

Original Article

Induced PDK1 kinase activity suppresses apoptosis in intestinal epithelial cells by activating Akt signaling following polyamine depletion

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Abstract: Apoptosis plays a critical role in the maintenance of gut mucosal homeostasis and is highly regulated by numerous factors including polyamines. Decreasing cellular polyamines promotes the resistance of intestinal epithelial cells (IECs) to apoptosis by increasing Akt kinase activity, but the exact mechanisms by which polyamine depletion activates Akt remain unknown. 3-phosphoinositide-dependent protein kinase-1 (PDK1), functions as a downstream of phosphatidylinositol-3 kinase (PI3K) and upstream of Akt and serves as a major regulator of Akt activity. The current study determined if polyamines regulate Akt activity by altering PDK1. Studies were conducted in IEC-6 cells, derived from rat small intestinal crypts. Depletion of cellular polyamines induced PDK1 phosphorylation and increased its kinase activity, which were prevented by exogenous polyamine putrescine. Induced PDK1 activation following polyamine depletion was associated with an increase in phosphorylated Akt (pAkt) and Akt kinase activity. In contrast, polyamine depletion did not alter levels of total PDK1 and Akt proteins. PDK1 silencing in polyamine-deficient cells not only prevented the induced Akt activation but also blocked the increased resistance to apoptosis. These results indicate that polyamine depletion enhanced Akt phosphorylation by increasing PDK1 kinase activity, thereby protecting IECs against apoptosis.

Keywords: Intestinal epithelium, Akt phosphorylation, apoptosis, siRNA, polyamines

Introduction

The epithelium of the intestinal mucosa has the most rapid turnover rate of any tissue in the body and its integrity depends on a dynamic balance between cell proliferation, growth arrest, and apoptosis [1-3]. Undifferentiated intestinal epithelial cells (IECs) continuously replicate in the proliferative zone within the crypt areas and differentiate as they move up the luminal surface of the intestine to replace lost cells [4, 5]. To maintain normal gut mucosal homeostasis, the rate of epithelial cell division is counter balanced by apoptosis that is regulated by various extrinsic and intrinsic factors including cellular polyamines. The natural polyamines spermidine, spermine and their precursor, putrescine, are the central convergence point for the multiple signaling pathways and regulate distinct cellular functions [6, 7]. We [8-11] and others [12, 13] have reported that cellular polyamines are implicated in the control of the

apoptotic response in IECs and that polyamine depletion induces a resistance to apoptosis, at least partially, by activating Akt activity. However, the exact mechanism by which polyamine depletion activates Akt remains unknown.

3-Phosphoinositide-dependent protein kinase-1 (PDK1) is a 63-kDa Ser/Thr protein kinase that phosphorylates and regulates several downstream signaling pathways including several "AGC" family kinases such as Akt/protein kinase B, protein kinase C (PKC), p70S6K, and serum and glucocorticoid-regulated kinase (SGK) [14, 15]. PDK1 exists in an active, phosphorylated form under basal conditions and appears to be refractive to additional activation and phosphorylation upon cell stimulation with agonists which activate phosphatidylinositol-3 kinase (PI3K) [16, 17]. PDK1 functions as a downstream of PI3K but upstream of Akt and serves as a major regulatory point in Akt signaling. PDK1 activity is primarily controlled through its

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phosphorylation and regulates a wide spectrum of cell functions [14, 18, 19].

Although the precise function of cellular polyamines in programmed cell death is highly dependent upon cell type and death stimulus, polyamine depletion by inhibiting ornithine decarboxylase (ODC, the first rate-limiting step in polyamine biosynthesis) enhances the resistance of normal IECs to Tumor necrosis factor- α /cycloheximide (TNF- α /CHX)-induced apoptosis [8, 9, 20, 21]. The current studies were to test the hypothesis that polyamines regulate Akt activity by altering PDK1, thus modulating apoptosis in IECs.

Materials and methods

Chemicals and supplies

Disposable culture ware was purchased from Corning Glass Works (Corning, NY). Tissue culture media and dialyzed fetal bovine serum (dFBS) were obtained from GIBCO-BRL (Gaithersburg, MD), and biochemicals were obtained from Sigma (St. Louis, MO). Antibodies against phosphorylated Akt (pAkt at Serine 473), total Akt, phosphorylated PDK1 (pPDK1), total PDK1, phosphorylated-PTEN (pPTEN), total PTEN, PI3K (p85, p110) were purchased from Cell Signaling Technology (Danvers, MA). Secondary antibodies conjugated to horseradish peroxidase (HRP) were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). [γ - 32 P]ATP was purchased from Perkin Elmer Life Sciences (San Jose, CA). PDK1 assay kit was purchased from Upstate Biotechnology (Lake Placid, NY) and DL- α -difluoromethylornithine (DFMO) was obtained from Genzyme (Cambridge, MA).

Cell culture

The IEC-6 cell line was purchased from the American Type Culture Collection (ATCC) at passage 13. The cell line was derived from normal rat intestine and was developed and characterized by Quaroni et al [22]. IEC-6 cells originated from intestinal crypt cells, as judged by morphological and immunological criteria. They are non-tumorigenic and retain the undifferentiated character of epithelial stem cells. Stock cells were maintained in T-150 flasks in Dulbecco's modified Eagle medium (DMEM) supplemented with 5% heat-inactivated FBS, 10 μ g/ml insulin, and 50 μ g/ml gentamicin sulfate. Flasks were incubated at 37°C in a humidified atmosphere

of 90% air-10% CO₂. Stock cells were subcultured once a week at 1:20; medium was changed three times weekly. The cells were restarted from original frozen stock every 7 passages. Tests for mycoplasma were routinely negative and passages 15-20 were used in the experiments.

RNA interference

The small interfering (si)RNA that were designed to specifically target the coding region of PDK1 (siPDK1) mRNA was synthesized and purchased from Dharmacon Inc (Lafayette, CO). Scrambled control siRNA (C-siRNA), which had no sequence homology to any known genes, was used as the control. The siPDK1, and C-siRNA were transfected into cells as described previously [9, 10, 23]. Briefly, for each 60-mm cell culture dish, 20 μ l of the 5 μ M stock siPDK1 or C-siRNA was mixed with 500 μ l of Opti-MEM medium (Invitrogen). This mixture was added to a solution containing LipofectAMINE 2000 in 500 μ l of Opti-MEM. The solution was incubated for 20 min at room temperature and gently overlaid onto monolayers of cells in 3 ml of medium, and cells were harvested for various assays after appropriate incubation times.

Western blot analysis

Cell samples, dissolved in ice-cold NP40 \times -buffer (25 mM Tris-HCl, pH 8.0, 137 mM NaCl, 1 mM EDTA, 205 mM sodium pyrophosphate, 1 mM Na₃VO₄, 10% glycerol, 1% Triton \times -100, 10 mg/ml aprotinin), were sonicated and centrifuged at 14,000 rpm for 15 min at 4°C. The protein concentration of the supernatant was measured by the methods described by Bradford [24], and each lane was loaded with 20 μ g of protein equivalent. The supernatant was boiled for 5 min and then subjected to electrophoresis on 10% acrylamide gels according to Laemmli [25]. Briefly, after the transfer of protein onto nitrocellulose filters, the filters were incubated for 1 h in 5% BSA in 1x TBS-T buffer (Tris-buffered saline, pH 7.4, with 0.1% Tween 20). Immunologic evaluation was then performed overnight at 4°C in 5% nonfat dry milk/TBS-T buffer containing specific antibodies against pPDK1, total PDK1, pPTEN, total PTEN, PI3K (p85, p110), pAkt, total Akt, proteins. The filters were subsequently washed with 1x TBS-T and incubated with the secondary antibodies conjugated with HRP for 1 h at room temperature. The immunocomplexes on the filters were reacted for

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1 min with Chemiluminescence Reagent (NEL-100 DuPont NEN).

Assessment of morphology and Annexin V staining

After various experimental treatments, cells were photographed with a Nikon inverted microscope before fixation. Annexin V staining of apoptosis was carried out by using a commercial apoptosis kit (Clontech Laboratories, Palo Alto, CA) and performed according to the protocol recommended by the manufacturer. Briefly, cells were rinsed with 1× binding buffer, and resuspended in 200 µl of 1× binding buffer. Five µl of Annexin V was added on a slide and incubated at room temperature for 10 min in the dark. Annexin-stained cells were visualized and photographed under fluorescence microscope using a dual filter set for FITC & rhodamine, and the percentage of “apoptotic” cells was determined.

PDK1 kinase activity assay

The PDK1 kinase activity was estimated by using a PDK1 kinase assay kit according to the manufacturer's instructions (Upstate Biotechnology). This assay was carried out in two stages. In stage one, the inactive SGK was incubated with active PDK1 in the presence of Mg²⁺/ATP to activate SGK. In the second stage, Mg²⁺ [γ-³²P]ATP and Akt/SGK substrate peptide (RPRAATF) were added to detect PDK1 dependent SGK activity.

Statistics

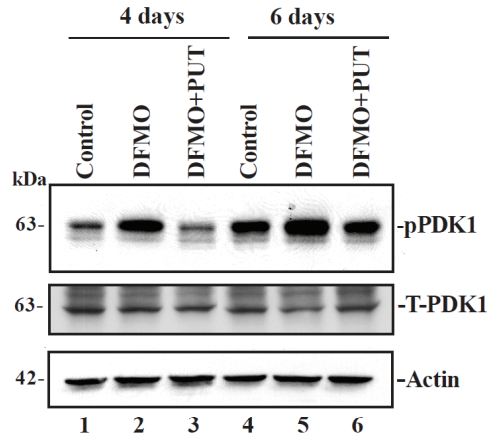
All data are expressed as means ± SE from three to six samples. Autoradiographic results were repeated three times. Apoptosis assay results were repeated separately three times. The significance of the difference between means was determined by analysis of variance. The level of significance was determined using Duncan's multiple range test [26].

Results

Effect of polyamine depletion on PDK1 kinase activity in IEC-6 cells

Consistent with our previous studies [20, 27, 28], inhibition of ODC activity by exposure to 5 mM DFMO almost completely depleted cellular

A



B

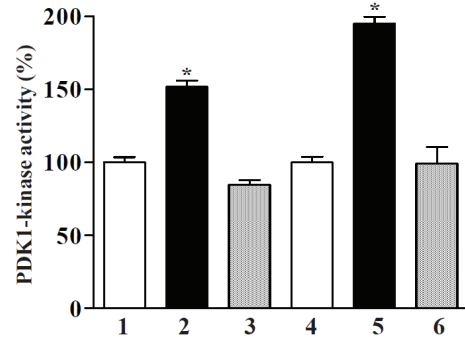


Figure 1. Changes in phosphorylated PDK1 (pPDK1) and its total protein levels after polyamine depletion. IEC-6 cells were grown in DMEM containing either DFMO (5 mM) alone or DFMO plus PUT (10 µM) for 4 and 6 days; whole cell lysates were harvested for various measurements. A: Representative immunoblots of Western blot analysis. Twenty micrograms of total protein were applied to each lane, and immunoblots were hybridized with the antibody specific for pPDK1 and total PDK1 (~63 kDa). After the blot was stripped, actin (~42 kDa) immunoblotting was performed as an internal control for equal loading. B: Levels of PDK1 kinase activity (% change) in cells described in A. Values are mean ± SE of data from three separate experiments. *P < 0.05 compared with control and DFMO + PUT.

polyamines in IEC-6 cells. Putrescine and spermidine were significantly decreased on day 2 and was undetectable on days 4 and 6. Spermine was less sensitive to DFMO but decreased by ~65% on days 4 and 6 after DFMO treatment (data not shown) [8, 20, 21]. Results presented in **Figure 1** show that polyamine depletion by DFMO increased the levels of phosphorylated

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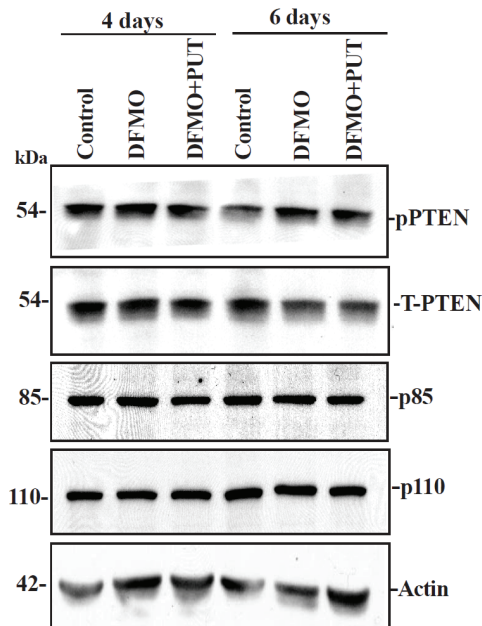


Figure 2. Changes in levels of phosphorylated PTEN (pPTEN), total PTEN, p85, and p110 proteins in cells described in Figure 1. Twenty micrograms of total protein were applied to each lane, and immunoblots were hybridized with the antibody specific for pPTEN (~54 kDa), total PTEN (~54 kDa), p85 (~85 kDa) and p110 (~110 kDa), and actin (~42 kDa) immunoblotting was performed as an internal control for equal loading. Three experiments were performed that showed similar results.

PDK1 (pPDK1) although it had no effect on expression of total PDK1 protein. The induction of pPDK1 occurred on day 4 after treatment with DFMO, and the levels of pPDK1 in DFMO-treated cells were ~5.0, and 6.0 times the normal values (without DFMO) on days 4 and 6 respectively (**Figure 1A**). Putrescine (10 μ M) added together with DFMO returned pPDK1 to near normal levels. Exogenous addition of spermidine at 5 μ M to cultures containing DFMO had an effect similar to putrescine on pPDK1 protein levels (data not shown). Consistently, increased levels of pPDK1 in DFMO-treated cells were paralleled by an increase in PDK1 kinase activity (**Figure 1B**), which was also completely prevented by putrescine given together with DFMO. In addition, polyamine depletion had no effect on the levels of other members of PI3K/Akt pathway such as pPTEN, PTEN, p85 and p110 (**Figure 2**). These results clearly indicate that depletion of cellular polyamines increases PDK1 phosphorylation and activates its

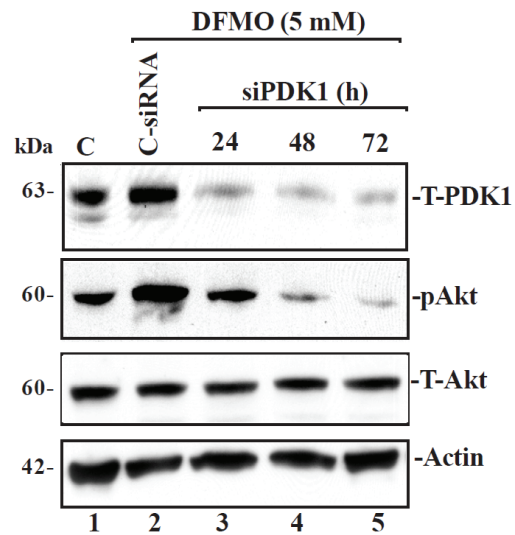


Figure 3. Effect of PDK1 silencing on pAkt and its total proteins after polyamine depletion. Cells were grown in DMEM containing DFMO (5 mM) for 4 days and then transfected with either control siRNA (C-siRNA) or siPDK1. Whole cell lysates were harvested at indicated times for Western blotting analysis.

kinase activity in IECs.

PDK1 silencing decreases Akt phosphorylation in polyamine-deficient IECs

Our previous studies show that polyamine depletion increases Akt phosphorylation [8]. The current study further determined whether increased Akt following polyamine depletion is mediated by PDK1 activation. As shown in **Figure 3**, transfection with the siRNA specifically targeting the coding region of PDK1 mRNA (siPDK1) decreased PDK1 protein by 85% in DFMO-treated cells. Decreased PDK1 by siPDK1 transfection reduced pAkt protein levels although it failed to alter total Akt protein content. These results strongly suggest that polyamine depletion induces Akt phosphorylation by activating PDK1 activity.

Effect of PDK1 silencing on TNF- α /CHX-induced apoptosis

To elucidate the function of increased PDK1 activity in IECs, the TNF- α /CHX-induced apoptosis model was used as reported previously [8, 20, 27, 29]. As shown in **Figure 4**, inhibition of PDK1 activation by siPDK1 enhanced the sensitivity to TNF- α /CHX-induced apoptosis in normal

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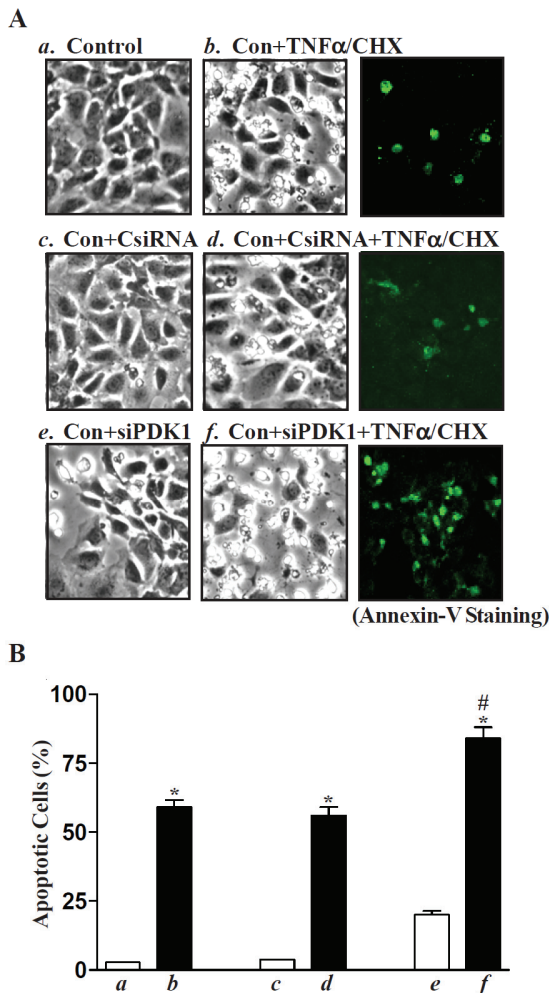


Figure 4. Apoptosis response to TNF- α /CHX in control IEC-6 cells after PDK1 silencing. **A:** Pictures of TNF- α /CHX-induced apoptosis after various treatments. Cells were transfected with siPDK1 or C-siRNA by the LipofectAMINE technique. Cells were exposed to TNF- α (20 ng/ml) and CHX (25 μ g/ml) after 48 h post-transfection. Apoptosis was measured by morphological analysis (middle) and annexin V staining (left) 3 h after the treatment with TNF- α /CHX. **a,** control; **b,** control cells exposed to TNF- α /CHX; **c,** cells transfected with C-siRNA; **d,** C-siRNA transfected cells exposed to TNF- α /CHX; **e,** cells transfected with siPDK1; **f,** siPDK1 transfected cells exposed to TNF- α /CHX. Original magnification X200. **B:** percentage of apoptotic cells in cells described in **A.** Values are mean \pm SE of data from three experiments. * $p < 0.05$ compared with cells treated without TNF- α /CHX (No-TNF- α /CHX).

IEC-6 cells (without DFMO treatment). When TNF- α /CHX was added to control cultures, it induced typical apoptotic cell death (**Figure 4A,**

a versus b, and **B, left panel**). Annexin V staining showed significant phosphatidylserine presence in the cell membrane, a classic indicator of apoptotic cells (**Figure 4A, b, right**). In siPDK1 transfected cells, treatment with the same doses of TNF- α /CHX increases number of apoptotic bodies significantly compared to IECs transfected with C-siRNA.

Interestingly, inhibition of PDK1 activation with siPDK1 also completely reversed the protective effect of polyamine depletion on TNF- α /CHX-induced apoptosis (**Figure 5**). When polyamine-deficient cells were exposed to TNF- α /CHX, they exhibited decreased cell death as reported previously [10, 20, 30]. There were no differences in morphological features and percentage of apoptotic cells between cells treated with DFMO alone and DFMO-treated cells exposed to TNF- α /CHX (**Figure 5A**). However, when PDK1 was silenced by siPDK1 the resistance of polyamine-deficient cells to TNF- α /CHX-induced apoptosis was abolished compared to the cells transfected with C-siRNA. These results indicate that activated PDK1 plays an important role in the induced resistance of polyamine-deficient cells to apoptosis.

Discussion

Our previous studies [8, 20, 21, 29] show that polyamines regulate apoptosis through multiple signaling pathways including Akt kinase. Polyamines negatively regulate Akt phosphorylation in normal IECs, and activated Akt kinase activity following polyamine depletion plays a critical role in the increased resistance to apoptosis [8]. In the current study we further showed that increased Akt signal results from induced PDK1 activation, thus advancing our understanding of polyamine-regulated apoptosis in IECs.

The results presented in **Figure 3** clearly show that inhibition of PDK1 by siPDK1 almost completely prevented the increased Akt phosphorylation in polyamine-deficient cells, suggesting that polyamine depletion induces Akt activity by increasing PDK1 activity. The most important findings reported here are that PDK1-mediated Akt phosphorylation regulates apoptosis after polyamine depletion. The activated PDK1 was associated with an increased resistance to TNF- α /CHX-induced apoptosis in polyamine-deficient IEC-6 cells, whereas inhibition of PDK1 activity by siPDK1 increased the sensitivity to apoptosis

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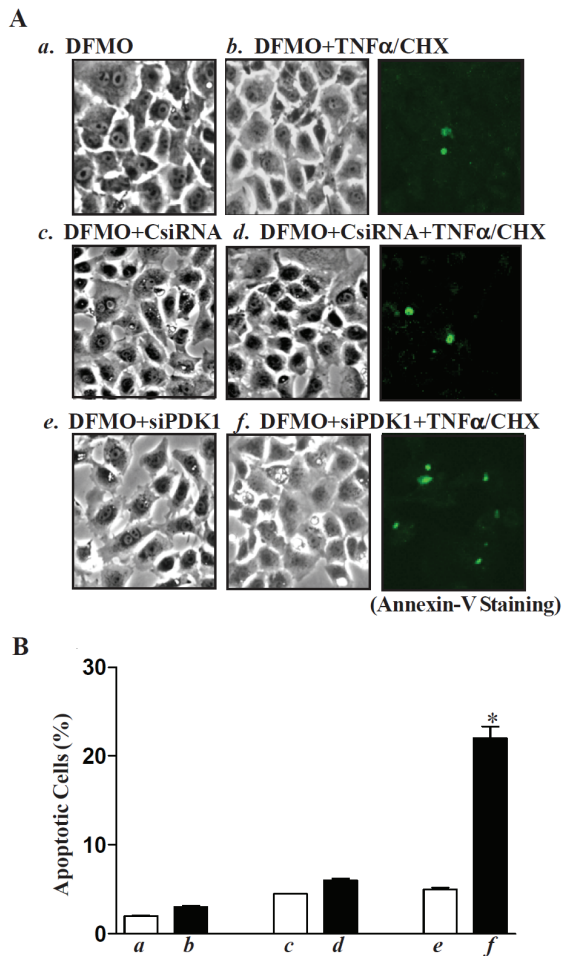


Figure 5. Apoptosis response in polyamine-depleted IEC-6 cells after PDK1 silencing. A: Pictures of TNF- α /CHX-induced apoptosis after various treatments. Cells were grown in the media containing 5 mM DFMO for 4 days, and then transfected with siPDK1 or C-siRNA. Cells were exposed to TNF- α /CHX after 48 h post-transfection. Apoptosis was measured by morphological analysis (middle) and annexin V staining (left) 3 h after TNF- α /CHX. a, cells treated with DFMO alone; b, DFMO-treated cells exposed to TNF- α /CHX; c, DFMO-treated cells transfected with C-siRNA; d, DFMO-treated cells transfected with C-siRNA and then exposed to TNF- α /CHX. e, DFMO-treated cells transfected with siPDK1; f, DFMO-treated cells transfected with siPDK1 and then exposed to TNF- α /CHX. Original magnification X200. B: percentage of apoptotic cells as described in A. Values are mean \pm SE of data from three experiments. * $p < 0.05$ compared with cells treated without TNF- α /CHX (No-TNF- α /CHX).

in control (Figure 4) as well as in DFMO-treated cells (Figure 5). These findings are consistent

with results from others [31-33], who have demonstrated that PDK1 activation and resulting Akt activation suppress apoptosis in a wide variety of cell types. It has been shown that overexpression of wild-type Akt protein prevents apoptosis in primary cultures of cerebellar neurons when survival factors were withdrawal or PI3K was inhibited [34]. Kikani et al [35] showed that the cell lines stably expressing wild type PDK1 displayed increased resistance to UV-induced apoptosis.

In summary, these results indicate that PDK1 activity is regulated by cellular polyamines in normal IECs. Decreasing cellular polyamines activates PDK1 phosphorylation, increases its kinase activity, thus activating Akt kinase activity and protecting IECs against TNF- α /CHX-induced apoptosis. Inactivation of PDK1 by siPDK1 decreased Akt phosphorylation and enhanced the susceptibility to apoptosis. These findings suggest that PDK1 is an important cell survival factor in the gut mucosa *in vivo* and plays an important role in biological regulation of epithelial homeostasis.

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References

- [1] van der Flier LG and Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu Rev Physiol* 2009; 71: 241-260.
- [2] Rao JN and Wang JY. Regulation of gastrointestinal mucosal growth. Edited by Granger ND and Granger J. Morgan and Claypool Publishers 2011; pp: 1-114.
- [3] Potten CS and Loeffler M. Stem cells; attributes, cycles, spirals, pitfalls, and uncertainties-lessons for and from the crypt. *Development* 1990; 110: 1001-1020.

PDK1 kinase activity suppresses apoptosis

- [4] Potten CS. Epithelial cell growth and differentiation 2: Intestinal apoptosis. *Am J physiol Gastrointest Liver Physiol* 1997; 36: G253-G257.
- [5] Potten CS, Wilson JW and Booth C. Regulation and significance of apoptosis in the stem cells of the gastrointestinal epithelium. *Stem* 1997; 15: 82-93.
- [6] Seiler N and Raul F. Polyamines and apoptosis. *J Cell Mol Med* 2005; 9: 623-642.
- [7] Babbar N and Gerner EW. Targeting polyamines and inflammation for cancer prevention. *Recent Results Cancer Res* 2011; 188: 49-64.
- [8] Zhang HM, Rao JN, Guo X, Liu L, Zou T, Turner DJ and Wang JY. Akt kinase activation blocks apoptosis in intestinal epithelial cells by inhibiting caspase-3 after polyamine depletion. *J Biol Chem* 2004; 279: 22539-22547.
- [9] Zhang HM, Keledjian KM, Rao JN, Zou T, Liu L, Marasa BS, Wang SR, Ru L, Strauch ED and Wang JY. Induced focal adhesion kinase expression suppresses apoptosis by activating NF- κ B signaling in intestinal epithelial cells. *Am J Physiol Cell Physiol* 2006; 290: C1310-C1320.
- [10] Wang PY, Rao JN, Zou T, Liu L, Xiao L, Yu TX, Turner DJ, Gorospe M and Wang JY. Post-transcriptional regulation of MEK-1 by polyamines through the RNA-binding protein HuR modulating intestinal epithelial apoptosis. *Biochem J* 2010; 426: 293-306.
- [11] Rathor N, Wang SR, Chang ET and Rao JN. Differentiated intestinal epithelial cells express high levels of TGF- β receptors and exhibit increased sensitivity to growth inhibition. *Int J Clin Exp Med* 2011; 4: 299-308.
- [12] Tirupathi Pichiah PB, Suriyakalaa U, Kamalakannan S, Kokilavani P, Kalaiselvi S, SankarGanesh D, Gowri J, Archunan G, Cha YS and Achiraman S. Spermidine may decrease ER stress in pancreatic beta cells and may reduce apoptosis via activating AMPK dependent autophagy pathway. *Med Hypotheses* 2011; 77: 677-679.
- [13] Bhattacharya S, Ray RM and Johnson LR. Decreased apoptosis in polyamine depleted IEC-6 cells depends on Akt-mediated NF- κ B activation but not GSK3 β activity. *Apoptosis* 2005; 10: 759-776.
- [14] Feldman RI, Wu JM, Polokoff MA, Kochanny MJ, Dinter H, Zhu D, Biroc SL, Alicke B, Bryant J, Yuan S, Buckman BO, Lentz D, Ferrer M, Whitlow M, Adler M, Finster S, Chang Z and Arnaiz DO. Novel small molecule inhibitors of 3-phosphoinositide-dependent kinase-1. *J Biol Chem* 2005; 280: 19867-19874.
- [15] Seong HA, Jung H, Kim KT and Ha H. 3-Phosphoinositide-dependent PDK1 negatively regulates transforming growth factor-beta-induced signaling in a kinase-dependent manner through physical interaction with Smad proteins. *J Biol Chem* 2007; 282: 12272-12289.
- [16] Duronio V. The life of a cell: apoptosis regulation by the PI3K/PKB pathway. *Biochem J* 2008; 415: 333-344.
- [17] Vanhaesebroeck B and Alessi DR. The PI3K-PDK1 connection: more than just a road to PKB. *Biochem J* 2000; 346: 561-576.
- [18] Hill KM, Kalifa S, Das JR, Bhatti T, Gay M, Williams D, Taliferro-Smith L and De Marzo AM. The role of PI3-kinase p110 β in AKT signaling, cell survival, and proliferation in human prostate cancer cells. *Prostate* 2010; 70: 755-764.
- [19] Rexhepaj R, Grahammer F, Völkl H, Remy C, Wagner CA, Sandulache D, Artunc F, Henke G, Nammi S, Capasso G, Alessi DR and Lang F. Reduced intestinal and renal amino acid transport in PDK1 hypomorphic mice. *FASEB J* 2006; 20: 2214-2222.
- [20] Li L, Li J, Rao JN, Li M-L, Bass BL and Wang JY. Inhibition of polyamine synthesis induces p53 gene expression but not apoptosis. *Am J Physiol Cell Physiol* 1999; 276: C946-C954.
- [21] Li L, Rao JN, Guo X, Liu L, Santora R, Bass BL and Wang JY. Polyamine depletion stabilizes p53 resulting in inhibition of normal intestinal epithelial cell proliferation. *Am J Physiol Cell Physiol* 2001; 281: C941-C953.
- [22] Quaroni A, Wands J, Trelstad RL and Isselbacher KJ. Epithelial cell cultures from rat small intestine. *J Cell Biol* 1979; 80: 248-265.
- [23] Rao JN, Rathor N, Zou T, Liu L, Xiao L, Yu TX, Cui YH and Wang JY. STIM1 translocation to the plasma membrane enhances intestinal epithelial restitution by inducing TRPC1-mediated Ca²⁺ signaling after wounding. *Am J Physiol Cell Physiol* 2010; 299: C579-C588.
- [24] Bradford MA. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of dye binding. *Anal Biochem* 1976; 72: 248-254.
- [25] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1990; 227: 680-685.
- [26] Harter JL. Critical values for Duncan's new multiple range test. *Biometrics* 1960; 16: 671-685.
- [27] Wang JY, McCormack SA, Viar MJ, Wang HL, Tzen CY, Scott RE and Johnson LR. Decreased expression of protooncogenes c-fos, c-myc, and c-jun following polyamine depletion in IEC-6 cells. *Am J Physiol Gastrointest Liver Physiol* 1993; 265: G331-G338.
- [28] Wang JY, McCormack SA, Viar MJ and Johnson LR. Stimulation of proximal small intestinal mucosal growth by luminal polyamines. *Am J Physiol Gastrointest Liver Physiol* 1991; 261: G504-G511.
- [29] Cui YH, Xiao L, Rao JN, Zou T, Liu L, Chen Yu, Turner DJ, Gorospe M and Wang JY. miR-503 represses CUG binding protein 1 translation by recruiting CUGBP1 mRNA to processing bodies. *Mol Biol Cell* 2012; 23: 151-162.
- [30] Zou T, Rao JN, Liu L, Xiao L, Yu TX, Jiang P, Gorospe M and Wang JY. Polyamines regulate

PDK1 kinase activity suppresses apoptosis

- the stability of JunD mRNA by modulating the competitive binding of its 3' untranslated region to HuR and AUF1. *Mol Cell Biol* 2010; 30: 5021-5032.
- [31] Shen X, Xi G, Radhakrishnan Y and Clemmons DR. PDK1 recruitment to the SHPS-1 signaling complex enhances insulin-like growth factor-stimulated AKT activation and vascular smooth muscle cell survival. *J Biol Chem* 2010; 285: 29416-29424.
- [32] Park SH, Lee JH, Lee GB, Byun HJ, Kim BR, Park CY, Kim HB and Rho SB. PDCD6 additively cooperates with anti-cancer drugs through activation of NF- κ B pathways. *Cell Signal* 2012; 24: 726-733.
- [33] Choi JH, Yang YR, Lee SK, Kim SH, Kim YH, Cha JY, Oh SW, Ha JR, Ryu SH and Suh PG. Potential inhibition of PDK1/Akt signaling by phenothiazines suppresses cancer cell proliferation and survival. *Ann N Y Acad Sci* 2008; 1138: 393-403.
- [34] de la Torre AV, Junyent F, Folch J, Pelegrí C, Vilaplana J, Auladell C, Beas-Zarate C, Pallàs M, Verdaguer E and Camins A. GSK3 β inhibition is involved in the neuroprotective effects of cyclin-dependent kinase inhibitors in neurons. *Pharmacol Res* 2012; 65: 66-73.
- [35] Kikani CK, Dong LQ and Liu F. "New"-clear functions of PDK1: beyond a master kinase in the cytosol? *J Cell Biochem* 2005; 96: 1157-1162.