RESEARCH ARTICLE

p21-activated kinase 5 inhibits camptothecin-induced apoptosis in colorectal carcinoma cells

Xia Wang · Wei Gong · Haitao Qing · Yan Geng ·
Xinying Wang · Yongsheng Zhang · Liang Peng ·
Hongquan Zhang · Bo Jiang

Abstract p21-activated kinase 5 (PAK5) is a recently identified member of the group B PAK family. The PAK proteins are effectors of the small GTPase Cdc42 and Rac1 and are known to regulate cell motility and activate cell-survival signaling pathways. Especially, the mitochondrial localization of PAK5 is vital to its effects on apoptosis and cell survival. Previously, we demonstrated that PAK5 expression increased significantly during the malignant progression of colorectal carcinoma (CRC) and that PAK5 promoted CRC metastasis by regulating CRC cell adhesion and migration. In the present study, we aim to investigate the role of PAK5 in camptothecin-induced apoptosis and its potential mechanism of action. Our results showed that overexpression of PAK5 inhibited camptothecin-induced apoptosis by inhibiting the activity of caspase-8 in CRC cells. Accordingly, knockdown of PAK5 in LoVo cells resulted in increased apoptosis. Mechanistically, we found that PAK5 directly phosphorylated Bad on serine 112 and indirectly led to phosphorylation of serine 136 via the Akt pathway. In conclusion, our study revealed previously unappreciated inhibitory role of PAK5 in camptothecin-induced apoptosis, thus suggesting PAK5 as a novel therapeutic target in CRC.

Xia Wang and Wei Gong contributed equally to this work.

X. Wang · W. Gong · H. Qing · Y. Geng · X. Wang · Y. Zhang ·
L. Peng · H. Zhang · B. Jiang (✉)
Guangdong Provincial Key Laboratory of Gastroenterology,
Department of Gastroenterology, Nanfang Hospital,
Southern Medical University,
Guangzhou 510515, People's Republic of China
e-mail: drjiang@163.com

H. Zhang
Key laboratory of Carcinogenesis and Translational Research,
Ministry of Education, Departments of Pathology and Histology
and Embryology, Peking University Health Science Center,
Beijing 100191, People's Republic of China

Introduction

The p21-activated protein kinases (PAKs) are serine/threonine protein kinases activated by binding to the Rho family small GTPases, Rac, and Cdc42. PAKs are categorized into two subgroups (A and B) based on their amino acid sequences and functions [1]. Group B PAKs (PAK4, 5, and 6) do not contain the highly conserved auto-inhibitory domain that is found in the Group A PAKs members (PAK1, 2, and 3). Thus, while Group A members have 80-90% sequence identity within their catalytic domains, Group B PAKs have only approximately 50% identity to the kinase domains of the Group A PAKs [2, 3]. Unlike Group A PAKs, which are activated upon binding to Cdc42 and Rac, Group B PAKs are not strongly activated by GTPase binding [4].

Apart from playing an important role in cytoskeleton organization and cell morphology by regulating the actin cytoskeleton [5–10], PAKs have also recently been shown to regulate apoptosis. Both group A and group B PAKs have been shown to play key roles in regulating the apoptotic response, although their effects may vary, depending on the circumstances and type of cells. For example, PAK2 has pro-apoptotic effects in cells that are sensitive to signaling through the c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein (MAPK) pathways [11]. It is likely that PAK2 is cleaved by caspase-3 during apoptosis, activating PAK2 by releasing its kinase domain. The activated kinase is thought to contribute to membrane and morphological changes that occur during apoptosis [12–14]. On the other hand, PAK1 and PAK4 have been reported to protect cells from apoptosis without

cleavage by caspases. PAKs have also been shown to increase the phosphorylation of Ser112 and Ser136 of Bad [15–17], to activate MAPK pathways [18], and to block the caspase cascade by acting upstream of the mitochondrial pathway and effector caspases [18]. Overexpression of PAK5 activates JNK pathway to decrease apoptosis [19]. In addition, it is found that PAK5 shuttles from the mitochondria to the nucleus, and that the mitochondrial localization of PAK5, which leads to phosphorylation of Ser112 and Ser136 of Bad, is vital to its effects on cell survival [20, 21]. PAK5 can also activate Raf-1, an important effector of Ras-mediated signaling, and target it to the mitochondria, regulating its kinase activity and controlling Raf-1 dependent signaling at mitochondria [22].

Given the fact that PAK5 can protect cells from apoptosis, we reason that PAK5 may be associated with human carcinogenesis. In a previous study, we found that PAK5 expression increased with colorectal carcinoma (CRC) progression from normal colon mucosa to metastatic carcinoma, with remarkable increases in invasive and metastatic CRCs. Furthermore, we found that PAK5 decreased CRC cell adhesion but promoted their migration, which may contribute to CRC metastasis [23]. However, the functional role of PAK5 in CRC progression remains to be further studied. In this study, we aim to examine whether PAK5 can protect chemotherapeutic drug-induced apoptosis and to uncover the signaling pathway involved. Our results showed that overexpression of PAK5 inhibited camptothecin-induced apoptosis in SW480 cells, while knockdown of PAK5 expression resulted in increased apoptosis in Lovo cells. Furthermore, we found that overexpression of PAK5 inhibited activity of Caspase-8. Consistent with the previous study, we also found that PAK5 directly phosphorylated Bad on serine 112 and indirectly led to phosphorylation of serine 136 via the Akt pathway in CRC cells. Taken together, in this study, we present data indicating that PAK5 inhibits apoptosis induced by chemotherapeutic agent and provide evidence of its potential mechanism of action.

Materials and methods

Cell transfection and selection

PEGFP-C3-PAK5 plasmid was constructed as described previously [23]. The PAK5-shRNA plasmid (pGFP/Neo-PAK5) and empty plasmid vector (pGFP/Neo-control) were purchased from GenePharma (Shanghai, China). Cells were seeded in six-well plate with 40–60% confluence 20 h before transfection and PEGFP-C3-PAK5 or control PEGFP-C3 were transfected by Lipofectamine 2000 (Invitrogen, Guangzhou, China) according to the user's protocol. For RNA interference, PAK5-shRNA and control vector (GenePharma,

Shanghai, China) were transfected into the targeted cells by Oligofectamine (Invitrogen) according to the user's protocol. To make stable cell line, transfected cells were selected by 1,000 μg/ml G418 (Merk, Guangzhou, China) for 2 weeks and positive clones were confirmed by Western blotting.

Reagents and antibodies

AKT Inhibitor IV was obtained from Merck. The anticancer agents used in this study were oxaliplatin (Sigma), 5-fluorouracil (5-Fu, KeyGEN), and camptothecin (Sigma). Anti rabbit PAK5 antibody was made as described previously [23]. Rabbit polyclonal anti-Bad, anti-phospho Ser112-Bad, anti-phospho Ser136-Bad (185D10), mouse monoclonal anti-Caspase-8 (1C12), rabbit monoclonal anti-poly (ADP-ribose) polymerase (PARP; 46D11), mouse monoclonal anti-GFP (4B10), and anti-phospho Ser 473-AKT antibodies were from Cell Signaling. The anti-actin antibody and secondary antibodies conjugated to horseradish peroxidase were from Santa Cruz Biotechnology.

Apoptosis assays

Cells were plated in six-well plates at 70–80% confluence 24 h before exposure to anticancer agents. DMSO stock solutions of anticancer agents were diluted with cell culture medium then added to cells. Cells were exposed to anticancer agents for 0, 12, 24, or 48 h, then were collected for flow cytometry or used to prepare lysates. Cells were collected by trypsinization, combined with the floating cells, and then labeled with Annexin V-PE and 7-AAD Viability Staining Solution according to the manufacturer's recommendations (BioVision) for fluorescence-activated cell sorting analysis. For analysis of apoptosis by nuclear staining with Hoechst 33258 (Sigma), cells were treated as above, washed once with phosphate-buffered saline (PBS) and then fixed with ice-cold methanol (500 ml/well) for 10 min. After fixing, cells were washed twice with PBS, stained with 1 mM Hoechst 33258 for 10 min, and then extensively washed twice with PBS and distilled water. Apoptosis was indicated by the presence of condensed or fragmented nuclei which bound the Hoechst dye with high affinity.

For analysis of cell morphology by photo-microscopy, coverslips were washed once with PBS and then inverted and mounted onto glass slides. Cells were visualized under an Olympus microscope. Two hundred cells in three randomly chosen fields were counted and scored for the incidence of apoptotic chromatin changes under fluorescence microscopy.

Western blotting analysis

Cells were exposed to 10, 50, or 100 μM camptothecin for 0, 12, 24, or 48 h, and then collected for Western blotting

analysis. Cells were washed twice with ice-cold PBS then lysed with ice-cold lysis buffer (20 mM Tris-HCl, 1 mM EDTA, 1 mM EGTA, 1 mM sodium vanadate, 0.2 mM phenylmethylsulph-ony1 fluoride, 0.5% NP-40, 1 mg/ml leupeptin, 1 mg/ml aprotinin, 1 mg/ml Pepstatin), and PMSF (1:100) for 30 min on ice. For the detection of phosphorylated Bad, cells were lysed with ice-cold lysis buffer supplemented with protease and phosphatase inhibitors. The lysates were transferred to 1.5 ml Eppendorf tubes to incubate on ice for 30 min, with occasional vortexing. Insoluble material was removed by centrifugation. Soluble protein was removed to a new tube and stored at −20°C until required. Protein samples (30-50 μg) were separated by SDS/PAGE (10% acrylamide gel) using a Bio-Rad Mini-Protean system (100 V for about 3 h). Proteins were transferred to PVDF membranes, 4 MA/cm^2 for 1 h. Then the membranes were blocked for 1 h at room temperature with 5% (w/v) skim milk powder in Tris buffered saline-Tween 20 (0.1% by volume, TBS-T). The membranes were then incubated for 1-2 h at room temperature with primary antibodies in 5% (w/v) skim milk powder dissolved in TBS-T (1:500 dilution). Primary antibodies were removed, and the membranes were extensively washed with TBS-T four times for 20 min. The membranes were then incubated for 1 h at room temperature with the secondary antibodies (goat anti-mouse or anti-rabbit antibody coupled to horseradish peroxidase, 1:3,000-5,000 dilution) in 5% (w/v) skim milk powder dissolved in TBS-T. Following removal of the secondary antibody, the membranes were extensively washed as above for an hour and developed using the Enhanced Chemiluminescence Detection System (Pierce).

Statistical analysis

All results were confirmed in multiple independent experiments, with each time point or condition assayed in triplicate within each experiment. Data were expressed as means±SD and analyzed by one-way ANOVA, followed by Dunnett's multiple comparison tests, with significance defined as $P<0.05$.

Results

Overexpression of PAK5 inhibits camptothecin-induced apoptosis

To investigate the function of PAK5 in apoptosis, we up-regulated PAK5 expression in CRC cell lines by gene transfection. We observed that exogeneous PAK5 protein increased significantly in SW480 cells transfected with the PEGFP-C3-PAK5 plasmid compared to untransfected cells

and cells transfected with the empty plasmid vector PEGFP-C3 (Fig. 1a). Nuclear morphological analysis revealed that exposure of cells to oxaliplatin or 5-FU induced apoptosis to a similar extent in the PAK5 over-expressing SW480 cells as it did in the parental cells. However, apoptosis following exposure to camptothecin was lower in the PAK5 overexpressing SW480 cells compared to the parental cells (Fig. 1b). To further examine the effect of PAK5 overexpression on the induction of apoptosis by camptothecin, parental, and PAK5-overexpressing SW480 cells were exposed to various concentrations of the agent (10, 50, 100 μM) for different durations (0, 12, 24, 48 h), and the extent of apoptosis were analyzed by flow cytometry. Analysis of apoptosis by AnnexinV-PE staining confirmed that camptothecin-induced apoptosis was abrogated by the overexpression of PAK5 in SW480 cells (Fig. 1c), with a significant decrease in apoptosis observed in cells exposed to 100 μM camptothecin for 48 h compared with empty vector SW480 cells and parental cells (Fig. 1d).

Camptothecin-induced activation of caspase-8 and PARP is inhibited in PAK5-overexpressing SW480 cells

Since PAK5 could protect cells from camptothecin-induced apoptosis as detected by flow cytometry; next, we were interested in determining what steps in the camptothecin-induced death pathway are abrogated by PAK5. We first examined the possibility that PAK5 could affect initiator caspases and thereby function upstream of, or independently of, the mitochondrial pathway. To test this possibility, cleavage of the initiator caspase-8 and the effector PARP was examined following camptothecin treatment. As expected, after a 24-h exposure to camptothecin, considerable amounts of intermediate and active caspase-8 fragments (p43/41) could be detected in the control cells (Fig. 2a). Activation of PARP was observed to follow a similar time course (Fig. 2b). In contrast, in cells overexpressing PAK5, cleavage of both caspase-8 and PARP was dramatically reduced and delayed (Fig. 2a and b). Caspase-8 and PARP both remained completely uncleaved after 24 h in the presence of camptothecin, and they were only partially cleaved at 48 h, indicating that induction of caspase-8-like activity was delayed in the PAK5-overexpressing SW480 cells. These results confirm the decrease (and the delay in induction) of apoptosis in cells overexpressing PAK5 (Fig. 2).

Camptothecin-induced apoptosis is increased in PAK5-deficient LoVo cells

To investigate whether the effects of PAK5 on apoptosis are exclusive to SW480 cells, or whether PAK5 exerts similar

Fig. 1 Chemotherapeutic agents-induced apoptosis in SW480 cells. **a** Overexpression of PAK5 in SW480 cells. SW480 cells were transfected with PEGFP-c3-PAK5 or empty plasmid vector PEGFP-c3, and PAK5 expression was assessed by Western blotting. **b** Assessment of apoptosis by Hoechst 33258 staining. Equal numbers of SW480 parental cells, empty vector-transfected cells, or cells overexpressing PAK5 were plated in six-well plates. The cells were exposed to oxaliplatin (120 μM), 5-Fu (50 μM), or camptothecin (100 μM) for 48 h, then subjected to fluorescence microscopy analysis. **c** Flow cytometric analysis of apoptosis induced by camptothecin. Equal numbers of stably transfected SW480 cells were plated in six-well plates 24 h before exposure to 100 μM camptothecin for 0, 12, 24, or 48 h. After treatment, cells were collected and evaluated by flow cytometric analysis of Annexin V-PE and 7-AAD Viability Staining. The percentages of positive cells were labeled. **d** SW480 cells were exposed to various concentrations of camptothecin (10, 50, and 100 μM) for 48 h, then evaluated for apoptosis. These results reflect the data from three independent experiments. *$p<0.05$
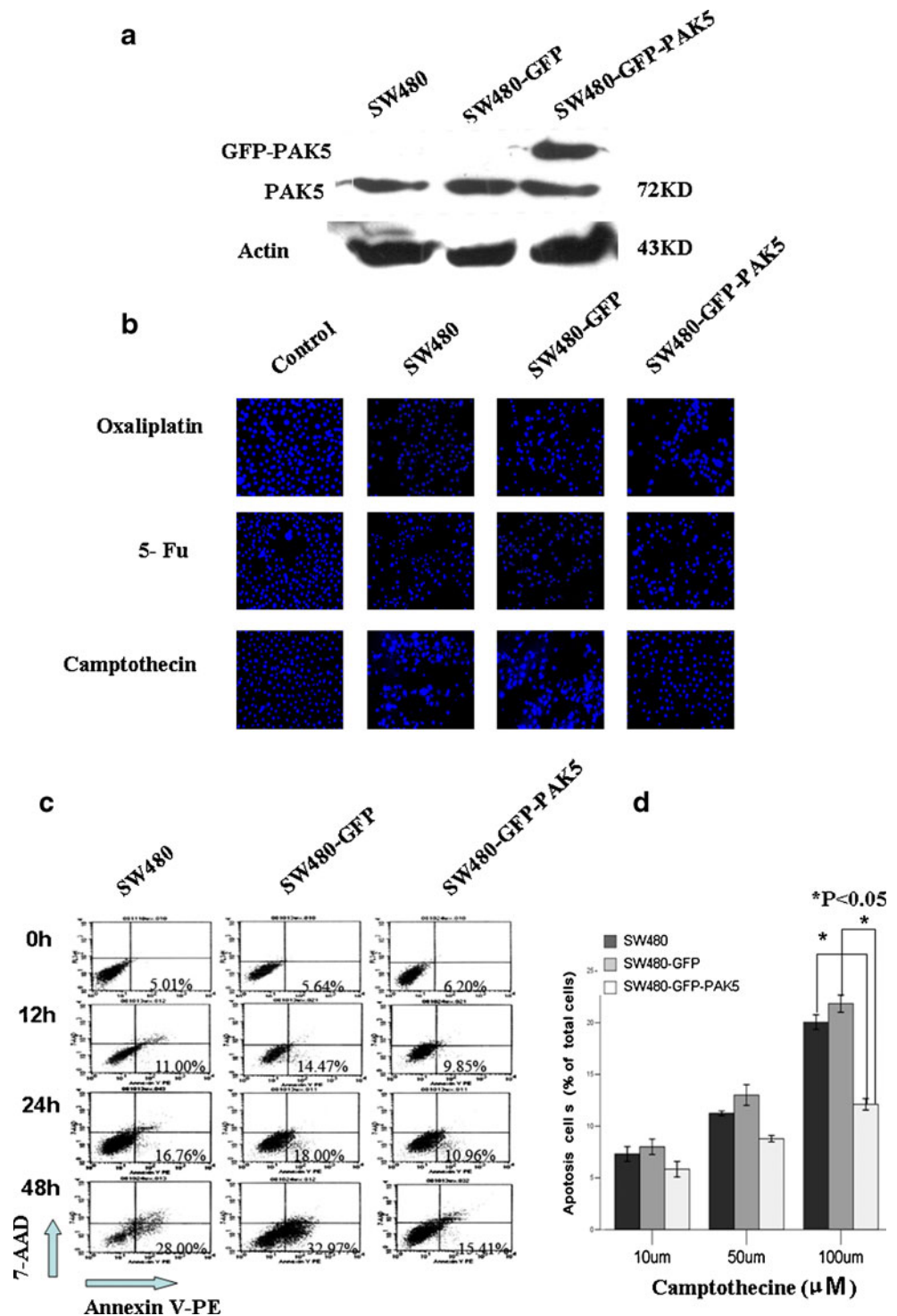

effects in other cell lines, LoVo cells were transfected with a PAK5-shRNA plasmid (Fig. 3a). When the PAK5-shRNA LoVo cells were exposed to 100 μM camptothecin for various times, there was an increase in apoptosis compared to parental cells (Fig. 3b). After 24 h of stimulation, considerable amounts of intermediate and active caspase-8 fragments (p43/41 and p18) could be detected in the

PAK5-siRNA LoVo cells, and similar effects were observed for PARP activation (Fig. 3c).

PAK5 phosphorylates Bad

Because other members of the PAK family have been shown to phosphorylate Bad, we examined the effect of

Fig. 2 PAK5 inhibits Camptothecin-induced activation of caspase-8 and PARP in SW480 cells. SW480 cells were exposed to 100 μM camptothecin for various times (0, 12, 24, and 48 h), then assessed for cleavage of caspase-8 (**a**) and PARP (**b**) by Western blotting. The results presented were representative of three different experiments
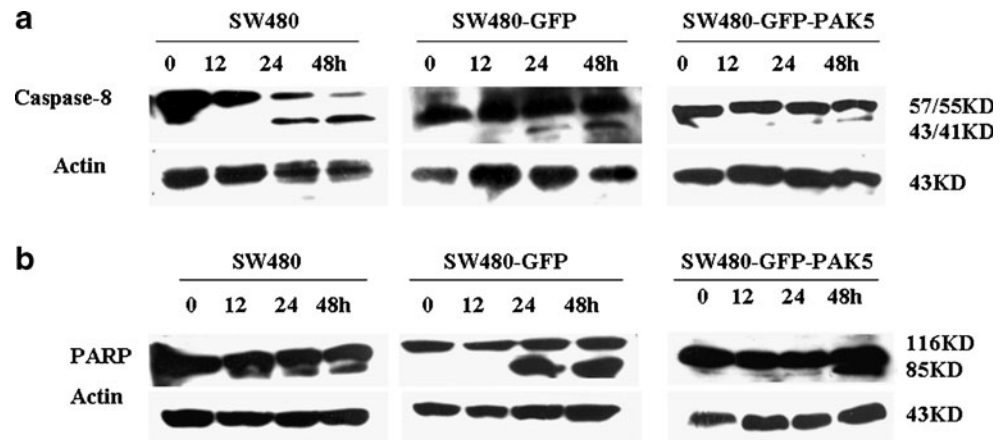
PAK5 on the phosphorylation status of Bad in SW480 cells transfected with either empty vector PEGFP-C3 or PEGFP-C3-PAK5. After treating the cells with 100 μM camptothecin for 48 h, we observed that PAK5 overexpression leads to an increased in the phosphorylation of Bad specifically on serine 112 and 136 (Fig. 4a). However, when cells were exposed to the AKT Inhibitor IV for 48 h, there was no increase in the phosphorylation of Ser 136 (Fig. 4b and c). These experiments indicate that PAK5 may directly increase the phosphorylation of Ser 112, but indirectly increase the phosphorylation of Ser 136 via the Akt pathway.
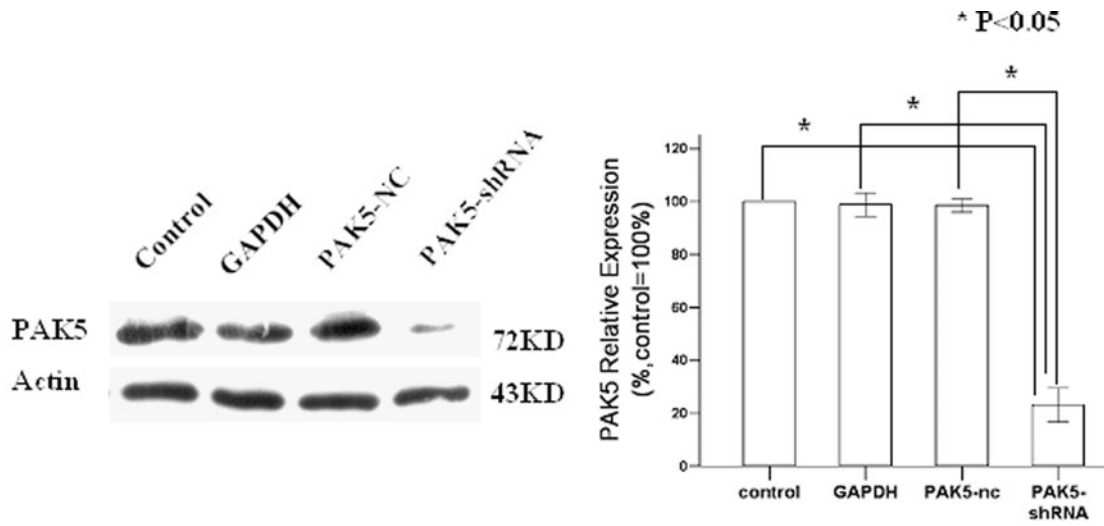
Discussion

PAK5 is a recently identified member of group B PAK kinase family. Group B PAKs are highly expressed in the brain [13, 14] and are localized in the mitochondria where they inhibit apoptosis by phosphorylating BAD in CHO cells [17, 18]. PAK5 shares some common activities with other group B PAKs. Like PAK4, PAK5 can confer protection against apoptotic stimuli and has the ability to phosphorylate Bad on Ser-112 [18, 20]. Both PAK1 and PAK5 interacted with Raf-1 and phosphorylated it at Ser 338, which targeted it to the mitochondria [22, 24]. In our prior study, we found that PAK5 was overexpressed in a variety of CRC cells, and that PAK5 expression was increased with CRC progression from the adenoma to carcinoma, with the highest expression observed in highly invasive and metastatic CRCs [23]. Expression of PAK5 was also found to be increased during the development of CRC from lower to higher Duke's stages. Moreover, the expression of PAK5 was increased from well to poorly differentiated CRCs. These findings imply that PAK5 plays an important role in the progression of CRC via its regulation of apoptosis and cell survival. Therefore, the aim of the present study was to determine whether PAK5 can protect cells from apoptosis and the signal pathway involved.
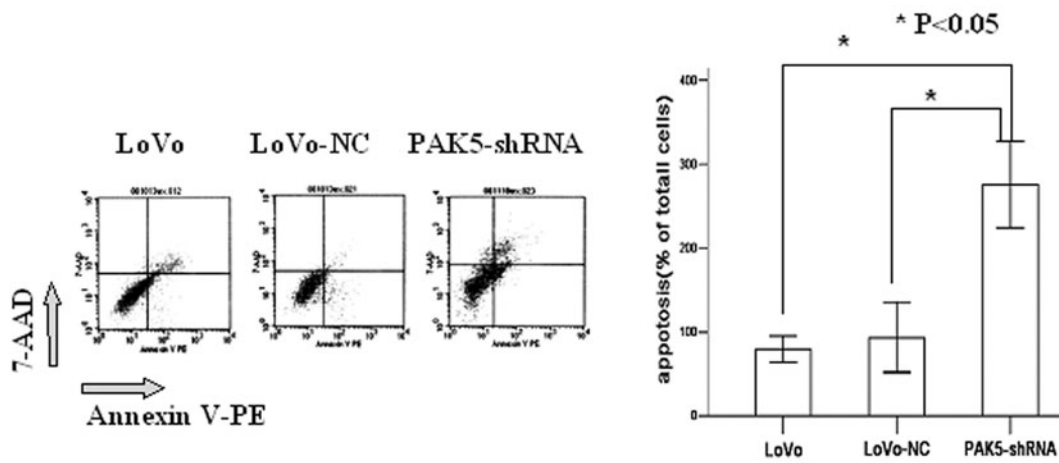
To determine whether PAK5 can protect cells from apoptosis, stably transfected cell lines with PEGFP–c3 empty vector, PEGFP-c3-PAK5 or PAK5-shRNA plasmids were developed. These cell lines provide a good model system to study the function of PAK5 in apoptosis and cell survival. In this study, we determined the response of the SW480 cells overexpressing PAK5 and the parental cells to a variety of anticancer agents, including oxaliplatin, 5-FU, and campto-thecin. Our results showed that the oxaliplatin/5-FU-induced apoptosis exhibited no significant difference between the PAK5 overexpressing cells and the parental cells after the same treatment. However, a moderate decrease in camptothecin-induced apoptosis was observed in the PAK5 overexpressing cells compared with parental cells. Interestingly, previous study showed that SW480 cell lines had low sensitivity to 5-Fu-induced apoptosis and high sensitivity to camptothecin-induced apoptosis [25], our present study further demonstrated that apoptosis induced by camptothecin was significantly affected in PAK5-overexpressing SW480 cells, suggesting that PAK5 activity was negatively correlated with camptothecin-induced apoptosis.

Our results showed that the overexpression of PAK5 in SW480 cells led to the inhibition of camptothecin-induced apoptosis and cleavage of the initiator caspase-8 and the effector PARP. Conversely, shRNA-mediated deletion of PAK5 in LoVo cells strongly increased camptothecin-induced apoptosis and cleavage of initiator caspase-8 and the effector PARP. Thus, PAK5 appears to be a novel regulator responsible for cellular resistance to apoptosis. It is still unclear how PAK5 inhibits caspase-8 activity and whether these effects are direct or indirect. The most attractive possibility is that PAK5 mediated phosphorylation of Bad promotes the interaction between Bad phosphorylated at either Ser-112 or Ser-136 and 14-3-3 proteins. As a result, Bad is sequestered in the cytoplasm and could not translocate into the mitochondria to trigger the apoptosis pathway and the activation of caspases including caspase-8. However, we could not rule out the possibility that PAK5 functions by
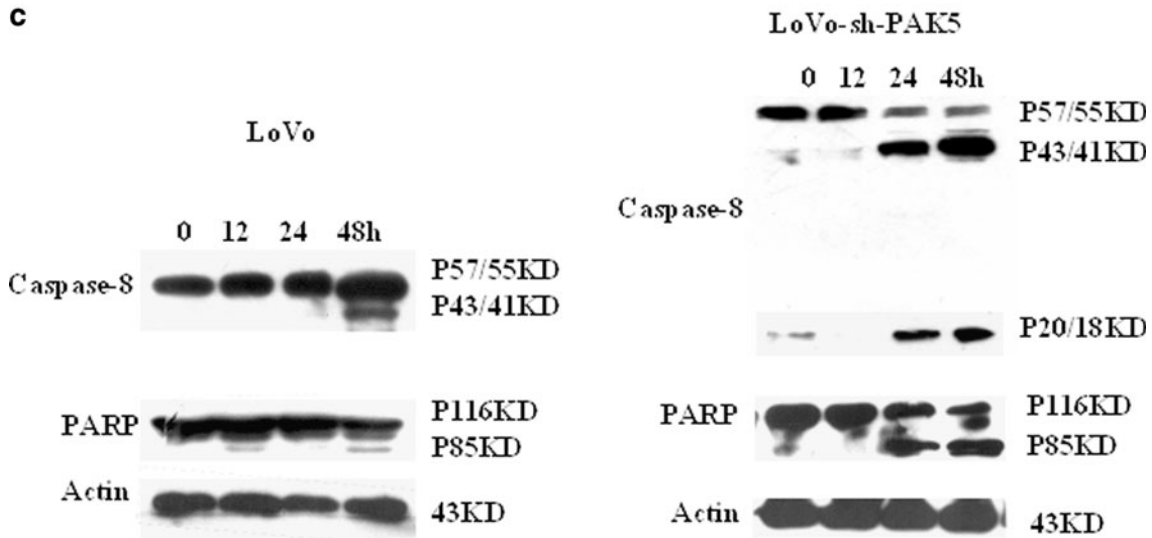
a

b

c

causing changes in the protein level of FLIP, which can affect the activity of caspase-8 [26–28]. On the other hand, PAK1 has been shown to promote osteoclast survival by modulating expression of the IAP family member survivin and the expression of survivin was found to be positively correlated to clinicopathologic parameters of colorectal carcinoma [29, 30]. It is therefore attractive to speculate that PAK5 may upregulate the expression of survivin to antagonize camptothecin-induced apoptosis. Regardless of the mechanism, the regulation of caspase-8 activity demonstrated here is a new function of PAK5, and our results provide novel insights into the mechanism by which colorectal carcinoma cells develop resistance to apoptosis.
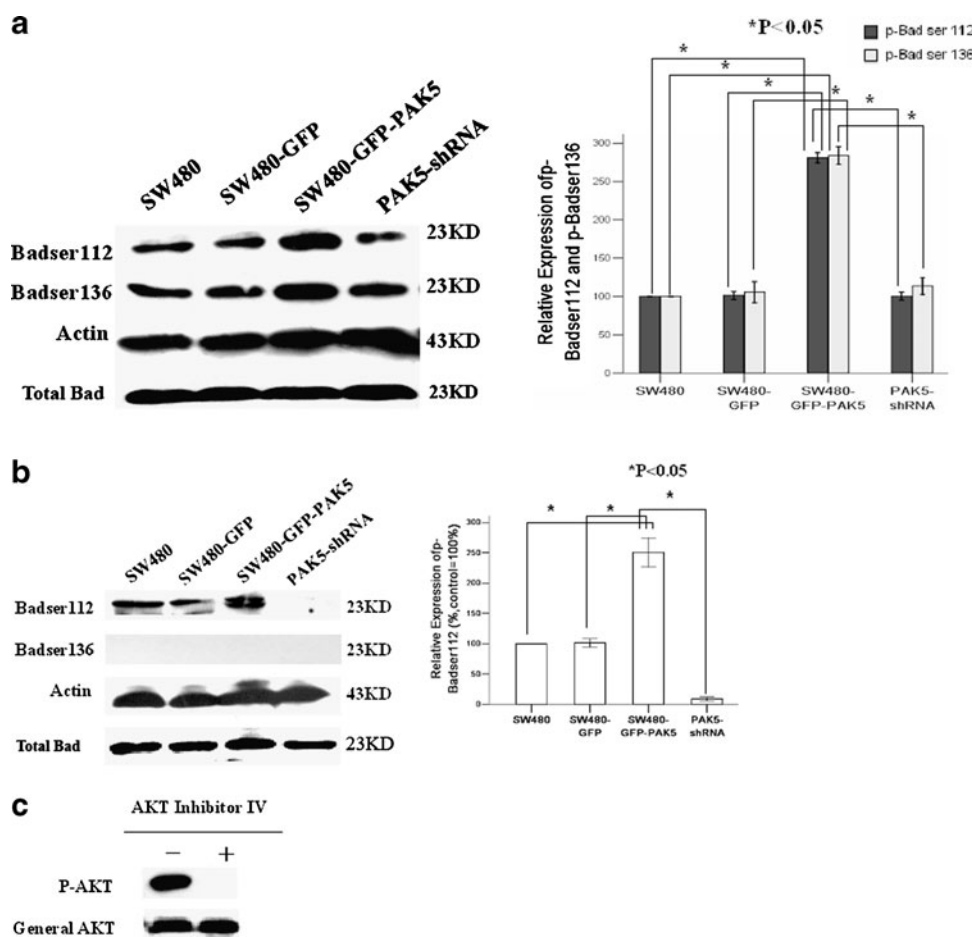

Fig. 4 PAK5 regulates Bad phosphorylation both directly and indirectly. **a** Parental, GFP control, GFP-PAK5, and PAK5-siRNA SW480 cells were treated with 100 μM camptothecin for 48 h, and total Bad and the phosphorylation of Bad (on Ser 112 and 136) were examined by Western blotting. Right panel: Photodensitometric analysis of relative amount of phosphorlated Bad with actin as internal control. The Bad/Actin ratio was set as 100 in parental SW480 cells. **b** Parental, GFP control, GFP-PAK5 and PAK5-siRNA SW480 cells were plated in six-well plates at 60–70% confluence 24 h before a 48 h treatment with Akt Inhibitor IV, and total Bad and the phosphorlation of Bad (on Ser 112 and 136) were examined by Western blotting. Right panel: Photodensitometric analysis of relative amount of phosphorlated Bad with actin as internal control. The Bad/Actin ratio was set as 100 in parental SW480 cells. **c** PAK5-overexpressing SW480 cells were incubated in the presence or absence of 50 μM AKT Inhibitor IV for 48 h, then expression of total (general) and phospho-AKT was evaluated by Western blotting. The results in A and B reflect the data from three independent experiments; *$p < 0.05$; **c** was representative of three different experiments

Current data support the concept that PAK5 appears to induce resistance to apoptosis via the phosphorylation of Bad. While PAK1 and PAK4 have been reported to directly phosphorylate Bad on Ser 112 and 136, and perhaps additional Ser residues [13, 17, 18, 31], our data suggest that the PAK5 phosphorylation of BAD at Ser112 was a direct action. In contrast, the PAK5-stimulated phosphorylation of BAD at Ser136 may be mediated through Akt. Future studies will examine the relationship between PAK5 and the Akt pathway, and how their expression and activity affect apoptosis and cell survival.

In summary, here we demonstrate that PAK5 is involved in cellular resistance to apoptosis, and that this effect likely occurs via alterations in Bad phosphorylation leading to a decrease in the activity of caspase 8. Further examination of PAK5 level and activity in human tumor and normal tissues will help determine whether PAK5 represents a novel target to improve the response to conventional cancer therapies.

References

1. Ching YP, Leong VY, Wong CM, Kung HF. Identification of an autoinhibitory domain of p21-activated protein kinase 5. J Biol Chem. 2003;278:33621–4.
2. Jaffer ZM, Chernoff J. p21-activated kinases: three more join the Pak. Int J Biochem Cell Biol. 2002;34:713–7.
3. Dan I, Watanabe NM, Kusumi A. The Ste20 group kinases as regulators of MAP kinase cascades. Trends Cell Biol. 2001;11:220–30.
4. Parrini MC, Lei M, Harrison SC, Mayer BJ. Pak1 kinase homodimers are autoinhibited in trans and dissociated upon activation by Cdc42 and Rac1. Mol Cell. 2002;9:73–83.
5. Edwards DC, Sanders LC, Bokoch GM, Gill GN. Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. Nat Cell Biol. 1999;1:253–9.
6. Frost JA, Khokhlatchev A, Stippec S, White MA, Cobb MH. Differential effects of PAK1-activating mutations reveal activity-dependent and -independent effects on cytoskeletal regulation. J Biol Chem. 1998;273:28191–8.
7. Kiosses WB, Daniels RH, Otey C, Bokoch GM, Schwartz MA. A role for p21-activated kinase in endothelial cell migration. J Cell Biol. 1999;147:831–44.
8. Manser E, Huang H-Y, Loo T-H, Chen XQ, Leung T, Lim L. Expression of constitutively active a-Pak reveals effects of the kinase on actin and focal complexes. Mol Cell Biol. 1997;17:1129–43.
9. Sells MA, Boyd JT, Chernoff J. p21-activated kinase 1 (Pak1) regulates cell motility in mammalian fibroblasts. J Cell Biol. 1999;145:837–49.
10. Sells MA, Knaus UG, Bagrodia S, Ambrose DM, Bokoch GM, Chernoff J. Human p21-activated kinase (Pak1) regulates actin organization in mammalian cells. Curr Biol. 1997;7:202–10.
11. Rudel T, Zenke FT, Chuang T-H, Bokoch GM. p21-activated kinase (PAK) is required for Fas-induced JNK activation in Jurkat cells. J Immunol. 1998;160:7–11.
12. Lee N, MacDonald H, Reinhard C, Halenbeck R, Roulston A, Shi T, et al. Activation of hPAK65 by caspase cleavage induces some

13. of the morphological and biochemical changes of apoptosis. Proc Natl Acad Sci USA. 1997;94:13642–7.
14. Rudel T, Bokoch GM. Membrane and morphological changes in apoptotic cells regulated by caspase-mediated activation of PAK2. Science. 1997;276:1571–4.
14. Walter BN, Huang Z, Jakobi R, Tuazon PT, Alnemri ES, Litwack G, et al. Cleavage and activation of p21-activated protein kinase gamma-PAK by CPP32 (caspase 3). Effects of autophosphorylation on activity. J Biol Chem. 1998;273:28733–9.
15. Daniels RH, Bokoch GM. p21-activated protein kinase: acrucial component of morphological signaling? Trends Biochem Sci. 1999;24:350–5.
16. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, et al. Akt phosphorylation of BAD couples survival signals to the cell intrinsic death machinery. Cell. 1997;91:231–41.
17. Schurmann A, Mooney AF, Sanders LC, Sells MA, Wang H-G, Reed JC, et al. p21-activated kinase 1 (PAK1) phosphorylates the death agonist BAD and protects cells from apoptosis. Mol Cell Biol. 2000;20:453–61.
18. Gnesutta N, Qu J, Minden AG. The serine/threonine kinase PAK4 prevents caspase activation and protects cells from apoptosis. J Biol Chem. 2001;276:14414–9.
19. Pandey A, Dan I, Kristiansen TZ, Watanabe NM, Voldby J, Kajikawa E, et al. Cloning and characterization of PAK5, a novel member of mammalian p21-activated kinase-II sub-family that is predominantly expressed in brain. Oncogene. 2002;21:3939–48.
20. Cotteret S, Jaffer ZM, Beeser A, Chernoff J. p21-activated kinase 5(Pak5) localizes to mitochondria and inhibits apoptosis by phosphorylating BAD. Mol Cell Biol. 2003;23:5526–39.
21. Cotteret S, Chernoff J. Nucleocytoplasmic shuttling of pak5 regulates its antiapoptotic properties. Mol Cell Biol. 2006;26:3215–30.
22. Wu X, Carr HS, Dan I, Ruvolo PP, Frost JA. p21 activated kinase 5 activates Raf-1 and targets it to mitochondria. J Cell Biochem. 2008;105:167–75.
23. Gong W, An Z, Wang Y, Pan X, Fang W, Jiang B, et al. P21-activated kinase 5 is overexpressed during colorectal cancer progression and regulates colorectal carcinoma cell adhesion and migration. Int J Cancer. 2009;125:548–55.
24. Jin S, Zhuo Y, Guo W, Field J. p21-activated kinase 1 (Pak1)-dependent phosphorylation of Raf-1 regulates its mitochondrial local-ization, phosphorylation of BAD, and Bcl-2 association. J Biol Chem. 2005;280:24698–705.
25. Mariadason JM, Arango D, Shi Q, Wilson AJ, Corner GA, Nicholas C, et al. Gene expression profiling-based prediction of response of colon carcinoma cells to 5-fluorouracil and campto-thecin. Cancer Res. 2003;63:8791–812.
26. Chang DW, Xing Z, Pan Y, Algeciras-Schimnich A, Barnhart BC, Yaish-Ohad S, et al. c-FLIP(L) is a dual function regulator for caspase-8 activation and CD95-mediated apoptosis. EMBO J. 2002;21:3704–14.
27. Irmler M, Thome M, Hahne M, Schneider P, Hofmann K, Steiner V, et al. Inhibition of death receptor signals by cellular FLIP. Nature. 1997;388:190–5.
28. Li H, Zhu H, Xy C-J, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the fas pathway of apoptosis. Cell. 1998;94:491–501.
29. Bradley EW, Ruan MM, Oursler MJ. PAK1 is a novel MEK-independent raf target controlling expression of the IAP survivin in M-CSF-mediated osteoclast survival. J Cell Physiol. 2008;217:752–8.
30. Liang QL, Wang BR, Li GH. DcR3 and survivin are highly expressed in colorectal carcinoma and closely correlated to its clinicopathologic parameters. J Zhejiang Univ Sci B. 2009;10:675–82.
31. Tang Y, Zhou H, Chen A, Pittman RN, Field J. The Akt proto-oncogene links Ras to Pak and cell survival signals. J Biol Chem. 2000;275:9106–9.