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# NIK is involved in constitutive activation of the alternative NF-κB pathway and proliferation of pancreatic cancer cells

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ABSTRACT

Pancreatic cancer has one of the poorest prognoses among human neoplasms. Constitutive activation of NF-κB is frequently observed in pancreatic cancer cells and is involved in their malignancy. However, little is known about the molecular mechanism of this constitutive NF-κB activation. Here, we show that the alternative pathway is constitutively activated and NF-κB-inducing kinase (NIK), a mediator of the alternative pathway, is significantly expressed in pancreatic cancer cells. siRNA-mediated silencing of NIK expression followed by subcellular fractionation revealed that NIK is constitutively involved in the processing of p100 and nuclear transport of p52 and RelB in pancreatic cancer cells. In addition, NIK silencing significantly suppressed proliferation of pancreatic cancer cells. These results clearly indicate that NIK is involved in the constitutive activation of the alternative pathway and controls cell proliferation in pancreatic cancer cells. Therefore, NIK might be a novel target for the treatment of pancreatic cancer.



## Introduction

Pancreatic cancer is a highly lethal malignancy with an overall 5-year survival rate of 5% [1]. This high mortality is due to the aggressive and rapidly metastatic nature of the disease and its ability to resist conventional chemotherapy [2]. Therefore, elucidation of the molecular mechanisms of pancreatic cancer development is essential for the discovery of new therapeutic strategies.

One of the signal transduction pathways that are deregulated to be involved in the malignancy of pancreatic cancer cells is the NF-κB pathway [3]. NF-κB proteins comprise a family that encompasses p50/p105, p52/p100, c-Rel, RelA (also known as p65) and RelB. To a limited extent, these proteins can form homo- or hetero-dimers with distinct DNA-binding specificities. Such dimers are inactive in the cytoplasm in most normal cells due to their interaction with the IκB family of proteins. Through membrane-bound receptors and a series of adaptor and modulator proteins, various extracellular signals activate NF-κB by stimulus-provoked phosphorylation of IκB by an IKK complex, which induces the nuclear translocation of NF-κB as a consequence of proteasome-mediated degradation of IκB [4]. Two distinct pathways lead to activation of NF-κB: a classical pathway and an alternative pathway. For the most part, the classical pathway is defined by activation of the p50-RelA complex upon degradation of an associated IκB, and the alternative pathway is characterized by the processing of an inactive NF-κB2 p100-RelB dimer to activate p52-RelB through degradation of C-terminal IκB-like sequences of p100, which is induced by IKKα [5–8].

Activation of NF-κB is tightly regulated by various stimuli in normal cells so that its activation is transient, whereas NF-κB is constitutively activated in pancreatic cancer cell lines [9–11]. Constitutive activation of the alternative pathway has also been reported recently [12]. Several reports have indicated that constitutive activation of NF-κB promotes angiogenesis, metastasis, and resistance to apoptosis and chemotherapy in pancreatic cancer cells [13–16]. Moreover, it has been reported that IKKα and RelB positively control proliferation of pancreatic cancer cell lines [17], indicating that constitutive activation of the alternative pathway is involved in the proliferation of pancreatic cancer cells. Although previous studies have shown that autocrine secretion of interleukin-1 contributes to constitutive activation of the classical pathway [18,19], how the alternative pathway is constitutively activated in pancreatic cancer cells remains to be elucidated.

*Abbreviations:* NF-κB, nuclear factor-κB; IκB, inhibitor of NF-κB; NIK, NF-κB-inducing kinase; IKK, IκB kinase; siRNA, small interfering RNA; TRAF, tumor necrosis factor receptor-associated factor; cIAP, cellular inhibitor of apoptosis; BAFF, B cell-activating factor belonging to the tumor necrosis factor family; GAPDH, glyceraldehydes-3-phosphate dehydrogenase.

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## Materials and methods

*Cell culture.* All pancreatic carcinoma cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA) and maintained in RPMI 1640 containing 10% FBS. Plat-A cells were maintained in DMEM containing 10% FBS, 2 μg/ml of puromycin (Sigma–Aldrich, St Louis, MO) and 4 μg/ml of blasticidin (Sigma–Aldrich).

*NIK siRNA transfection experiments.* Transfection of NIK stealth siRNA (5′-GCCAGUCCGAGAGUCUUGAUCAGAU-3′) or control siRNA (Invitrogen, Carlsbad, CA, USA) was performed with Lipofectamine RNAiMAX (Invitrogen). For fractionation analysis, cells were incubated with siRNA/RNAiMAX solution (siRNA concentration: 6 pmol). In the proliferation assay, PANC-1 ($5 \times 10^4$ cells/well) and PK-45H ($2.5 \times 10^4$ cells/well) were incubated with siRNA/RNAiMAX solution (siRNA concentration: 4.7 fmol), and six days after siRNA transfection, cells were trypsinized and the viable cell number was counted with the trypan blue exclusion method.

*Retrovirus-mediated gene transfer.* A mutant NIK cDNA that carries six silent mutations within the target region for a siRNA for NIK (siNIK) was generated by PCR-mediated site-directed mutagenesis to change the sequence from cagtccgagagtcttgat (nt 328–345) to caAtcGgaAagCctAgaC. The NIK cDNAs were introduced into the retroviral vector pMXs-puro (kindly provided by Dr. T. Kitamura). The packaging Plat-A cells were transfected with the pMXs plasmids by the calcium phosphate method, and virus stocks were prepared by collecting the culture medium. PANC-1 and PK-45H were infected with the virus stock in the presence of 6 μg/ml polybrene (Sigma–Aldrich) and selected with puromycin.

*Cell fractionation and immunoblotting.* Cytoplasmic and nuclear fractions were prepared as described [20]. Immunoblotting was performed as described [21]. Primary antibodies were anti-RelA, anti-RelB and anti-PARP-1 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-p100/p52 antibody (Millipore, Billerica, MA, USA), anti-NIK and anti-phospho-p100 antibody (Cell Signaling Technology, Beverly, MA, USA), and anti-α-Tubulin antibody (Calbiochem, La Jolla, CA, USA).

*Semiquantitative reverse transcription-PCR (RT-PCR) and quantitative real-time PCR.* Cells were grown for 48 h after siRNA transfection. Methods for RNA isolation, cDNA preparation, RT-PCR were described previously [21]. Primers for *NIK* were forward, 5′-CTCAGCA TCGACAGCCTCTC-3′ and reverse, 5′-GTGCACTGCAGGTCGATGC-3′. Primers for *GAPDH* were forward, 5′-CCACATCGCTCAGACACCAT-3′ and reverse, 5′-TGACAAGCTTCCCGTTCTCA-3′. The PCR cycle was as follows: 37 cycles (*NIK*) or 27 cycles (*GAPDH*) at 95 °C for 30 s, 64 °C (*NIK*) or 57 °C (*GAPDH*) for 30 s, 72 °C for 30 s with a 2-min initial denaturation at 95 °C and a 2-min final extension at 72 °C. Quantitative real-time PCR were preformed as described [21].

## Results and discussion

*The alternative pathway is constitutively activated in pancreatic cancer cell lines*

To understand the frequency and extent of the constitutive activation of the alternative pathway and to elucidate the molecular mechanisms of the activation in pancreatic cancer cells, we collected eight pancreatic cancer cell lines, including QGP-1, PANC-1, PK-45P, PK-45H, PK-1, KP-1N, KP-2, and PK-59. We then performed subcellular fractionation of each cell line to check the processing of p100 to p52 and nuclear localization of p52 and RelB. Processing of p100 to p52 and nuclear translocation of both proteins are mediated by the alternative pathway. Relatively large amounts of p52 and RelB were found in nuclear fractions of PANC-1, PK-1, and KP-1N, whereas less, but still significant amounts of these two proteins were localized in the nuclei of other pancreatic cancer cell lines (Fig. 1). These results strongly suggest that the alternative pathway is constitutively activated in all eight

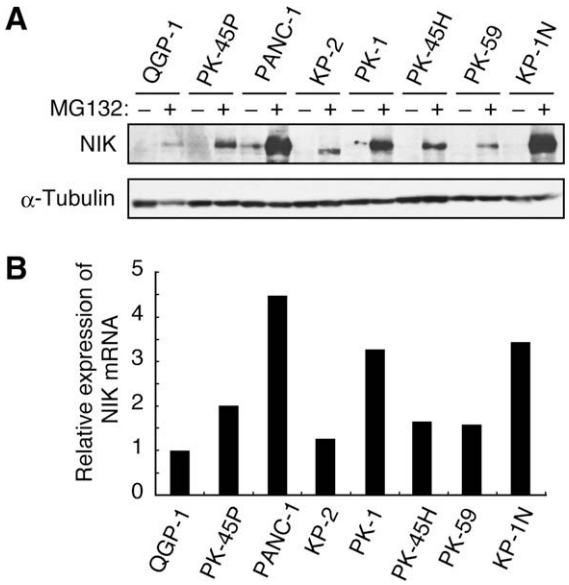


**Fig. 2.** Expression levels of NIK protein are correlated with those of its mRNA in various pancreatic cancer cells. (A) Cells were pretreated with (+) or without (–) MG132 (20 μM) for 4 h. Whole cell lysates were then prepared and subjected to immunoblotting. (B) Total RNA was isolated from cells, and cDNA synthesis and quantitative real-time PCR analysis were performed. The relative NIK mRNA level of each cell is shown as a fold increase relative to that of the QGP-1. Results shown in (B) are representative of two independent experiments.

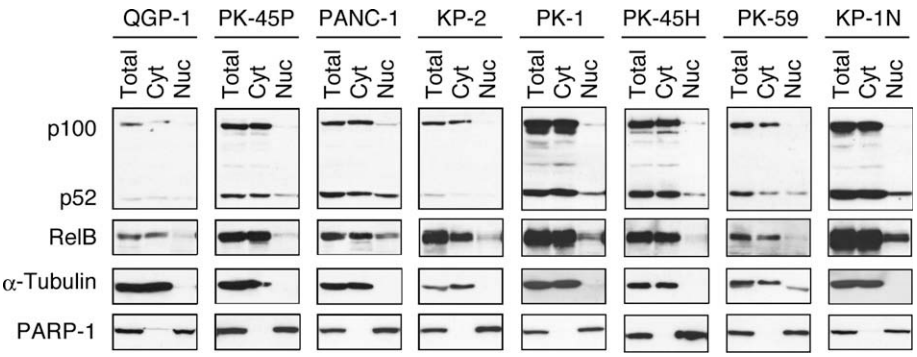


**Fig. 1.** The alternative pathway is constitutively activated in various pancreatic cancer cells. Whole cell lysates (Total) and lysates prepared from cytoplasmic (Cyt) and nuclear (Nuc) fractions of various pancreatic cell lines were analyzed by immunoblotting using anti-p100/p52, anti-RelB, anti-α-Tubulin (cytoplasmic marker) and anti-PARP-1 (nuclear marker). Each lane is prepared from $3 \times 10^4$ cells.

pancreatic cancer cell lines, although the extent of the activation varied among cell lines.

*Expression levels of NIK mRNA and protein are correlated with nuclear amounts of p52 and RelB proteins in pancreatic cancer cell lines*

The alternative pathway is mediated by the NIK protein because *Alymphoplasia* (*aly*) mice, which carry an inactive mutation in *NIK* gene, display impaired alternative pathways [8,22]. Activity of NIK is regulated by a ubiquitin ligase complex that comprises TRAF2, TRAF3, cIAP1, and cIAP2. In unstimulated cells, NIK is inactivated through its proteasome-mediated degradation triggered by polyubiquitination of NIK, which is catalyzed by cIAP1/cIAP2. CD40 ligand or BAFF stimulation induces degradation of TRAF3 and prevents the polyubiquitination of NIK by the TRAF2–cIAP1–cIAP2 complex, allowing NIK accumulation and activation through autophosphorylation [23,24]. Accumulated NIK then activates IKKα and induces activation of the alternative pathway [25]. Interestingly, inactivation of the TRAF3 and cIAP1/2 proteins by mutations causes stabilization of NIK proteins, which leads to constitutive activation of both classical and alternative NF-κB activation in some multiple myeloma cell lines [26,27]. In addition, NIK is over-expressed at the pre-translational level and induces constitutive NF-κB activation in adult T-cell leukemia (ATL) [28]. These links between pathologically deregulated expression of NIK and carcinogenesis led us to speculate that NIK might be involved in the constitutive activation of NF-κB and in the malignant phenotypes of pancreatic cancer cell lines. Thus, we next determined whether NIK is accumulated in pancreatic cancer cell lines. Endogenous NIK protein was hardly detected, but could be observed when cells were treated with proteasome inhibitor MG132, as reported previously [25]. Relatively high levels of NIK protein were detected in PANC-1, PK-1, and KP-1N (Fig. 2A), whereas lower levels of NIK protein were observed in the other cell lines. Together

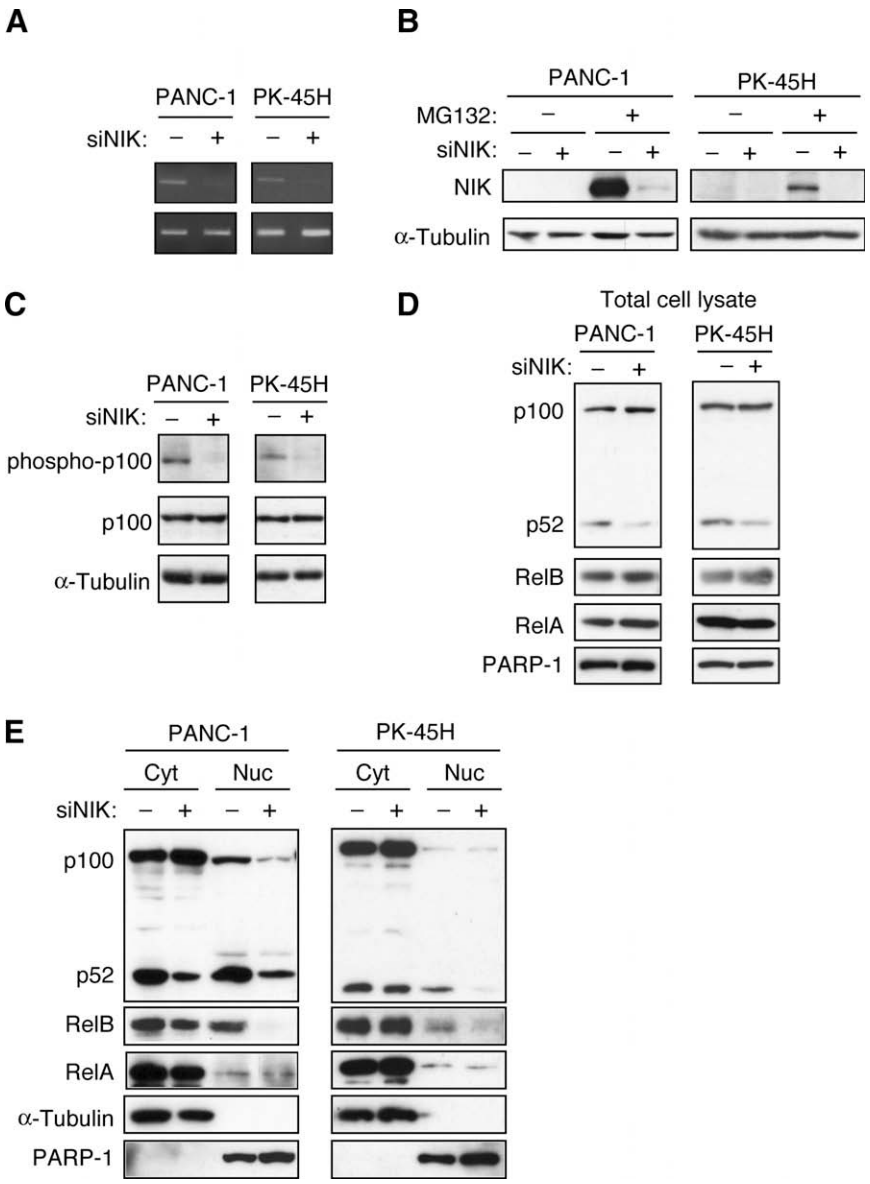


**Fig. 3.** NIK is involved in the constitutive activation of the alternative pathway, but not in that of the classical pathway. (A) Total RNA was isolated from mock- or siNIK-transfected cells, and cDNA synthesis and semiquantitative RT-PCR analysis were performed. (B) Cells were mock-transfected or transfected with siNIK for 44 h. Cells were then treated with DMSO or MG132 (20 μM) for 4 h. Lysates were analyzed by immunoblotting. (C) Cells were mock-transfected or transfected with siNIK for 48 h. Lysates were then analyzed by immunoblotting. (D) Cells were transfected as in (C) and lysates were then analyzed by immunoblotting. (E) Cells were transfected as in (C) and lysates were fractionated. Cytoplasmic fractions (prepared from $7 \times 10^3$ cells) and nuclear fractions (prepared from $2.8 \times 10^4$ cells) were analyzed by immunoblotting.

with the results shown in Fig. 1, expression levels of the NIK protein are correlated with the extent of activation of the alternative pathway, as judged by the nuclear amounts of p52 and RelB. To analyze the relation between expression levels of NIK protein and those of NIK mRNA, we analyzed amounts of NIK mRNA through real-time PCR (Fig. 2B). Expression levels of NIK mRNA varied among the eight cell lines and they were high in PANC-1, PK-1, and KP-1N, as in the case of NIK protein. NIK mRNA levels are well correlated with expression levels of NIK protein, suggesting that the expression of NIK protein is likely to be determined by mRNA levels, but not by protein stability. Expression of NIK is up-regulated in pancreatic cancer cells because the expression of NIK mRNA is higher than that in Jurkat (T-cell leukemia) and HeLa cells (cervical cancer) (data not shown), and NIK protein was not detected in MCF7 (breast cancer) and Jurkat cells even when they were treated with MG132 (data not shown). These results suggest that up-regulation of NIK mRNA and protein may result in activation of the alternative pathway in pancreatic cancer cell lines.

*NIK regulates the constitutive activation of the alternative pathway but not that of the classical pathway*

To obtain evidence that NIK contributes to the constitutive activation of the alternative pathway, siNIK was used to suppress NIK expression in PANC-1 and PK-45H, which were chosen as representatives of the NIK high and low expressers, respectively. The efficiency of NIK suppression in pancreatic cancer cells was checked by semiquantitative RT-PCR analysis. More than 80% reduction of *NIK* mRNA was observed in PANC-1 and PK-45H at 2 days after siRNA transfection (Fig. 3A). We also performed immunoblotting analysis of lysates prepared from cells treated with or without MG132 and confirmed that NIK protein expression was barely

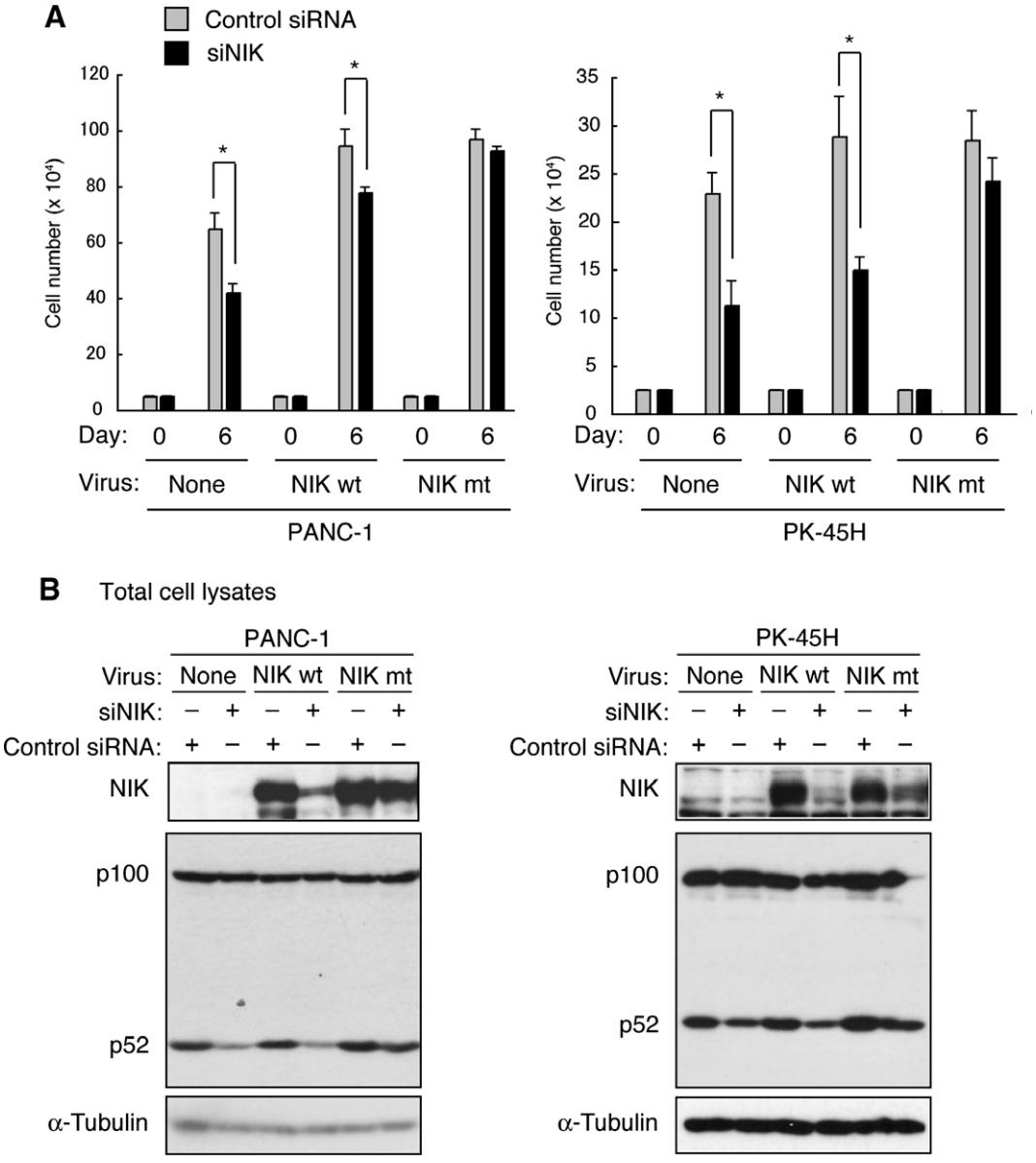


**Fig. 4.** NIK is involved in proliferation of pancreatic cancer cell lines. (A) PANC-1 and PK-45H retrovirally infected with the empty vector (None) or vectors expressing NIKwt or NIKmt were transfected with siNIK or control siRNA. Six days after transfection, the numbers of viable cells were counted. Data are presented as means ± SD of triplicate determinations and are representative of two independent experiments. $^{*}p < 0.05$. (B) PANC-1 and PK-45H retrovirally infected as in (A) were transfected with siNIK or control siRNA. After 48 h incubation, cell lysates were analyzed by immunoblotting.

detectable in siNIK-transfected cells compared with mock-transfected cells (Fig. 3B). NIK suppression resulted in a severe reduction of p100 phosphorylation (Fig. 3C), which is catalyzed by IKKα activated by NIK and triggers processing of p100 to p52. Furthermore, NIK suppression also reduced processing of p100 to p52 (Fig. 3D) and nuclear translocation of p52 and RelB in both PANC-1 and PK-45H (Fig. 3E). Interestingly, NIK suppression did not affect nuclear translocation of RelA. These results clearly indicate that NIK is involved in the constitutive activation of the alternative pathway, but not the classical pathway, in pancreatic cancer cells.

Constitutive activation of the alternative pathway occurs under a number of pathological conditions. In various lymphomas associated with *nfkb2* gene rearrangement, which results in the generation of a C-terminal truncated p100 protein lacking the processing inhibitory domain, processing occurs constitutively in an IKKα-dependent but NIK-independent manner [29]. In addition, the NIK protein is accumulated in multiple myeloma cells due to its stabilization induced by mutations that inactivate TRAF3 or cIAP1/2 [26,27], which leads to constitutive activation of both classical and alternative pathways. Because constitutive activation of the alternative pathway, but not that of the classical pathway, is NIK-dependent in pancreatic cancer cells, molecular mechanisms underlying constitutive activation of the alternative pathway in pancreatic cancer cells are distinct from those in lymphoma and myeloma cells described above.

Because the alternative pathway is mediated by p100 and RelB, these two genes must be sufficiently expressed to activate the alternative pathway. *p100* gene is a target of classical NF-κB and Notch signaling pathways, and RelB expression is up-regulated by the classical pathway [30]. Both the Notch pathway and the classical pathway are constitutively activated in pancreatic cancer cells [11,18,31]. Therefore, constitutive activation of the alternative pathway is likely to be maintained by continuous expression of p100 and RelB by the classical and Notch pathways.

*NIK is involved in proliferation of pancreatic cancer cell lines*

Both IKKα and RelB are involved in the proliferation of pancreatic cancer cells by inducing the expression of skp2, which is required for G1/S transition [17]. Therefore, we checked whether NIK contributes to the cell proliferation of pancreatic cancer cells because NIK plays a critical role in the constitutive activation of the alternative pathway. PANC-1 and PK-45H were transfected with siNIK or control siRNA and cell numbers were counted 6 days after transfection. Proliferation of each cell line after siNIK transfection was significantly lower than that after transfection of control siRNA (Fig. 4A), suggesting the involvement of NIK in proliferation. To rule out the possibility that siNIK transfection-induced growth inhibition may result from suppression of growth promoting gene(s) other than NIK, growth experiments were performed with cells that were engineered to exogenously express a wild-type NIK cDNA (NIKwt) or NIK cDNA containing a silent mutation in the siRNA target region (NIKmt) (Fig. 4A). In both PANC-1 and PK-45H, siNIK treatment resulted in severe reduction of the NIK protein in cells exogenously expressing NIKwt, whereas the same treatment only partially reduced NIK protein in cells expressing NIKmt (Fig. 4B). Meanwhile, transfection of siNIK resulted in significant reduction of p100 processing in NIKwt-expressing cells, whereas generation of p52 was barely affected by siNIK transfection into NIKmt-expressing cells (Fig. 4B). More importantly, siNIK-induced growth inhibition was observed only in NIKwt-expressing cells but not in NIKmt-expressing cells (Fig. 4A). These results clearly indicate that siNIK transfection-induced growth inhibition was due to suppression of NIK protein, but not due to suppression of other growth promoting gene(s). In addition, proliferation of NIKwt or NIKmt-expressing cells was significantly enhanced compared to that of control cells, especially in PANC-1 (Fig. 4A). The enhanced NIK expression barely changed the processing of p100 (Fig. 4B), probably because the proteasome-mediated processing of p100 was saturated. However, amounts of p52 and RelB in nuclear fraction were increased in these cells (data not shown). These results strongly suggest that overexpression of NIK enhances activation of the alternative pathway without increasing the p100 processing, thereby enhancing cell growth. Taken together, we conclude that NIK is involved in the regulation of pancreatic cancer cell line proliferation.

In this study, we clearly provide evidence that NIK is involved in the constitutive activation of the alternative NF-κB pathway and also in the proliferation of pancreatic cancer cell lines. Deregulation of NIK activity and its involvement in tumorigenesis have been reported in multiple myeloma [26,27], ATL, and Hodgkin's lymphoma [28]. We have also recently demonstrated that NIK is involved in the constitutive activation of the alternative pathway in basal-like subtype breast cancer cells [21]. Therefore, NIK could be a potential therapeutic target in various cancers.

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## References

[1] A. Jemal, R. Siegel, E. Ward, Y.P. Hao, J.Q. Xu, T. Murray, M.J. Thun, Cancer statistics, 2008, CA Cancer J. Clin. 58 (2008) 71–96.
[2] D.H. Li, K.P. Xie, R. Wolff, J.L. Abbruzzese, Pancreatic cancer, Lancet 363 (2004) 1049–1057.
[3] B. Holcom, M. Yip-Schneider, C.M. Schmidt, The role of nuclear factor kB in pancreatic cancer and the clinical applications of targeted therapy, Pancreas 36 (2008) 225–235.
[4] M. Karin, Y. Ben-Neriah, Phosphorylation meets ubiquitination: the control of NF-kB activity, Ann. Rev. Immunol. 18 (2000) 621–663.
[5] P. Ramakrishnan, W.X. Wang, D. Wallach, Receptor-specific signaling for both the alternative and the canonical NF-kappa B activation pathways by NF-kB-inducing kinase, Immunity 21 (2004) 477–489.
[6] U. Senftleben, Y.X. Cao, G.T. Xiao, F.R. Greten, G. Krahn, G. Bonizzi, Y. Chen, Y.L. Hu, A. Fong, S.C. Sun, M. Karin, Activation by IKKa of a second, evolutionary conserved, NF-kB signaling pathway, Science 293 (2001) 1495–1499.
[7] G.T. Xiao, A. Fong, S.C. Sun, Induction of p100 processing by NF-κB-inducing kinase involves docking IκB kinase a (IKKα) to p100 and IKKα-mediated phosphorylation, J. Biol. Chem. 279 (2004) 30099–30105.
[8] G.T. Xiao, E.W. Harhaj, S.C. Sun, NF-κB-inducing kinase regulates the processing of NF-κB2 p100, Mol. Cell 7 (2001) 401–409.
[9] N.M. Chandler, J.J. Canete, M.P. Callery, Increased expression of NF-κB subunits in human pancreatic cancer cells, J. Surg. Res. 118 (2004) 9–14.
[10] L. Li, B.B. Aggarwal, S. Shishodia, J. Abbruzzese, R. Kurzrock, Nuclear factor-κB and IκB kinase are constitutively active in human pancreatic cells, and their down-regulation by curcumin (diferuloylmethane) is associated with the suppression of proliferation and the induction of apoptosis, Cancer 101 (2004) 2351–2362.
[11] S. Liptay, C.K. Weber, L. Ludwig, M. Wagner, G. Adler, R.M. Schmid, Mitogenic and antiapoptotic role of constitutive NF-κB/Rel activity in pancreatic cancer, Int. J. Cancer 105 (2003) 735–746.
[12] C.E. Wharry, K.M. Haines, R.G. Carroll, M.J. May, Constitutive non-canonical NFκB signaling in pancreatic cancer cells, Cancer Biol. Ther. 8 (2009) 58–67.
[13] A. Arlt, A. Gehrz, S. Muerkoster, J. Vorndamm, M.L. Kruse, U.R. Folsch, H. Schafer, Role of NF-κB and Akt/PI3K in the resistance of pancreatic carcinoma cell lines against gemcitabine-induced cell death, Oncogene 22 (2003) 3243–3251.
[14] S. Fujioka, G.M. Sclabas, C. Schmidt, J.G. Niu, W.A. Frederick, Q.G. Dong, J.L. Abbruzzese, D.B. Evans, C. Baker, P.J. Chiao, Inhibition of constitutive NF-κB activity by IκBαM suppresses tumorigenesis, Oncogene 22 (2003) 1365–1370.
[15] X. Pan, T. Arumugam, T. Yamamoto, P.A. Levin, V. Ramachandran, B. Ji, G. Lopez-Berestein, P.E. Vivas-Mejia, A.K. Sood, D.J. McConkey, C.D. Logsdon, Nuclear factor-κB p65/relA silencing induces apoptosis and increases gemcitabine effectiveness in a subset of pancreatic cancer cells, Clin. Cancer Res. 14 (2008) 8143–8151.

[16] H.Q. Xiong, J.L. Abbruzzese, E. Lin, L.W. Wang, L.Z. Zheng, K.P. Xie, NF-κB activity blockade impairs the angiogenic potential of human pancreatic cancer cells, Int. J. Cancer 108 (2004) 181–188.
[17] G. Schneider, D. Saur, J.T. Siveke, R. Fritsch, F.R. Greten, R.M. Schmid, IKKα controls p52/RelB at the skp2 gene promoter to regulate G1- to S-phase progression, EMBO J. 25 (2006) 3801–3812.
[18] A. Arlt, J. Vorndamm, S. Muerkoster, H. Yu, W.E. Schmidt, U.R. Folsch, H. Schafer, Autocrine production of interleukin 1b confers constitutive nuclear factor κB activity and chemoresistance in pancreatic carcinoma cell lines, Cancer Res 62 (2002) 910–916.
[19] D. Melisi, J. Niu, Z. Chang, Q. Xia, B. Peng, S. Ishiyama, D.B. Evans, P.J. Chiao, Secreted interleukin-1a induces a metastatic phenotype in pancreatic cancer by sustaining a constitutive activation of nuclear factor-κB, Mol. Cancer Res. 7 (2009) 624–633.
[20] N.C. Andrews, D.V. Faller, A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian-cells, Nucleic Acids Res. 19 (1991) 2499.
[21] N. Yamaguchi, T. Ito, S. Azuma, E. Ito, R. Honma, Y. Yanagisawa, A. Nishikawa, M. Kawamura, J.I. Imai, S. Watanabe, K. Semba, J.I. Inoue, Constitutive activation of nuclear factor-κB is preferentially involved in the proliferation of basal-like subtype breast cancer cell lines, Cancer Sci (in press), doi:10.1111/j1349-7006.2009.01228.x.
[22] T. Yamada, T. Mitani, K. Yorita, D. Uchida, A. Matsushima, K. Iwamasa, S. Fujita, M. Matsumoto, Abnormal immune function of hemopoietic cells from alymphoplasia (aly) mice, a natural strain with mutant NF-κB-inducing kinase, J. Immunol. 165 (2000) 804–812.
[23] S. Vallabhapurapu, A. Matsuzawa, W.Z. Zhang, P.H. Tseng, J.J. Keats, H.P. Wang, D.A.A. Vignali, P.L. Bergsagel, M. Karin, Nonredundant and complementary functions of TRAF2 and TRAF3 in a ubiquitination cascade that activates NIK-dependent alternative NF-κB signaling, Nat. Immunol. 9 (2008) 1364–1370.
[24] B.J. Zarnegar, Y.Y. Wang, D.J. Mahoney, P.W. Dempsey, H.H. Cheung, J. He, T. Shiba, X.L. Yang, W.C. Yeh, T.W. Mak, R.G. Korneluk, G.H. Cheng, Noncanonical NF-κB activation requires coordinated assembly of a regulatory complex of the adaptors cIAP1, cIAP2, TRAF2 and TRAF3 and the kinase NIK, Nat. Immunol. 9 (2008) 1371–1378.
[25] G.L. Qing, Z.X. Qu, G.T. Xiao, Stabilization of basally translated NF-κB-inducing kinase (NIK) protein functions as a molecular switch of processing of NF-κB2 p100, J. Biol. Chem. 280 (2005) 40578–40582.
[26] C.M. Annunziata, R.E. Davis, Y. Demchenko, W. Bellamy, A. Gabrea, F. Zhan, G. Lenz, I. Hanamura, G. Wright, W. Xiao, S. Dave, E.M. Hurt, B. Tan, H. Zhao, O. Stephens, M. Santra, D.R. Williams, L. Dang, B. Barlogie, J.D. Shaughnessy, W.M. Kuehl, L.M. Staudt, Frequent engagement of the classical and alternative NF-κB pathways by diverse genetic abnormalities in multiple myeloma, Cancer Cell 12 (2007) 115–130.
[27] J.J. Keats, R. Fonseca, M. Chesi, R. Schop, A. Baker, W.J. Ching, S. Van Wier, R. Tiedemann, C.X. Shi, M. Sebag, E. Braggio, T. Henry, Y.X. Zhu, H. Fogle, T. Price-Troska, G. Ahmann, C. Mancini, L.A. Brents, S. Kumar, P. Greipp, A. Dispenzieri, B. Bryant, G. Mulligan, L. Bruhn, M. Barrett, R. Valdez, J. Trent, A.K. Stewart, J. Carpten, P.L. Bergsagel, Promiscuous mutations activate the noncanonical NF-κB pathway in multiple myeloma, Cancer Cell 12 (2007) 131–144.
[28] Y. Saitoh, N. Yamamoto, M.Z. Dewan, H. Sugimoto, V.J.M. Bruyn, Y. Iwasaki, K. Matsubara, X.H. Qi, T. Saitoh, I. Imoto, J. Inazawa, A. Utsunomiya, T. Watanabe, T. Masuda, S. Yamaoka, Overexpressed NF-κB-inducing kinase contributes to the tumorigenesis of adult T-cell leukemia and Hodgkin Reed–Sternberg cells, Blood 111 (2008) 5118–5129.
[29] G.L. Qing, G.T. Xiao, Essential role of IκB kinase alpha in the constitutive processing of NF-κB2 p100, J. Biol. Chem. 280 (2005) 9765–9768.
[30] G.D. Bren, N.J. Solan, H. Miyoshi, K.N. Pennington, L.J. Pobst, C.V. Paya, Transcription of the RelB gene is regulated by NF-κB, Oncogene 20 (2001) 7722–7733.
[31] Y. Miyamoto, A. Maitra, B. Ghosh, U. Zechner, P. Argani, C.A. Iacobuzio-Donahue, V. Sriuranpong, T. Iso, I.M. Meszoely, M.S. Wolfe, R.H. Hruban, D.W. Ball, R.M. Schmid, S.D. Leach, Notch mediates TGF alpha-induced changes in epithelial differentiation during pancreatic tumorigenesis, Cancer Cell 3 (2003) 565–576.