SPANDIDOS PUBLICATIONS

Expression of the tumor suppressor ARHI inhibits the growth of pancreatic cancer cells by inducing G_1 cell cycle arrest

XINQING LU[1], JIAMING QIAN[1], YINHUA YU[2], HONG YANG[1] and JINGNAN LI[1]

[1]Department of Gastroenterology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100730; [2]Department of Gynecology, Ob/Gyn Hospital of Fudan University, Shanghai 200011, P.R. China

Received April 6, 2009; Accepted May 27, 2009

DOI: 10.3892/or_00000483

Abstract. A Ras homologue member I (ARHI) is an imprinted tumor suppressor gene whose expression is frequently lost in pancreatic cancers. This small GTP-binding protein is a member of the Ras superfamily with significant homology to Ras. In contrast to the Ras oncogene, ARHI has been shown to have anti-proliferative effects, but the mechanisms by which it inhibits pancreatic cancer cell proliferation and induces cell cycle arrest remain unclear. By generating stable transfectants, ARHI was reexpressed in pancreatic cancer cells that had lost its expression. Flow cytometry analysis indicated that ARHI blocked cell cycle progression at the G_1 phase in pancreatic cancer cells. In ARHI transfectants, phosphorylated AKT protein expression decreased compared to that of vector transfectants. Reexpression of ARHI increased the expression of the cyclin-dependent kinase (CDK) inhibitor (CKI) $p21^{WAF1}$, through the accumulation of p53 protein by the inhibition of PI-3K/AKT signaling. In addition, ARHI enhances expression of CKI $p27^{kip1}$ through the inhibition of PI-3K/AKT signaling. The expression of cyclins A and D1 decreased, while cyclin E was not affected under the same conditions. The activities of cyclin-dependent kinases 2 (CDK2) and 4 (CDK4) were reduced in ARHI transfectants. These results suggest that the PI-3K/AKT pathway plays a pivotal role in the pathogenesis of pancreatic cancer and ARHI exerts its growth-inhibitory effects through modulation of several key G_1 regulatory proteins, such as $p21^{WAF1}$, $p27^{kip1}$, CDK2, CDK4 and cyclins A and D1. ARHI represents a modulator of cancer cell proliferation and may play an important role in the development of pancreatic cancer.

Correspondence to: Dr Jiaming Qian, Department of Gastroenterology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100730, P.R. China
E-mail: yuanjiah@gmail.com

Abbreviations: ARHI, A Ras homologue member I; CDK, cyclin-dependent kinase; CKI, CDK inhibitor; DMEM, Dulbecco's modified Eagle's medium; EGF, epidermal growth factor; PBS, phospho-buffered saline

Key words: A Ras homologue member I, PI-3K/AKT signaling, cell cycle, $p21^{WAF1}$, $p27^{kip1}$, pancreatic cancer

Introduction

Pancreatic cancer is one of the most lethal cancers in the industrialized world and the sixth leading cause of cancer-related death in China (1). The incidence of pancreatic cancer almost equals that of pancreatic cancer-related mortality, with fewer than 20% of patients surviving the first year and with only 3% surviving 5 years after the diagnosis (2-4). This poor outcome motivates ongoing molecular research to understand tumor biology. A variety of regulatory mechanisms contribute to the development and growth of pancreatic cancer. The up-regulation of numerous oncogenic genes and the down-regulation of tumor suppressor genes are responsible, in part, for the uncontrolled growth, survival and metastasis of pancreatic cancer cells during the development of this disease. The genetic abnormalities found in pancreatic cancer affect the mechanisms controlling G_1 to S cell cycle progression and cellular proliferation.

A Ras homologue member I (ARHI) is a maternally imprinted human tumor suppressor gene that encodes a 26-kDa small G protein with 50-60% amino acid homology to Rap and Ras. In ovarian and breast cancers, reexpression of ARHI inhibits cancer cell growth *in vitro* and *in vivo*, induces $p21^{WAF1}$ and down-regulates cyclin D1 promoter activity (5). Recent studies showed that ARHI regulates autophagy and tumor dormancy in ovarian cancer (6).

ARHI is expressed in normal pancreatic epithelial cells, but its expression is lost or down-regulated in pancreatic cancers (7). The induction of this gene into pancreatic cancer cells that lack ARHI expression can block epidermal growth factor (EGF)-mediated signaling through RAS/mitogen-activated protein kinase, suppress STAT3 activity and induce apoptosis in pancreatic cancers (Hu and Lu, unpublished data). In this study, we further demonstrated that ARHI reexpression suppresses the proliferation of pancreatic cancer cells by modulating expression of cell cycle regulatory proteins.

Materials and methods

Cell culture and treatment. The human pancreatic carcinoma cell lines PANC-1 (anaplastic adenocarcinoma) and MiaPaCa-2

(poorly differentiated adenocarcinoma) were obtained from Professor Zhang Wei (Cancer Research Institute, Chinese Academy of Medical Sciences, Beijing, China). PANC-1 and MiaPaCa-2 cells and their G418-resistant stable transfectants were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL) supplemented with 10% fetal bovine serum (Gibco BRL), without any antibiotics in an incubator with 5% CO_2 at 37˚C. To analyze the effect of EGF on AKT expression and cell proliferation, pancreatic cancer cell lines were grown to 70-80% confluency in the corresponding medium containing 10% fetal bovine serum. At that point, cells were starved for 24 h in serum-free medium and finally treated for the specified time with 50 ng/ml EGF in serum or with medium alone.

Generation, selection and analysis of stable transfectants. PANC-1 or MiaPaCa-2 cell lines were transfected with the pIRES2-EGFP-ARHI constructs or with an empty vector pIRES2-EGFP plasmid as a control, using Lipofectamine 2000 (Invitrogen). Two days after transfection, the cells were placed into the selection medium containing 900 μg/ml G418 (Gibco BRL) for PANC-1 cells; 800 μg/ml G418 for MiaPaCa-2. Fourteen days after selection, stable G418-resistant cell colonies had formed. Stable pools were used for experiments. ARHI protein expression was analyzed by Western blotting using the ARHI antibody.

Cell growth assay. Pancreatic cancer cells and their stable transfectants (ARHI or vector) ($1x10^5$) were seeded in 6-well plates. At different intervals, cells were trypsinized and viable cells that excluded trypan blue dye were counted in a hemocytometer. The average numbers of cells per milliliter were calculated and plotted as a function of time.

Cell cycle arrest analysis. Cells were plated at a density of $5x10^5$ cells/plate in 60-mm tissue culture dishes. Cell cycle distribution was analyzed by flow cytometry. Briefly, pancreatic cancer cells and their stable pool transfectants were washed once with phosphate-buffered saline (PBS), harvested by trypsinization, collected and then resuspended in 300 μl of PBS and fixed with 5 ml of ice-cold 75% ethanol overnight. The cells were sedimented by centrifugation and resuspended in PBS containing 1 mg/ml each of glucose and RNaseA and incubated at room temperature for 30 min. Then, propidium iodide solution (1 mg/ml in distilled water) was added to each sample and incubated in the dark for an additional 30 min. The samples were analyzed via flow cytometry (Coulter Epics XL). Cell cycle analysis software was used to analyze the percentage of cells in the different phases of the cell cycle. Three different experiments were performed and the results are reported as means ± standard error.

Western blot analysis. Cells were washed twice with ice-cold PBS followed by lysis for 15 min in a buffer (20 mM sodium phosphate buffer, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, pH 7.4) supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF), sodium orthovanadate (1 mM, pH 7.4) and a mixture of phosphatase inhibitors. Cells were then scraped from the plates and the cell lysates were clarified by centrifugation for 15 min at 12,000 x g and 4˚C. The concentration of total protein was quantified using the Bicinchoninic Acid (BCA) method (Applygen). Total cell protein (50 μg) was separated via 10 or 12% SDS-PAGE, transferred to a PVDF membrane and blocked for 2 h at room temperature or overnight at 4˚C with 5% non-fat milk/TBS-Tween (TBS-T). Primary antibodies were diluted in blocking buffer and incubated overnight at 4˚C. To detect target proteins, we incubated the membranes with antibodies against: ARHI (from the laboratory of Dr Yu), p53 (Santa Cruz), $p27^{Kip1}$ (Santa Cruz), $p21^{WAF1}$ (Santa Cruz), cyclins A, D1 and E (Santa Cruz), CDK2, 4 (Santa Cruz), phospho-AKT polyclonal, AKT (Cell Signaling Technology, Beverly, MA) and actin (Santa Cruz), respectively. HRP-conjugated secondary antibodies (Santa Cruz, anti-mouse and anti-rabbit) were diluted 1:2000 in TBS-T and incubated for 1 h at room temperature with gentle rocking. Proteins were visualized using ECL detection reagents (Applygen) and exposure to film (Kodak Biomax MR, Rochester, NY).

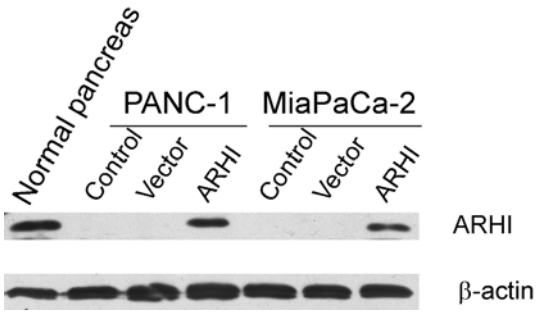

Figure 1. ARHI expression in stable transfectants. Western blot analysis was performed on PANC-1 or MiaPaCa-2 stable transfectants with pIRES2-EGFP-ARHI (ARHI) or pIRES2-EGFP (vector). Normal pancreas was used as positive control. ß-actin was included as the loading control. The data shown are representative of three independent experiments.

Results

ARHI reexpression inhibited growth of pancreatic cancer cells. Two pancreatic cancer cell lines (PANC-1, MiaPaCa-2) were transfected with pIRES2-EGFP-ARHI. Stable transfectants were selected by G418. They exhibited clear expression of ARHI (Fig. 1). Normal pancreas tissue was used as a positive control. The parental cell line and transfectants with vector did not have detectable ARHI expression and served as negative controls.

Previous studies have shown that ARHI inhibits the proliferation of ovarian and breast cancer cells (8). Thus, we examined whether ARHI reexpression inhibits the proliferation of pancreatic cancer cells. As shown in Fig. 2, stable transfectants of ARHI markedly inhibited anchorage-dependent growth of PANC-1 pancreatic cancer cells (Fig. 2A) and MiaPaCa-2 pancreatic cancer cells (Fig. 2B). The difference between ARHI transfectants vs. vector transfectants was statistically significant at 3, 4 and 5 days ($P<0.05$ or $P<0.01$).

Reexpression of ARHI causes cell cycle G_1 arrest. Since ARHI reexpression exerted marked effects on cellular proliferation as revealed by growth curvature assays (Fig. 2), we analyzed the effects of this suppression on cell cycle progression.

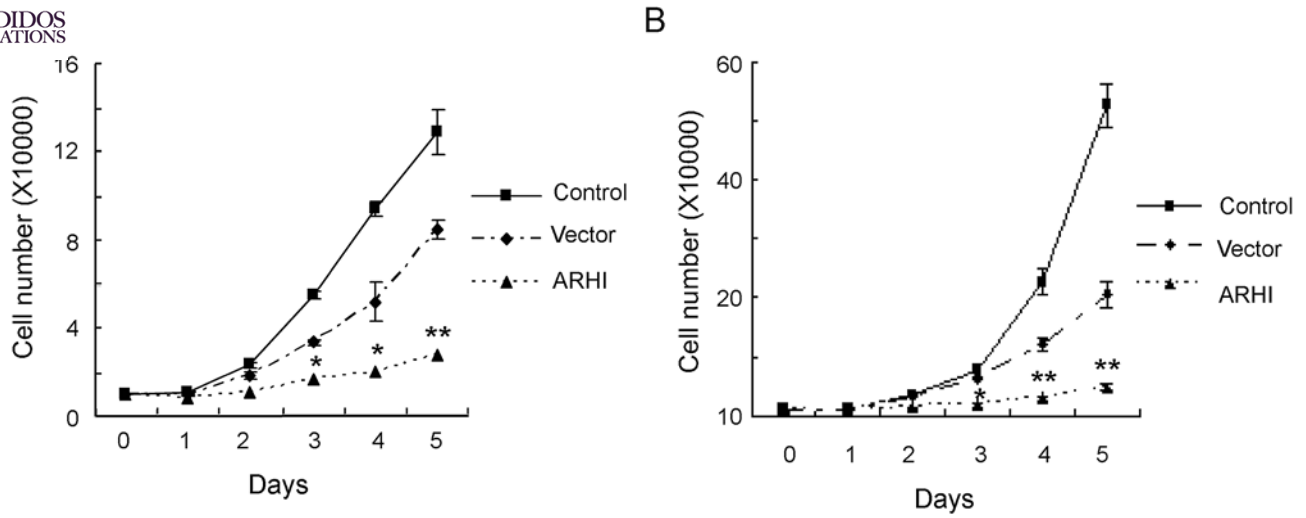

Figure 2. Effects of ARHI reexpression on growth of the pancreatic cancer cell lines (A) PANC-1, (B) MiaPaCa-2. Stable transfectants with pIRES2-EGFP-ARHI (ARHI) or pIRES2-EGFP (vector) were used in the proliferation assays. Parental cells were used as the control. ARHI transfectants markedly inhibited growth of pancreatic cancer cells compared with vector control ($^{*}P<0.05$, $^{**}P<0.01$). Values reflect the mean number of cells ± SE for three replicate at different intervals.

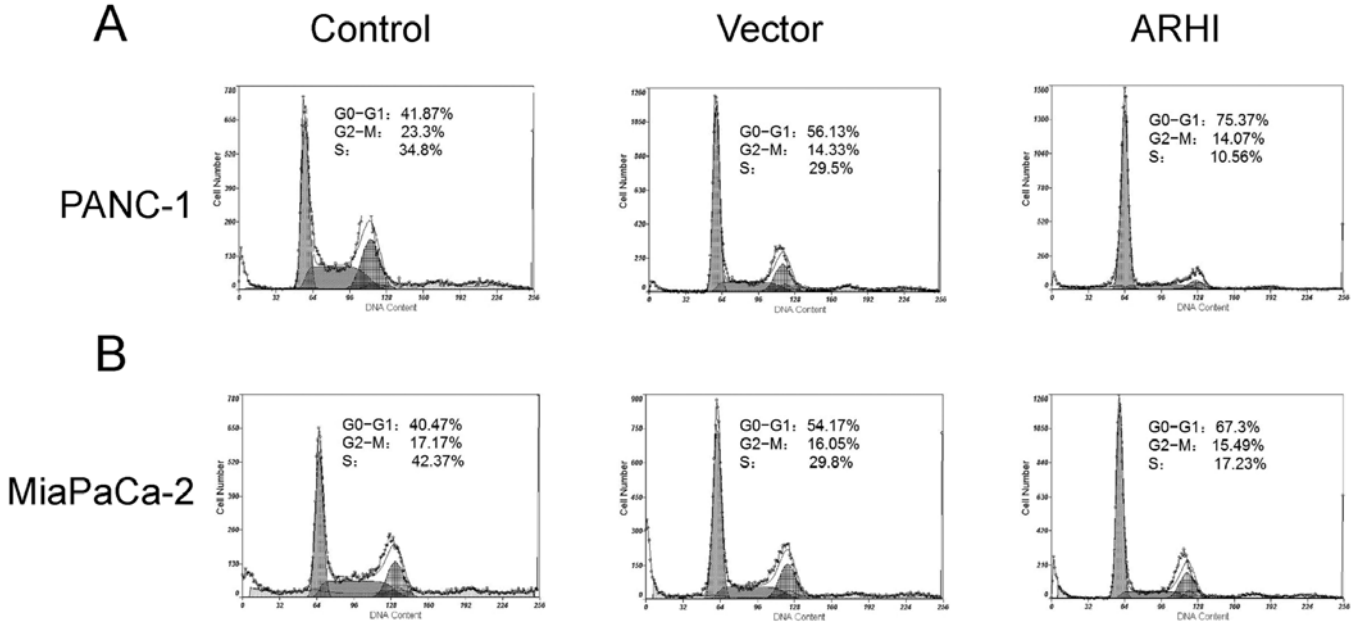

Figure 3. Effects of reexpressing ARHI on cell cycle. PANC-1 (A), MiaPaCa-2 (B) and their stable transfectants were examined with regard to cell cycle distribution. The proportion of cells in G_0-G_1, S and G_2-M phases were measured via flow cytometry. The fraction of cells in G_0-G_1 and S phases in ARHI transfectants differed from that of the parental cell line and from cells transfected with vector. The data shown are representative of at least three independent experiments.

PANC-1 or MiaPaCa-2 cell lines, stably transfected with the ARHI constructs or with an empty vector, were examined by flow cytometric analysis and the percentages of cells in the S and G_1 phases were determined. As shown in Fig. 3, cells reexpressing ARHI were arrested in the G_1 phase of the cell cycle. The fraction of cells in the G_1 phase increased from 56 to 75% (PANC-1) or 54 to 67% (MiaPaCa-2) compared with vector transfectants. No change in G_2-M cells was observed. These results indicated that reexpression of ARHI strongly impacted cellular proliferation, which led to a reduction of S phase cells and cellular arrest in the G_1 phase.

Down-regulation of phosphorylated Akt (p-AKT) in ARHI transfectants. The PI-3K/AKT pathway is an important regulatory pathway governing cell cycle progression and apoptotic response (9,10). We therefore investigated whether ARHI expression blocks the activation of PI-3K/AKT signaling in pancreatic cancer cells. As the activity of AKT is regulated by phosphorylation, we examined the level of p-AKT in PANC-1 or MiaPaCa-2 cells stably transfected with ARHI or vector at different time points. As shown in Fig. 4, the level of p-AKT in ARHI transfectants was down-regulated compared with vector controls, but total levels of AKT protein remained unchanged.

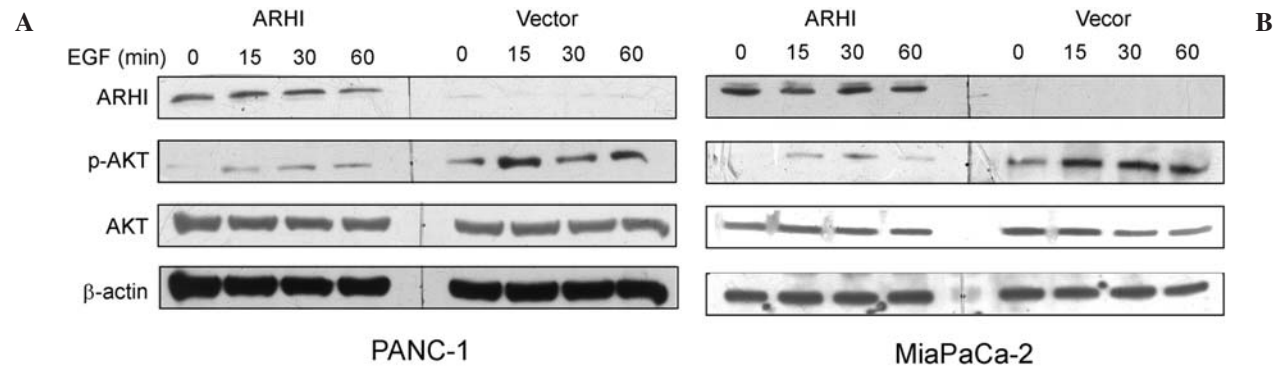

Figure 4. Effect of ARHI reexpression on AKT phosphorylation in PANC-1 (A) or MiaPaCa-2 (B) pancreatic cancer cell lines. The stable ARHI transfectants were treated with 50 ng/ml EGF for 0, 15, 30 and 60 min. Vector transfectants served as the control. Western blot analysis was performed with antibodies against human ARHI, p-AKT and AKT. ß-actin was included as the loading control. The data shown are representatives from three independent experiments.

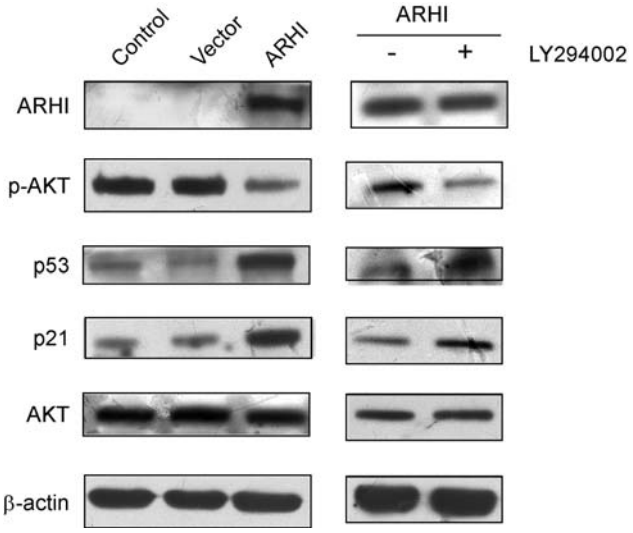

Figure 5. Inhibition of the PI-3K/AKT pathway and the increase of p53 and $p21^{WAF1}$ protein by ARHI reexpression and PI-3K inhibitor LY294002 in PANC-1 pancreatic cancer cells. The experiment was performed on PANC-1 cells (control), ARHI transfectants or vector transfectants (left), ARHI transfectants were also treated by LY294002 for 12 h (right). Western blot analysis was performed with antibodies against human ARHI, p-AKT, AKT, p53 and $p21^{WAF1}$. ß-actin was included as an internal control. The data shown are representative of three independent experiments.

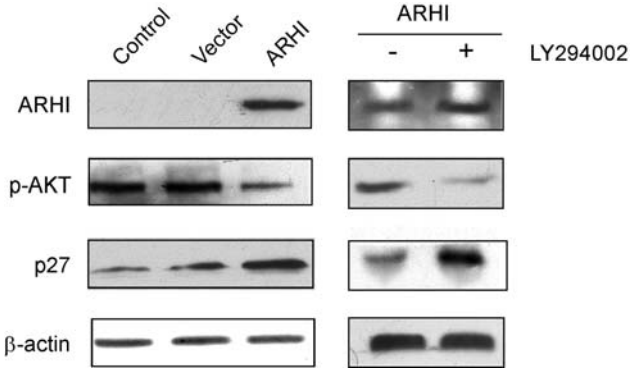

Figure 6. The enhanced expression of $p27^{kip1}$ by ARHI reexpression and treatment with PI-3K inhibitor LY294002 in PANC-1 pancreatic cancer cells. The experiment was performed on PANC-1 cells (control), ARHI transfectants or vector transfectants (left), ARHI transfectants were also treated by LY294002 for 12 h (right). Western blot analysis was performed with antibodies against human ARHI, p-AKT and $p27^{kip1}$. ß-actin was included as an internal control. The results shown are representative of three independent experiments.

ARHI reexpression increased the expression of $p21^{WAF1}$ and p53 through the inhibition of the PI-3K/AKT signaling pathway. Our present data show that ARHI down-regulated p-AKT expression. Previous studies have reported that the activation of PI-3K/AKT induces translocation of MDM2 from the cytoplasm to the nucleus, where it negatively regulates p53 (11). To provide further evidence that AKT may be a crucial target of ARHI, we further investigated the effects of ARHI on another AKT target, p53, using Western blot analysis on PANC-1 pancreatic cancer cells. In agreement with the observed cell cycle arrest induced by ARHI, we observed up-regulation of p53 protein expression via the PI-3K/AKT pathway in ARHI transfectants, as compared with vector transfectants or untreated controls (Fig. 5). Since p53 could induce G_1 cell cycle arrest through transcriptional activation of $p21^{WAF1}$ (12), we examined the effect of ARHI on the expression of $p21^{WAF1}$. As expected, $p21^{WAF1}$ expression was clearly up-regulated in ARHI transfectants. To better understand the role of the PI-3K/AKT pathway in ARHI-reactivation, we treated cells with PI-3K inhibitor LY294002. This specific inhibitor was able to block AKT activation, increasing $p21^{WAF1}$ expression via up-regulation of p53. ARHI expression displayed no change (Fig. 5). These results suggest that the reexpression of ARHI increases $p21^{WAF1}$, induces cell cycle G_1 arrest by up-regulation of p53 and blocks the PI-3K/AKT pathway.

ARHI reexpression increased the expression of $p27^{kip1}$. Many studies show that blocking the PI-3K/AKT signaling pathway modulates G_1 cell cycle progression and one critical target of this signaling process is the cyclin-dependent kinase (CDK) inhibitor (CKI) $p27^{kip1}$ (13). Thus, we investigated whether ARHI up-regulates expression of $p27^{kip1}$ through the inhibition of PI-3K/AKT activation. As shown in Fig. 6, $p27^{kip1}$ expression was increased in ARHI transfectants compared with vector transfectants and parental control cells. Additionally, PI-3K inhibitor LY294002 resulted in the

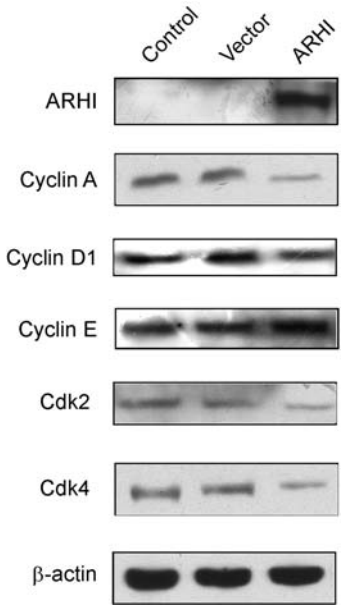

Figure 7. The modulation of cell cycle regulatory proteins involved in G_1 cell cycle arrest by ARHI reexpression in PANC-1 cells. Western blots were performed with antibodies against cyclins A, D1, E, CDK2 and CDK4. ß-actin served as a loading control. The data shown are representative of three independent experiments.

induction of $p27^{kip1}$ expression by regulating AKT activation. These results indicated that ARHI reactivation increases $p27^{kip1}$ expression by inactivating the PI-3K/AKT pathway.

The inhibitory effect of ARHI on the levels of cell cycle regulatory proteins. The cell cycle is regulated through the sequential activation and inactivation of CDKs that drive cell cycle progression through the phosphorylation and dephosphorylation of several regulatory proteins (14). Additionally, progression from G_1 to S phase in cells is regulated by the accumulation of cyclins D1, E and A, which bind to and activate different CDK catalytic subunits. The activation of the CDK4-cyclin D1 complex is necessary for transition from early to mid-G_1 phase. Transition from mid-G_1 to S phase is regulated by activation of the CDK2-cyclin E complex. Progression through late G_1 to S phase also requires the presence of the CDK2-cyclin A complex (15). Thus, we investigated whether ARHI modulates expression of cell cycle regulatory proteins (CDK2, CDK4, cyclin D1, cyclin A and cyclin E) involved in G_1 cell cycle arrest. In comparison with vector transfectants or parental control cells, the protein levels of cyclin A, cyclin D1, CDK2 and CDK4, but not cyclin E, were reduced by ARHI reexpression in pancreatic cancer cells (Fig. 7).

Discussion

The existing data suggest that pancreatic oncogenesis is a multi-step process, during which oncogenes are activated and the function of tumor suppressor genes is lost (16). ARHI is a tumor suppressor gene that is expressed by normal pancreatic cells (7). Expression of ARHI is lost in a majority of pancreatic cancers (Yang *et al*, unpublished data). Although the inhibition of cell proliferation is modulated by ARHI, the effects and mechanisms in pancreatic cancer have not been fully explored.

Past studies have shown that ARHI, an imprinted tumor suppressor gene, negatively regulates the PI-3K/AKT pathway in ovarian and breast cancers (17). As reported previously, the PI-3K/AKT pathway plays a key role in the proliferation and survival of subsets of pancreatic epithelial cancer cells that are characterized by basal AKT activity (18,19). The activation of PI-3K/AKT in response to nerve growth factor, insulin-like growth factor-1, PDGF, interleukin-3 and the extracellular matrix promotes cell survival (20-22). In this study, we demonstrate that ARHI reexpression suppresses the growth of pancreatic cancer cells, decreases AKT phosphorylation and arrests cell cycle progression in the G_1 phase. Hence, our data indicate that the PI-3K/AKT pathway is negatively regulated by ARHI (Fig. 4); this tumor suppressor might play an important role in cell proliferation of human pancreatic cancer cells.

To provide additional evidence for AKT as a crucial target of ARHI, we investigated the effects of ARHI on another AKT target, p53. AKT is known to promote cell survival through activation of the p53-binding protein MDM2, resulting in enhanced p53 degradation and negative regulation of the transcriptional functions of p53 (23). The tumor suppressor gene p53 controls the G_1 and G_2/M cell cycle checkpoints that mediate growth arrest (24). Among the transcriptional targets of p53, the CDK inhibitor $p21^{WAF1}$ plays a key role in mediating G_1 arrest (25). In this study, as expected, the findings showed that the levels of p53 and $p21^{WAF1}$ are increased due to ARHI reexpression (Fig. 5). ARHI significantly reduced p-AKT protein expression and the PI-3K inhibitor LY294002 increased p53 and $p21^{WAF1}$ expression in ARHI transfectants, suggesting that ARHI is effective in promoting p53 and $p21^{WAF1}$ accumulation by inactivating PI-3K/AKT signaling in pancreatic cancer cells.

Based on previous findings in our lab, we further examined cell cycle distribution using flow cytometry analysis in ARHI- or vector transfectants. As shown in Fig. 3, we demonstrated that ARHI reexpression resulted in G_1 cell cycle arrest. Our data also showed elevated $p27^{kip1}$ expression due to ARHI reexpression through the suppression of the PI-3K/AKT signaling pathway (Fig. 6). It is well known that G_1-to-S cell cycle progression is controlled by several CDK complexes such as cyclin A-CDK2, cyclin E-CDK2 and cyclin D1-CDK4 (26); the activities of CDK complexes are dependent on the balance of cyclins and CKIs such as $p27^{kip1}$ and $p21^{WAF1}$ (27). To determine whether ARHI-induced G_1 cell cycle arrest is due to the down-regulation of cyclins and CDKs, we analyzed the expression of these cell cycle regulators. The experiments indicated that ARHI reexpression down-regulated cyclin A-CDK2 and cyclin D1-CDK4, but not cyclin E-CDK2 (Fig. 7).

In conclusion, we show that expression of ARHI blocks PI-3K/AKT survival signaling in pancreatic cancer cells. ARHI reexpression upregulates p53, $p21^{WAF1}$ and $p27^{kip1}$ through PI-3K/AKT inactivation. Therefore, ARHI inhibits proliferation of pancreatic cancer cells by modulating the expression of cell cycle regulation factors. ARHI plays an important role in the development of pancreatic cancer.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (30670963).

References

1. Guo X and Cui Z: Current diagnosis and treatment of pancreatic cancer in China. Pancreas 31: 13-22, 2005.
2. Poston GJ, Gillespie J and Guillou PJ: Biology of pancreatic cancer. Gut 32: 800-812, 1991.
3. Warshaw AL and Fernandez-del Castillo C: Pancreatic carcinoma. N Engl J Med 326: 455-465, 1992.
4. Bardeesy N and DePinho RA: Pancreatic cancer biology and genetics. Nat Rev Cancer 2: 897-909, 2002.
5. Bao JJ, Le XF, Wang RY, *et al*: Reexpression of the tumor suppressor gene ARHI induces apoptosis in ovarian and breast cancer cells through a caspase-independent calpain-dependent pathway. Cancer Res 62: 7264-7272, 2002.
6. Lu Z, Luo RZ, Lu Y, *et al*: The tumor suppressor gene ARHI regulates autophagy and tumor dormancy in human ovarian cancer cells. J Clin Invest 118: 3917-3929, 2008.
7. Lu ZH, Chen J, Gu LJ, Luo YF and Gu CF: ARHI mRNA and protein expression in pancreatic cancers. Zhong guo Yi Xue Ke Xue Yuan Xue Bao 23: 324-327, 2001.
8. Yu Y, Xu F, Peng H, *et al*: NOEY2 (ARHI), an imprinted putative tumor suppressor gene in ovarian and breast carcinomas. Proc Natl Acad Sci USA 96: 214-219, 1999.
9. Semba S, Moriya T, Kimura W, *et al*: Phosphorylated AKT/PKB controls cell growth and apoptosis in intraductal papillary-mucinous tumor and invasive ductal adenocarcinoma of the pancreas. Pancreas 26: 250-257, 2003.
10. Asano T, Yao Y, Zhu J, Li D, Abbruzzese JL and Reddy SA: The PI3-kinase/AKT signaling pathway is activated due to aberrant Pten expression and targets transcription factors NF-kappaB and c-Myc in pancreatic cancer cells. Oncogene 23: 8571-8580, 2004.
11. Mayo LD, Dixon JE, Durden DL, Tonks NK and Donner DB: PTEN protects p53 from Mdm2 and sensitizes cancer cells to chemotherapy. J Biol Chem 277: 5484-5489, 2002.
12. Harper JW, Adami GR, Wei N, Keyomarsi K and Elledge SJ: The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 75: 805-816, 1993.
13. Viglietto G, Motti ML, Bruni P, *et al*: Cytoplasmic relocalization and inhibition of the cyclin-dependent kinase inhibitor p27Kip1 by PKB/Akt-mediated phosphorylation in breast cancer. Nat Med 8: 1136-1144, 2002.
14. Morgan DO: Principles of CDK regulation. Nature 374: 131-134, 1995.
15. Sherr CJ: Cancer cell cycles. Science 274: 1672-1677, 1996.
16. Mimeault M, Brand RE, Sasson AA and Batra SK: Recent advances on the molecular mechanisms involved in pancreatic cancer progression and therapies. Pancreas 31: 301-316, 2005.
17. Yu Y, Luo R, Lu Z, *et al*: Biochemistry and biology of ARHI (DIRAS3), an imprinted tumor suppressor gene whose expression is lost in ovarian and breast cancers. Methods Enzymol 407: 455-468, 2006.
18. Xiong HQ: Molecular targeting therapy for pancreatic cancer. Cancer Chemother Pharmacol 54: S69-S77, 2004.
19. Fujioka S, Sclabas GM, Schmidt C, *et al*: Function of nuclear factor kappaB in pancreatic cancer metastasis. Clin Cancer Res 9: 346-354, 2003.
20. Ahmed NN, Grimes HL, Bellacosa A, Chan TO and Tsichlis PN: Transduction of interleukin-2 antiapoptotic and proliferative signals via AKT protein kinase. Proc Natl Acad Sci USA 94: 3627-3632, 1997.
21. Hemmings BA: AKT signaling: linking membrane events to life and death decisions. Science 275: 628-630, 1997.
22. Songyang Z, Baltimore D, Cantley LC, Kaplan DR and Franke TF: Interleukin 3-dependent survival by the AKT protein kinase. Proc Natl Acad Sci USA 94: 11345-11350, 1997.
23. Zhou BP, Liao Y, Xia W, Zou Y, Spohn B and Hung MC: HER-2/neu induces p53 ubiquitination via AKT-mediated MDM2 phosphorylation. Nat Cell Biol 3: 973-982, 2001.
24. Agarwal ML, Agarwal A, Taylor WR and Stark GR: p53 controls both the G2/M and the G1 cell cycle checkpoints and mediates reversible growth arrest in human fibroblasts. Proc Natl Acad Sci USA 92: 8493-8497, 1995.
25. Waldman T, Kinzler KW and Vogelstein B: p21 is necessary for the p53-mediated G_1 arrest in human cancer cell. Cancer Res 55: 5187-5190, 1995.
26. Assoian RK: Anchorage-dependent cell cycle progression. J Cell Biol 136: 1-4, 1997.
27. Grana X and Reddy EP: Cell cycle control in mammalian cells: role of cyclins, cyclin dependent kinases (CDKs), growth suppressor genes and cyclin-dependent kinase inhibitors (CKIs). Oncogene 11: 211-219, 1995.