D, Chen Y, Cabrelle A, Sarno S and Pinna LA: Programmed cell death protein 5 (PDCD5) is phosphorylated by CK2 in vitro and in 293T cells. Biochem Biophys Res Commun 387: 606-610, 2009.
10. Li G, Ma D and Chen Y: Cellular functions of programmed cell death 5. Biochim Biophys Acta 1863: 572-580, 2016.
11. Wang Y, Li X, Wang L, Ding P, Zhang Y, Han W and Ma D: An alternative form of paraptosis-like cell death, triggered by TAJ/TROY and enhanced by PDCD5 overexpression. J Cell Sci 117: 1525-1532, 2004.
12. Zhang S, Li G, Fu X, Qi Y, Li M, Lu G, Hu J, Wang N, Chen Y, Bai Y and Cui M: PDCD5 protects against cardiac remodeling by regulating autophagy and apoptosis. Biochem Biophys Res Commun 461: 321-328, 2015.
13. Li H, Wang Q, Gao F, Zhu F, Wang X, Zhou C, Liu C, Chen Y, Ma C, Sun W and Zhang L: Reduced expression of PDCD5 is associated with high-grade astrocytic gliomas. Oncol Rep 20: 573-579, 2008.
14. Du YJ, Xiong L, Lou Y, Tan WL and Zheng SB: Reduced expression of programmed cell death 5 protein in tissue of human prostate cancer. Chin Med Sci J 24: 241-245, 2009.
15. Chen C, Zhou H, Xu L, Liu X, Liu Z, Ma D, Chen Y and Ma Q: Prognostic significance of downregulated expression of programmed cell death 5 in chondrosarcoma. J Surg Oncol 102: 838-843, 2010.
16. Zhang X, Wang X, Song X, Wei Z, Zhou C, Zhu F, Wang Q, Ma C and Zhang L: Clinical and prognostic significance of lost or decreased PDCD5 expression in human epithelial ovarian carcinomas. Oncol Rep 25: 353-358, 2011.
17. Wang Y, Wang GH and Zhang QY: Determination of PDCD5 in peripheral blood serum of cancer patients. Chin J Cancer Res 23: 224-228, 2011.
18. Gao M, Gao W, Wang Z, Liu Y, Li Y, Wei C, Sun Y, Guo C, Zhang L, Wei Z and Wang X: The reduced PDCD5 protein is correlated with the degree of tumor differentiation in endometrioid endometrial carcinoma. Springerplus 5: 988, 2016.
19. Chen Y, Zou Z, Xu A, Liu Y, Pan H and Jin L: Serum programmed cell death protein 5 (PDCD5) levels is upregulated in liver diseases. J Immunoassay Immunochem 34: 294-304, 2013.
20. Xu F, Wu K, Zhao M, Qin Y and Xia M: Expression and clinical significance of the programmed cell death 5 gene and protein in laryngeal squamous cell carcinoma. J Int Med Res 41: 1838-1847, 2013.
21. Wang D, Wang W, Song CL and Xia P: The roles of serum PDCD5 in circulating CD133 positive cells of the patients with gastric cancer. Tumour Biol 37: 11799-11804, 2016.
22. Wang W, Song XW and Zhao CH: Roles of programmed cell death protein 5 in inflammation and cancer (Review). Int J Oncol 49: 1801-1806, 2016.
23. Shi L, Song Q, Zhang Y, Lou Y, Wang Y, Tian L, Zheng Y, Ma D, Ke X and Wang Y: Potent antitumor activities of recombinant human PDCD5 protein in combination with chemotherapy drugs in K562 cells. Biochem Biophys Res Commun 396: 224-230, 2010.
24. Yin A, Jiang Y, Zhang X, Zhao J and Luo H: Transfection of PDCD5 sensitizes colorectal cancer cells to cisplatin-induced apoptosis in vitro and in vivo. Eur J Pharmacol 649: 120-126, 2010.
25. Xu HY, Chen ZW, Pan YM, Fan L, Guan J and Lu YY: Transfection of PDCD5 effect on the biological behavior of tumor cells and sensitized gastric cancer cells to cisplatin-induced apoptosis. Dig Dis Sci 57: 1847-1856, 2012.
26. Han XR, Sun Y and Bai XZ: The anti-tumor role and mechanism of integrated and truncated PDCD5 proteins in osteosarcoma cells. Cell Signal 24: 1713-1721, 2012.
27. Li Y, Zhou G, La L, Chi X, Cao Y, Liu J, Zhang Z, Chen Y and Wu B: Transgenic human programmed cell death 5 expression in mice suppresses skin cancer development by enhancing apoptosis. Life Sci 92: 1208-1214, 2013.
28. Zhu W, Li Y and Gao L: Cisplatin in combination with programmed cell death protein 5 increases antitumor activity in prostate cancer cells by promoting apoptosis. Mol Med Rep 11: 4561-4566, 2015.
29. Fu DZ, Cheng Y, He H, Liu HY and Liu YF: Recombinant human PDCD5 exhibits an antitumor role in hepatocellular carcinoma cells via clathrin-dependent endocytosis. Mol Med Rep 12: 8135-8140, 2015.
30. Gao L, Ye X, Ma RQ, Cheng HY, Han HJ, Cui H, Wei LH and Chang XH: Low programmed cell death 5 expression is a prognostic factor in ovarian cancer. Chin Med J (Engl) 128: 1084-1090, 2015.
31. Xiao J, Li G, Hu J, Qu L, Ma D and Chen Y: Anti-inflammatory effects of recombinant human PDCD5 (rhPDCD5) in a rat collagen-induced model of arthritis. Inflammation 38: 70-78, 2015.
32. Xiao J, Liu W, Chen Y and Deng W: Recombinant human PDCD5 (rhPDCD5) protein is protective in a mouse model of multiple sclerosis. J Neuroinflammation 12: 117, 2015.
33. Lu J, Jiang Z, Chen Y, Zhou C and Chen C: Knockout of programmed cell death 5 (PDCD5) gene attenuates neuron injury after middle cerebral artery occlusion in mice. Brain Res 1650: 152-161, 2016.
34. Spinola M, Meyer P, Kammerer S, Falvella FS, Boettger MB, Hoyal CR, Pignatiello C, Fischer R, Roth RB, Pastorino U, *et al*: Association of the PDCD5 locus with lung cancer risk and prognosis in smokers. J Clin Oncol 24: 1672-1678, 2006.
35. Nanba K, Toyooka S, Soh J, Tsukuda K, Yamamoto H, Sakai A, Ouchida M, Kobayashi N, Matsuo K, Koide N, *et al*: The allelic distribution of a single nucleotide polymorphism in the PDCD5 gene locus of Japanese non-small cell lung cancer patients. Mol Med Rep 1: 667-671, 2008.
36. Xu L, Chen Y, Song Q, Xu D, Wang Y and Ma D: PDCD5 interacts with Tip60 and functions as a cooperator in acetyltransferase activity and DNA damage-induced apoptosis. Neoplasia 11: 345-354, 2009.
37. Yao H, Feng Y, Zhou T, Wang J and Wang ZX: NMR studies of the interaction between human programmed cell death 5 and human p53. Biochemistry 51: 2684-2693, 2012.
38. Xu L, Hu J, Zhao Y, Hu J, Xiao J, Wang Y, Ma D and Chen Y: PDCD5 interacts with p53 and functions as a positive regulator in the p53 pathway. Apoptosis 17: 1235-1245, 2012.
39. Tracy CM, Gray AJ, Cuéllar J, Shaw TS, Howlett AC, Taylor RM, Prince JT, Ahn NG, Valpuesta JM and Willardson BM: Programmed cell death protein 5 interacts with the cytosolic chaperonin containing tailless complex polypeptide 1 (CCT) to regulate β-tubulin folding. J Biol Chem 289: 4490-4502, 2014.
40. Essers PB, Klasson TD, Pereboom TC, Mans DA, Nicastro M, Boldt K, Giles RH and MacInnes AW: The von Hippel-Lindau tumor suppressor regulates programmed cell death 5-mediated degradation of Mdm2. Oncogene 34: 771-779, 2015.
41. Park SY, Choi HK, Jo SH, Seo J, Han EJ, Choi KC, Jeong JW, Choi Y and Yoon HG: YAF2 promotes TP53-mediated genotoxic stress response via stabilization of PDCD5. Biochim Biophys Acta 1853: 1060-1072, 2015.
42. Choi HK, Choi Y, Park ES, Park SY, Lee SH, Seo J, Jeong MH, Jeong JW, Jeong JH, Lee PC, *et al*: Programmed cell death 5 mediates HDAC3 decay to promote genotoxic stress response. Nat Commun 6: 7390, 2015.
43. Zhuge C, Sun X, Chen Y and Lei J: PDCD5 functions as a regulator of p53 dynamics in the DNA damage response. J Theor Biol 388: 1-10, 2016.
44. Murshed F, Farhana L, Dawson MI and Fontana JA: NF-κB p65 recruited SHP regulates PDCD5-mediated apoptosis in cancer cells. Apoptosis 19: 506-517, 2014.
45. Park SY, Choi HK, Choi Y, Kwak S, Choi KC and Yoon HG: Deubiquitinase OTUD5 mediates the sequential activation of PDCD5 and p53 in response to genotoxic stress. Cancer Lett 357: 419-427, 2015.
46. Cui X, Choi HK, Choi YS, Park SY, Sung GJ, Lee YH, Lee J, Jun WJ, Kim K, Choi KC and Yoon HG: DNAJB1 destabilizes PDCD5 to suppress p53-mediated apoptosis. Cancer Lett 357: 307-315, 2015.
47. Ouyang L, Shi Z, Zhao S, Wang FT, Zhou TT, Liu B and Bao JK: Programmed cell death pathways in cancer: A review of apoptosis, autophagy and programmed necrosis. Cell Prolif 45: 487-498, 2012.
48. Wong RS: Apoptosis in cancer: From pathogenesis to treatment. J Exp Clin Cancer Res 30: 87, 2011.
49. Evan GI and Vousden KH: Proliferation, cell cycle and apoptosis in cancer. Nature 411: 342-348, 2001.
50. Pucci B, Kasten M and Giordano A: Cell cycle and apoptosis. Neoplasia 2: 291-299, 2000.

51. Leng C, Li Y, Qin J, Ma J, Liu X, Cui Y, Sun H, Wang Z, Hua X, Yu Y, *et al*: Relationship between expression of PD-L1 and PD-L2 on esophageal squamous cell carcinoma and the anti-tumor effects of $CD8^+$T cells. Oncol Rep 35: 699-708, 2016.
52. Dong Y, Sun Q and Zhang X: PD-1 and its ligands are important immune checkpoints in cancer. Oncotarget 8: 2171-2186, 2017.
53. Bardhan K, Anagnostou T and Boussiotis VA: The PD1:PD-L1/2 pathway from discovery to clinical implementation. Front Immunol 7: 550, 2016.
54. Zhang J, Wei W, Jin HC, Ying RC, Zhu AK and Zhang FJ: Programmed cell death 2 protein induces gastric cancer cell growth arrest at the early S phase of the cell cycle and apoptosis in a p53-dependent manner. Oncol Rep 33: 103-110, 2015.
55. Granier CJ, Wang W, Tsang T, Steward R, Sabaawy HE, Bhaumik M and Rabson AB: Conditional inactivation of PDCD2 induces p53 activation and cell cycle arrest. Biol Open 3: 821-831, 2014.
56. Sun Z, Li S, Kaufmann AM and Albers AE: miR-21 increases the programmed cell death 4 gene-regulated cell proliferation in head and neck squamous carcinoma cell lines. Oncol Rep 32: 2283-2289, 2014.
57. Xia L, Wen H, Han X, Tang J and Huang Y: Luteinizing hormone inhibits cisplatin-induced apoptosis in human epithelial ovarian cancer cells. Oncol Lett 11: 1943-1947, 2016.
58. Gao F, Ding L, Zhao M, Qu Z, Huang S and Zhang L: The clinical significance of reduced programmed cell death 5 expression in human gastrointestinal stromal tumors. Oncol Rep 28: 2195-2199, 2012.
59. McDermott DF and Atkins MB: PD-1 as a potential target in cancer therapy. Cancer Med 2: 662-673, 2013.
60. Mo Z, Liu J, Zhang Q, Chen Z, Mei J, Liu L, Yang S, Li H, Zhou L and You Z: Expression of PD-1, PD-L1 and PD-L2 is associated with differentiation status and histological type of endometrial cancer. Oncol Lett 12: 944-950, 2016.
61. Wen YH, Shi X, Chiriboga L, Matsahashi S, Yee H and Afonja O: Alterations in the expression of PDCD4 in ductal carcinoma of the breast. Oncol Rep 18: 1387-1393, 2007.
62. Fassan M, Cagol M, Pennelli G, Rizzetto C, Giacomelli L, Battaglia G, Zaninotto G, Ancona E, Ruol A and Rugge M: Programmed cell death 4 protein in esophageal cancer. Oncol Rep 24: 135-139, 2010.
63. González-Villasana V, Nieves-Alicea R, McMurtry V, Gutiérrez-Puente Y and Tari AM: Programmed cell death 4 inhibits leptin-induced breast cancer cell invasion. Oncol Rep 27: 861-866, 2012.
64. Barboza N, Minakhina S, Medina DJ, Balsara B, Greenwood S, Huzzy L, Rabson AB, Steward R and Schaar DG: PDCD2 functions in cancer cell proliferation and predicts relapsed leukemia. Cancer Biol Ther 14: 546-555, 2013.
65. Zidovetzki R, Johnson DA, Arndt-Jovin DJ and Jovin TM: Rotational mobility of high-affinity epidermal growth factor receptors on the surface of living A431 cells. Biochemistry 30: 6162-6166, 1991.
66. Wu SL, Taylor AD, Lu Q, Hanash SM, Im H, Snyder M and Hancock WS: Identification of potential glycan cancer markers with sialic acid attached to sialic acid and up-regulated fucosylated galactose structures in epidermal growth factor receptor secreted from A431 cell line. Mol Cell Proteomics 12: 1239-1249, 2013.
67. Zhang F, Wang S, Yin L, Yang Y, Guan Y, Wang W, Xu H and Tao N: Quantification of epidermal growth factor receptor expression level and binding kinetics on cell surfaces by surface plasmon resonance imaging. Anal Chem 87: 9960-9965, 2015.
68. Stanton P, Richards S, Reeves J, Nikolic M, Edington K, Clark L, Robertson G, Souter D, Mitchell R, Hendler FJ, *et al*: Epidermal growth factor receptor expression by human squamous cell carcinomas of the head and neck, cell lines and xenografts. Br J Cancer 70: 427-433, 1994.
69. Ozaki T and Nakagawara A: Role of p53 in cell death and human cancers. Cancers (Basel) 3: 994-1013, 2011.
70. Drosten M, Sum EY, Lechuga CG, Simón-Carrasco L, Jacob HK, García-Medina R, Huang S, Beijersbergen RL, Bernards R and Barbacid M: Loss of p53 induces cell proliferation via Ras-independent activation of the Raf/Mek/Erk signaling pathway. Proc Natl Acad Sci USA 111: 15155-15160, 2014.
71. Zhang HT, Wang YL, Zhang J and Zhang QX: Artemisinin inhibits gastric cancer cell proliferation through upregulation of p53. Tumour Biol 35: 1403-1409, 2014.
72. Ranganathan S, Joseph J and Mehta JL: Aspirin inhibits human coronary artery endothelial cell proliferation by upregulation of p53. Biochem Biophys Res Commun 301: 143-146, 2003.
73. Taylor WR and Stark GR: Regulation of the G2/M transition by p53. Oncogene 20: 1803-1815, 2001.
74. Chipuk JE, Kuwana T, Bouchier-Hayes L, Droin NM, Newmeyer DD, Schuler M and Green DR: Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. Science 303: 1010-1014, 2004.
75. Nakazawa K, Dashzeveg N and Yoshida K: Tumor suppressor p53 induces miR-1915 processing to inhibit Bcl-2 in the apoptotic response to DNA damage. FEBS J 281: 2937-2944, 2014.
76. Wang H, Ye Y, Chui JH, Zhu GY, Li YW, Fong DW and Yu ZL: Oridonin induces G2/M cell cycle arrest and apoptosis through MAPK and p53 signaling pathways in HepG2 cells. Oncol Rep 24: 647-651, 2010.
77. Ou X, Lu Y, Liao L, Li D, Liu L, Liu H and Xu H: Nitidine chloride induces apoptosis in human hepatocellular carcinoma cells through a pathway involving p53, p21, Bax and Bcl-2. Oncol Rep 33: 1264-1274, 2015.
78. Zhou Y and Ho WS: Combination of liquiritin, isoliquiritin and isoliquirigenin induce apoptotic cell death through upregulating p53 and p21 in the A549 non-small cell lung cancer cells. Oncol Rep 31: 298-304, 2014.