Biochemical and Biophysical Research Communications 423 (2012) 38–44

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Pyruvate kinase M2 promotes the growth of gastric cancer cells via regulation of Bcl-xL expression at transcriptional level

Oh-Hyung Kwon [a], Tae-Wook Kang [a], Jeong-Hwan Kim [a], Mirang Kim [a], Seung-Moo Noh [b], Kyu-Sang Song [c], Hyang-Sook Yoo [a], Woo-Ho Kim [d], Zhi Xie [e], David Pocalyko [e], Seon-Young Kim [a,*], Yong Sung Kim [a,*]

[a] Medical Genomics Research Center, KRIBB, Daejeon 305-806, Republic of Korea
[b] Department of General Surgery, College of Medicine, Chungnam National University, Daejeon 301-747, Republic of Korea
[c] Department of Pathology, College of Medicine, Chungnam National University, Daejeon 301-747, Republic of Korea
[d] Department of Pathology, College of Medicine, Seoul National University, Seoul 110-799, Republic of Korea
[e] Pfizer Worldwide Research and Development, Oncology Research Unit, 10724 Science Center Drive, San Diego, CA 92121, United States

ARTICLE INFO

Article history:
Received 2 May 2012
Available online 22 May 2012

Keywords:
PKM2
NF-κB
Bcl-xL
Gastric cancer

ABSTRACT

PKM2 is an isoenzyme of the glycolytic enzyme pyruvate kinase that promotes aerobic glycolysis. Here, we describe an important role for PKM2 in regulating the survival of gastric cancer (GC) cells. We showed that PKM2 was overexpressed in gastric tumor tissues compared to normal tissues and its expression level was associated with poor survival of gastric cancer patients. We also showed that PKM2 affected cell survival by regulating Bcl-xL at the transcriptional level. PKM2 knockdown partially affected the stability of NF-kB subunit p65, suggesting that post-translational regulation of p65 by PKM2 is one of plausible mechanisms for the increased cell growth. Therefore, PKM2 may function as an upstream molecule that regulates p65 function and thus enhances the growth of tumor cells.

1. Introduction

Metabolism is an essential cellular function that enables cells to grow, reproduce, maintain their structures, and respond to their environments. It is well known that the metabolism of tumor cells shifts from oxidative phosphorylation to aerobic glycolysis, which is known as the Warburg effect [1]. Pyruvate kinase (PK) is one of the molecules that are important for aerobic glycolysis [2,3]. Mammalian cells have four PK isoforms: PKL in the liver and kidney, PKR in red blood cells, PKM1 in muscle and the brain, and PKM2 (a splice variant of PKM1) in embryonic and tumor cells [1]. Whereas the pyruvate kinase isoenzyme types L, R and M1 are present as enzymatically highly active tetrameric forms, PKM2 may occur in both tetrameric and nearly inactive dimeric forms [2]. PKM2 plays a central role in the metabolism of cancer cells and is expressed in a broad range of human cancers [4]. Knocking down PKM2 expression by shRNA and replacing it with PKM1 significantly reduces the ability of tumor cells to form tumors in nude mice [5]. Clinical studies have demonstrated that PKM2 is released into the blood, and PKM2 in EDTA plasma samples is increased in gastrointestinal cancers [6]. As PKM2 is necessary for aerobic glycolysis in cancer cells, PKM2 is a potential molecular target for disrupting glucose metabolism in cancer cells.

NF-κB is an inducible transcription factor that controls the expression of a number of proteins involved in the regulation of cell survival and the immune response [7]. The NF-κB family is composed of five members, p65 (RelA), RelB, c-Rel, p50 and p52, which are sequestered in the cytoplasm as an inactive complex associated with members of the inhibitor of κB (IκB) protein family. In addition to the well-established role of NF-κB in both immunity and inflammation, deregulation of NF-κB signaling is also associated with oncogenesis and cancer malignancies [8,9]. Activated NF-κB in cancer cells has been shown to increase the expression of many genes involved in cell proliferation, metastasis, angiogenesis, and anti-apoptosis. Moreover, NF-κB activation has been shown to correlate with higher malignancies and poor prognosis [8,9]. Therefore, it is important to find the mechanisms that regulate NF-κB activity in cancer.

In this study, we identified PKM2 as an overexpressed gene in gastric cancer (GC) patients at both the transcriptional and protein levels and showed that PKM2 expression level affects the survival of GC cells. In addition, we found that high PKM2 expression was associated with poor prognosis in GC patients. We also showed that PKM2-mediated NF-κB stabilization may underlies the molecular basis for increased survival in GC cells, in part, by regulating the expression of Bcl-xL, one of apoptosis related genes.

* Corresponding authors. Fax: +82 42 879 8119.
E-mail addresses: kimsy@kribb.re.kr (S.-Y. Kim), yongsung@kribb.re.kr (Y.S. Kim).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.05.063

2. Materials and methods

2.1. Cell lines and tissue samples

GC cell lines were cultured in complete RPMI 1640 medium. The 293T cell line was maintained in complete DMEM media. All cell lines were obtained from the Korean Cell Line Bank, and all complete media contained 10% fetal bovine serum (Hyclone), 100 U/ml penicillin/streptomycin (Invitrogen, Carlsbad, CA), 2 mM L-glutamine, and 0.5 mM HEPES. One hundred eighty-eight tumors were collected from Chungnam National University Hospital. All samples were obtained with informed consent, and their use was approved by the Internal Review Board at Chungnam National University Hospital.

2.2. Real-time reverse transcription-polymerase chain reaction

Real-time RT-PCR has been done as described previously (for details see Supplementary data) [10].

2.3. Tissue array and immunohistochemistry

To investigate PKM2 protein expression in cancer tissues, we immunostained tissue array slides with tissues from GC cases treated at the Seoul National University College of Medicine. Core tissue biopsies (2 mm in diameter) were taken from individual paraffin-embedded gastric tumors (donor blocks) and arranged in a new recipient paraffin block (tissue array block) using a trephine apparatus (Superbiochips Laboratories, Seoul, Republic of Korea). An adequate case was defined as a tumor occupying more than 10% of the core area. Sections (4 μm) were cut from each tissue array block, deparaffinized with xylene, and dehydrated using ethanol. Normal serum was used to block nonspecific protein binding. Immunohistochemical staining for PKM2 (Cell Signaling Technology) was performed using a standard avidin–biotin immunoperoxidase complex method (Vectastatin Elite ABC peroxidase kit; Vector Laboratories, Burlingame, CA) after microwave antigen retrieval.

2.4. Lentiviral packaging and transduction of PKM2 small hairpin RNA (shRNA)

Lentiviral packaging and transduction of PKM2 shRNA was done as described previously (for details see Supplementary data) [10].

2.5. siRNA transfection

A non-targeting siRNA control (catalog No.: SN1003) and siRNAs for targeting human Bcl-xL mRNA (siRNA No. for siBcl-xL-1: 1011922 and siBcl-xL-2: 1011925) were purchased from Bioneer (Daejeon, Korea). For siRNA transfection, AGS cells were transfected with siRNAs (100 nM) using Lipofectamine Plus (Invitrogen), and the Bcl-xL knockdown was quantified 48 h post transfection by qRT-PCR. To assess the effect of Bcl-xL siRNA on cell proliferation, AGS cells were seeded in 96-well plates and transfected with siRNAs (100 nM) using Lipofectamine Plus (Invitrogen). Cell growth was measured 48 and 72 h after transfection using CCK-8 reagent (Dojindo).

2.6. Cell proliferation and anchorage-dependent colony formation assays

Cell proliferation and anchorage-dependent colony formation assays were done as described previously (for details see Supplementary data) [10].

2.7. Caspase-3/7 assay and TUNEL assay

Cells transduced with either shCTL or shPKM2 lentivirus were harvested, and then resuspended in PBS. The cells were mixed with the caspase-Glo-3/7 reagent (Promega) and incubated for 1 h at room temperature with agitation. The luciferase activity was measured using a luminometer and was normalized by protein concentration. Apoptotic cells were detected with the TUNEL method using an *in situ* cell death detection kit (Roche). The assay was performed according to the manufacturer's instructions. Apoptotic cells were visualized on a fluorescence microscope.

2.8. Western blot and preparation of nuclear fraction

Western blot and preparation of nuclear fraction were done as described previously (for details see Supplementary data) [10].

2.9. Statistical analysis

Statistical analysis of group differences was performed using Student's *t*-test. A P value <0.05 was considered significant. The following parameters were obtained from the medical records of the 188 patients studied: age, gender, histology, lymph node metastasis, and tumor stage. Estimates of cumulative survival were calculated using the Kaplan–Meier method, and the observed differences in survival were analyzed by the log-rank test. Statistical analyses were performed with the R statistical programming language (http://cran.r-project.org/).

3. Results

3.1. PKM2 is a reliable prognostic marker for GC patients

We assessed PKM2 expression levels in various cancers by searching the GENT database [11]. PKM2 is up-regulated in many cancer tissues compared to the corresponding normal tissues including GC (data not shown). PKM1 and PKM2 are produced by alternative splicing of transcripts of the PKM gene (exon 9 for PKM1 and exon 10 for PKM2) [1]. Thus, we designed specific primers that distinguish PKM2 from PKM1 (Supplementary Fig. S2A). We then assessed PKM2 mRNA levels in 188 paired gastric tumor and adjacent normal tissues. As expected, a majority of patients (146 of 188) showed higher expression levels of PKM2 in cancer tissues than in adjacent normal tissues (Fig. 1A). We then analyzed the correlation between PKM2 expression and several clinicopathological parameters of GC patients and observed a positive correlation between PKM2 expression and the tumor size (Fig. 1B). However, PKM2 expression was not correlated with other examined parameters such as age, gender, histology, lymph node metastasis, and tumor stage (Supplementary Table S1).

To examine PKM2 protein levels in gastric tumors and adjacent normal tissues, we performed tissue microarray (TMA) using PKM2-specific antibody on 46 paired normal and tumor tissues. We found that 37 of 46 tumor tissues were positive (80%), whereas 22 of 46 normal tissues were positive (48%) for PKM2 staining (Fig. 1C). We further performed TMA for PKM2 on gastric tumor tissues ($n = 380$) to examine the relationship between PKM2 expression and patients' survival. We separated the patients into two groups, low and high PKM2 expression groups, respectively, and then compared overall survival between the two groups. Kaplan–Meier survival analysis showed that there was a significant difference in survival between the two groups (Fig. 1D), suggesting that PKM2 may be a useful prognostic marker for GC patients.

We checked the expression level of PKM2 mRNA and protein in GC cell lines (Supplementary Figs. S1A and B). We also compared

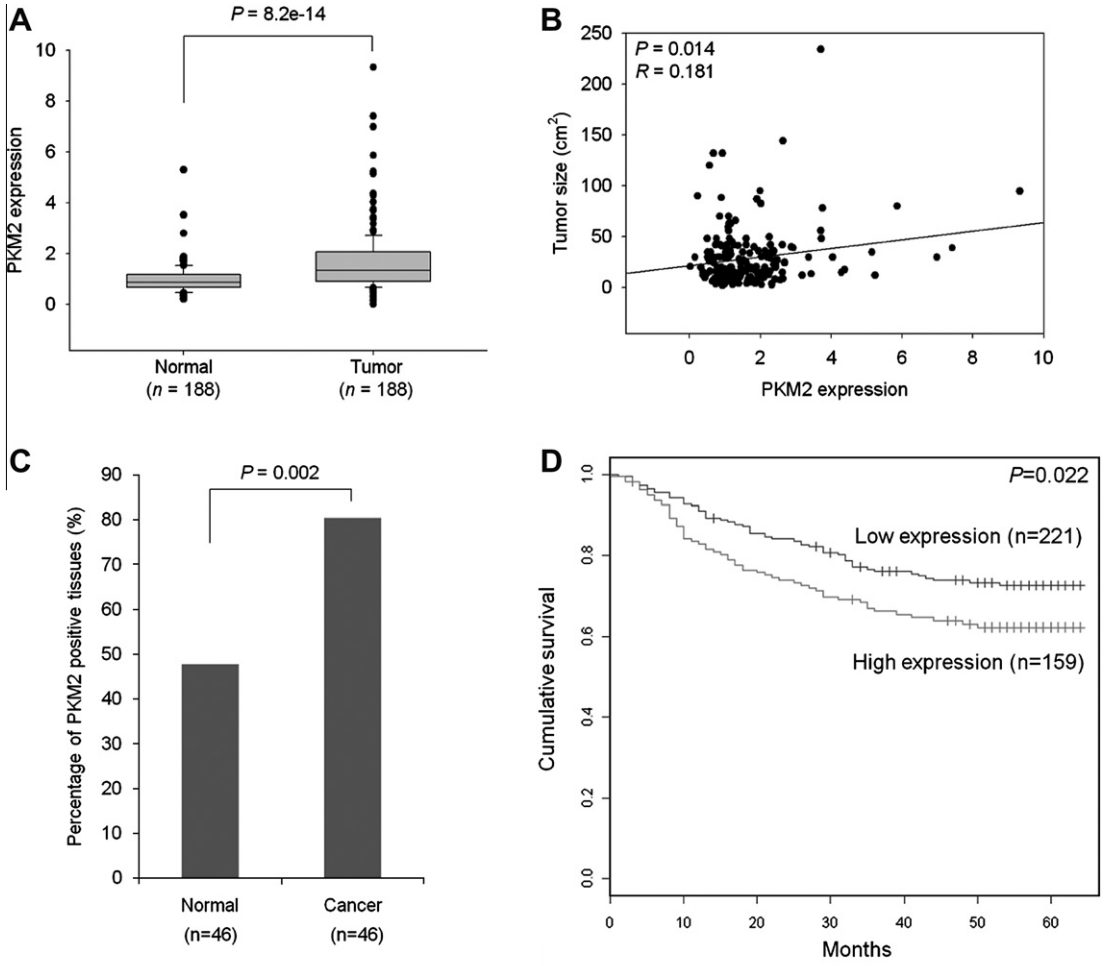

Fig. 1. The expression of PKM2 in gastric tumor and adjacent normal tissues and its correlation with the overall survival of patients with GC. (A) The expression levels of PKM2 were measured by qRT-PCR in 188 tumor and adjacent normal tissues. Expression levels were normalized to the β-actin level in each sample. (B) The relationship between tumor size and relative PKM2 mRNA level. (C) The expression pattern of PKM2 protein in gastric tumor and adjacent normal tissues by immunohistochemistry. (D) Survival of patient groups with high and low expression levels of PKM2 protein in gastric tumor tissues.

the expression levels of PKM1 and PKM2 in GC cell lines by qRT-PCR, and found that all the cell lines expressed PKM1 at low level, while expressing PKM2 at high level (Supplementary Fig. S2B). Furthermore, the levels of total PKM expression, which were obtained using the primers covering the common regions of PKM1 and PKM2 (Supplementary Fig. S2A), were similar to the pattern of PKM2 expression, suggesting that PKM2 is mainly responsible for the expression of the pyruvate kinase (both from M1 and M2 form) in GC cell lines.

To investigate whether the constant expression of PKM2 is associated with chromatin remodeling, we performed chromatin immunoprecipitation assays (ChIP). We designed primer sets covering two regions of the promoter. The active histone methylation marker, H3K4me3, was amplified in all examined cell lines (Supplementary Fig. S1C), indicating that an open chromatin structure is involved in maintaining the constant expression of PKM2.

3.2. Reduced expression of PKM2 decreased GC cell survival

Given that PKM2 is constantly expressed in GC cells and that it has been implicated in the survival of other tumors [5,12], we assessed its importance for the survival of GC cells. Using a PKM2 shRNA-expressing lentiviral vector, we observed reduced expression levels of PKM2 mRNA and protein in AGS cells by qRT-PCR and immunoblotting, respectively (Fig. 2A, *top* and *bottom*). Although PKM2 shRNA also reduced expression levels of PKM1 mRNA (data not shown), as shown in Supplementary Fig. S2, the expression levels of PKM2 exhibited about 100-fold increase compared to those of PKM1 in AGS cells, suggesting that PKM1 might have little effect on the cell phenotype. As expected, AGS cells transduced with PKM2 shRNA grew much more slowly than control shRNA-treated cells (Fig. 2B). An anchorage-dependent colony formation assay also revealed a significant reduction in the size and number of colonies (Fig. 2C). The growth inhibition was also observed in other GC cell lines (Supplementary Fig. S3). It was reported that PKM2 siRNA increased caspase-3/7 activity and reduced the survival of glioma cells [12]. We investigated whether the reduced cell growth mediated by PKM2 knockdown was caused by apoptosis. AGS cells stably expressing PKM2 shRNA were examined by TUNEL assay. Under fluorescence microscopy, many TUNEL-positive (green fluorescent) PKM2 shRNA-treated cells were observed, whereas fewer control cells were TUNEL-positive (Fig. 2D). In addition, caspase-3/7 activity and cleaved PARP-1 levels were increased significantly in PKM2 shRNA-treated cells compared to control cells (Figs. 2E and F). These results indicate that the apoptotic pathway may be involved in the reduced cell growth after PKM2 knockdown.

3.3. PKM2 affects the survival of GC cells by regulating Bcl-xL expression

We then asked whether the apoptosis of GC cells caused by the PKM2 knockdown was associated with Bcl-related genes. Using the GENT database, we first examined the pattern of correlation in

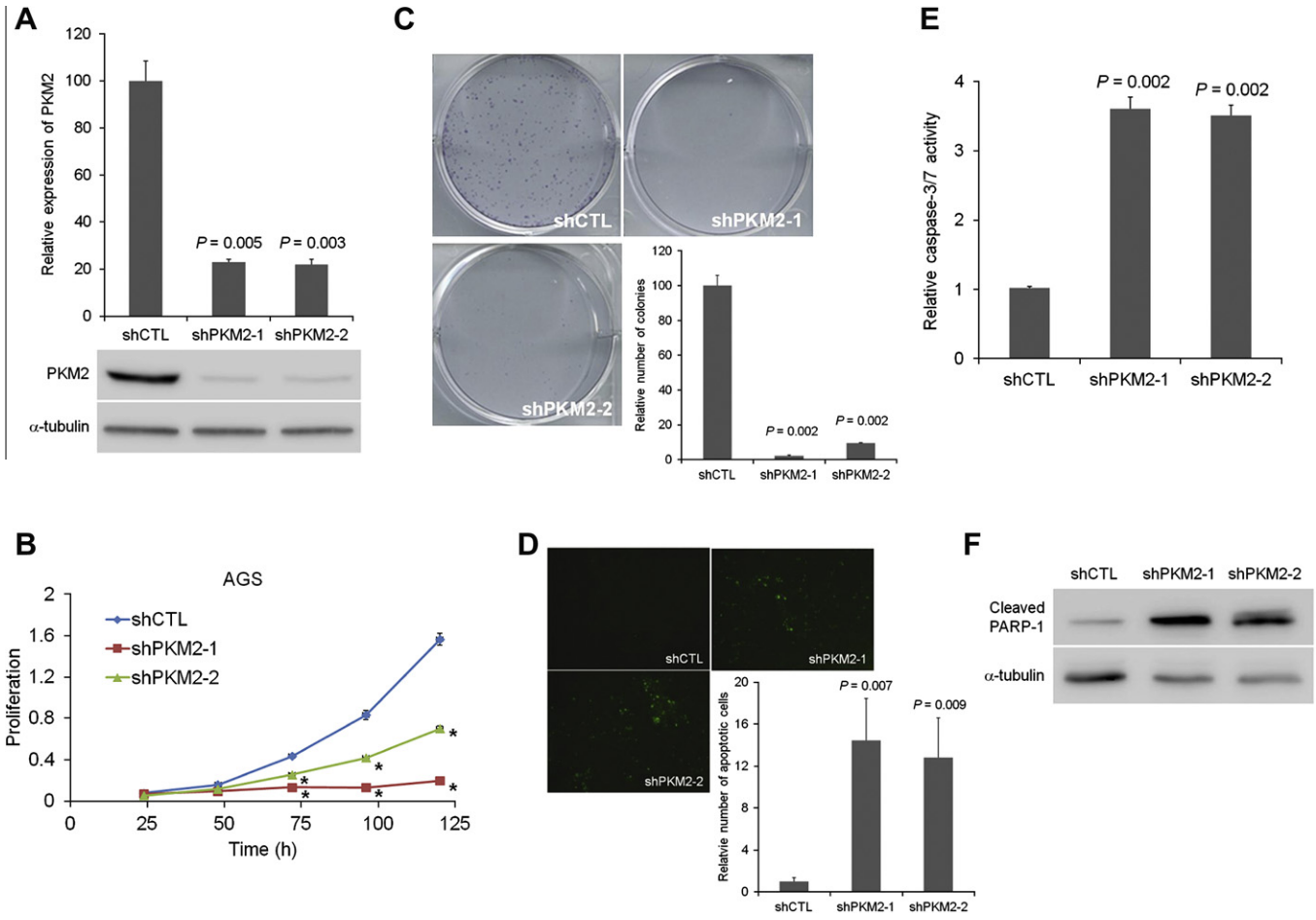

Fig. 2. Effect of PKM2 knockdown on the growth of AGS cells. (A) AGS cells were transduced with PKM2 shRNA (shPKM2-1 or shPKM2-2) or control shRNA (shCTL). PKM2 expression in each cell line was analyzed by qRT-PCR (*top*) and western blotting (*bottom*). (B) Proliferation of AGS cells was measured at 24, 48, 72, 96, and 120 h. The results from triplicate experiments are shown; error bars, SD. (C) Anchorage-dependent colony formation assay in monolayer culture. AGS cells were plated in 6-well plates. After two weeks of incubation, the cells were stained with crystal violet. The graph shows the number of colonies. Columns, results of triplicate experiments; error bars, SD. (D) TUNEL assay of AGS cells. The relative apoptotic cells were determined by counting TUNEL-positive cells in five random fields for each sample. (E) A plot of caspase-3/7 activity normalized to protein concentration in AGS cells. (F) The level of PARP-1 was determined by western blots using whole cell lysates.

expression between Bcl-xL and PKM2 in GC cell lines. A significant positive correlation was observed between PKM2 and Bcl-xL in 10 GC cell lines (Fig. 3A). The PKM2 knockdown in AGS cells decreased Bcl-xL expression (Fig. 3B, *top* and *bottom*). We further examined the correlations between PKM2 and Bcl-xL genes in gastric tumor and adjacent normal tissues. We performed qRT-PCR in paired normal and tumor tissues obtained from 188 individuals with GC (the same as the tissues used in qRT-PCR for PKM2 expression) and examined the correlation between PKM2 and Bcl-xL expression. Positive correlations were observed between PKM2 and Bcl-xL in both normal and tumor tissues (Figs. 3C and D). Our previous study revealed that Bcl-xL is overexpressed in tumor tissues compared to normal tissues [13]. These results suggest that PKM2 may be an important upstream regulator of the Bcl-xL gene in GC. We further examined whether Bcl-xL gene knockdown affects cell growth. Bcl-xL knockdown by siRNA significantly inhibited the growth (Figs. 3E and F) and increased caspase-3/7 activity of AGS cells (Fig. 3G). These results suggest that PKM2 expression in GC cells may affect cell survival through the regulation of the Bcl-xL gene.

3.4. PKM2 may regulate the expression of Bcl-xL gene via alteration of p65 expression at protein level

p65 activity is known to be modulated by the glycolytic pathway [14,15]. To investigate whether PKM2 regulates NF-κB activity, we performed western blot analysis with nuclear fraction of p65 and found that the nuclear p65 level was lower in PKM2-knockdown cells compared to control cells (Fig. 4A). To investigate whether the reduced nuclear p65 was due to a blockade of the nuclear translocation of p65, we examined the p65 level in the whole cell lysates. Interestingly, the whole cell p65 level was also lower in PKM2-knockdown cells compared to control cells (Fig. 4B). mRNA levels were similar between PKM2-knockdown cells and control cells (Fig. 4B), suggesting that p65 might be regulated by post-translational mechanisms, such as protein stability. To further characterize whether PKM2 affected p65 stability, we carried out a cycloheximide chase assay. We found that the half-life of p65 in shPKM2-transduced cells was considerably shorter than that in shCTL-transduced cells (Fig. 4C and D), suggesting that PKM2 might affect p65 stability. To determine whether down-regulation of the Bcl-xL gene was caused by reduced p65 binding to the promoters of the gene, we performed ChIP assays. We used a previously published set of primers for the amplification of p65 DNA binding sites in the ChIP assays [16]. ChIP assays showed that the reduced nuclear levels of p65 were paralleled with reduced DNA binding activity (Fig. 4E), indicating that PKM2 regulation of p65 stability affects the expression of its target genes. To confirm whether NF-κB regulates Bcl-xL expression, we treated AGS cells with the IKK inhibitor, PS1145. As expected, PS1145 reduced the expression levels of Bcl-xL (Fig. 4F), and increased caspase-3/7 activity (Fig. 4G).

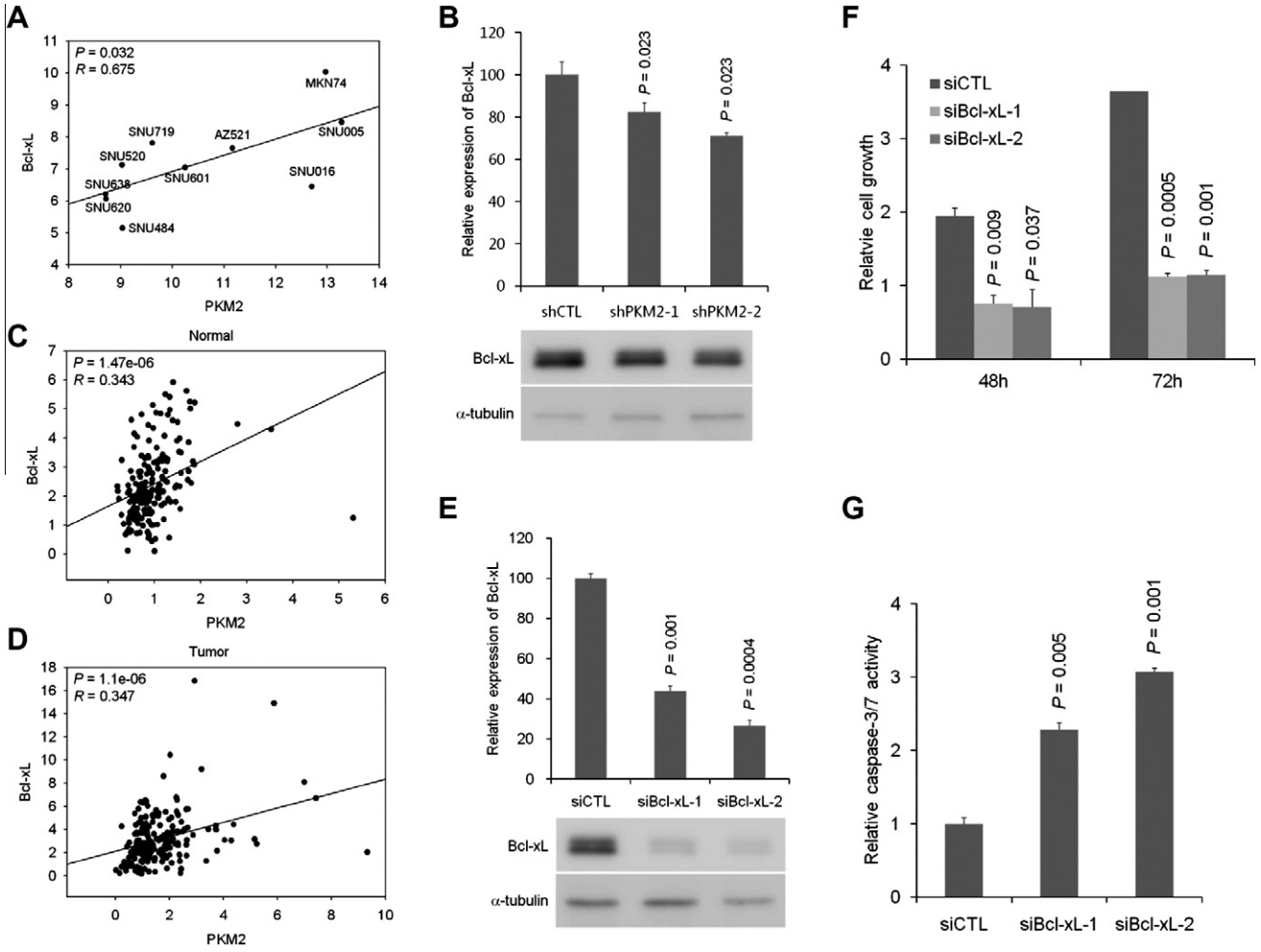

Fig. 3. PKM2 expression is significantly correlated with Bcl-xL expression in gastric normal and tumor tissues. (A) The relationship between expression levels of PKM2 and Bcl-xL in 10 GC cell lines. (B) Bcl-xL expression was analyzed by qRT-PCR using total RNA (*top*) and western blot using whole cell lysates (*bottom*) from AGS cells. (C) and (D) Correlation of mRNA expression levels of PKM2 and Bcl-xL in gastric normal (C) and tumor (D) tissues. (E) AGS cells were transfected with 100 nM siRNAs, and Bcl-xL expression was analyzed by qRT-PCR (*top*) and western blot (*bottom*) using total RNA and whole lysates, respectively, from control siRNA-transfected (siCTL) or Bcl-xL siRNA-transfected (siBcl-xL-1 and siBcl-xL-2) AGS cells. (F) AGS cells were seeded in 96-well plates and transfected with 100 nM siRNAs. Cell growth was measured 48 and 72 h after transfection. (G) A plot of caspase-3/7 activity normalized with protein concentration in AGS cells.

4. Discussion

Aerobic glycolysis is proposed to increase the availability of macromolecules needed for biosynthesis and cell growth [17]. Aerobic glycolysis is also involved in anti-apoptotic pathways. Increased glucose metabolism protects cells against the pro-apoptotic Bim and attenuates the degradation of the anti-apoptotic protein Mcl-1 [18]. PKM2 is believed to promote aerobic glycolysis to support biosynthesis and maintain ATP levels. Mice injected with PKM1-overexpressing cells showed a delay in tumor development compared with those injected with PKM2-overexpressing cells [5]. Reduced expression of PKM2 protein in lung tumors is associated with inhibited tumor growth and survival both *in vitro* and *in vivo* [19]. Recently, it was reported that miRNA-326 targets PKM2 in glioma cells and induces their apoptosis and reduces their metabolic activity [12]. We showed that shRNA-mediated inhibition of PKM2 down-regulated the anti-apoptotic gene Bcl-xL at the transcriptional level, resulting in increased apoptosis and reduced cell growth. Correlation analysis showed that PKM2 expression was positively correlated with Bcl-xL expression in GC cell lines and tumor tissues.

NF-κβ activity affects proliferation, death, cell motility, and sensitivity to anti-tumor agents. Therefore, the inhibition of NF-κβ signaling may be a potential therapeutic strategy for treating cancer [20–21]. In terms of cell survival, NF-κB regulates its target genes at the transcriptional level, either negatively or positively. For example, p65 binds to the κB site of the Bax promoter and negatively regulates Bax gene expression, resulting in the prevention of cell apoptosis [22–23]. In contrast, NF-κβ p65 binding to the Bcl-xL promoter up-regulates Bcl-xL gene expression and protects cells from radiation-induced apoptosis [16].

Some reports have linked NF-κB activation with glycolysis. Increased glycolysis can enhance the *O*-glycosylation of IKKβ that subsequently triggers NF-κB signaling [14]. It was recently reported that lactate triggers the phosphorylation/degradation of IκBα and then stimulates an autocrine NF-κB/IL-8 pathway driving cell migration [15]. For these reasons, we investigated the relation between PKM2 and p65 in GC. We found that PKM2 knockdown decreased p65 proteins (both in the nuclear and the whole cell lysates) but not mRNAs, suggesting that PKM2 might modulate p65 stability. It is well known that p65 is posttranslationally modified by diverse mechanisms such as phosphorylation, ubiquitination, or acetylation, and these modifications influence its transcriptional activity. Some reports showed that p65 is polyubiquitinated and degraded [24–25]. Thus, we do not exclude possibilities that reduced nuclear p65 level may be caused by other modifications in addition to protein stability. It is known that pyruvate and lactate, which are end products of glycolytic metabolism, promote HIF-1α

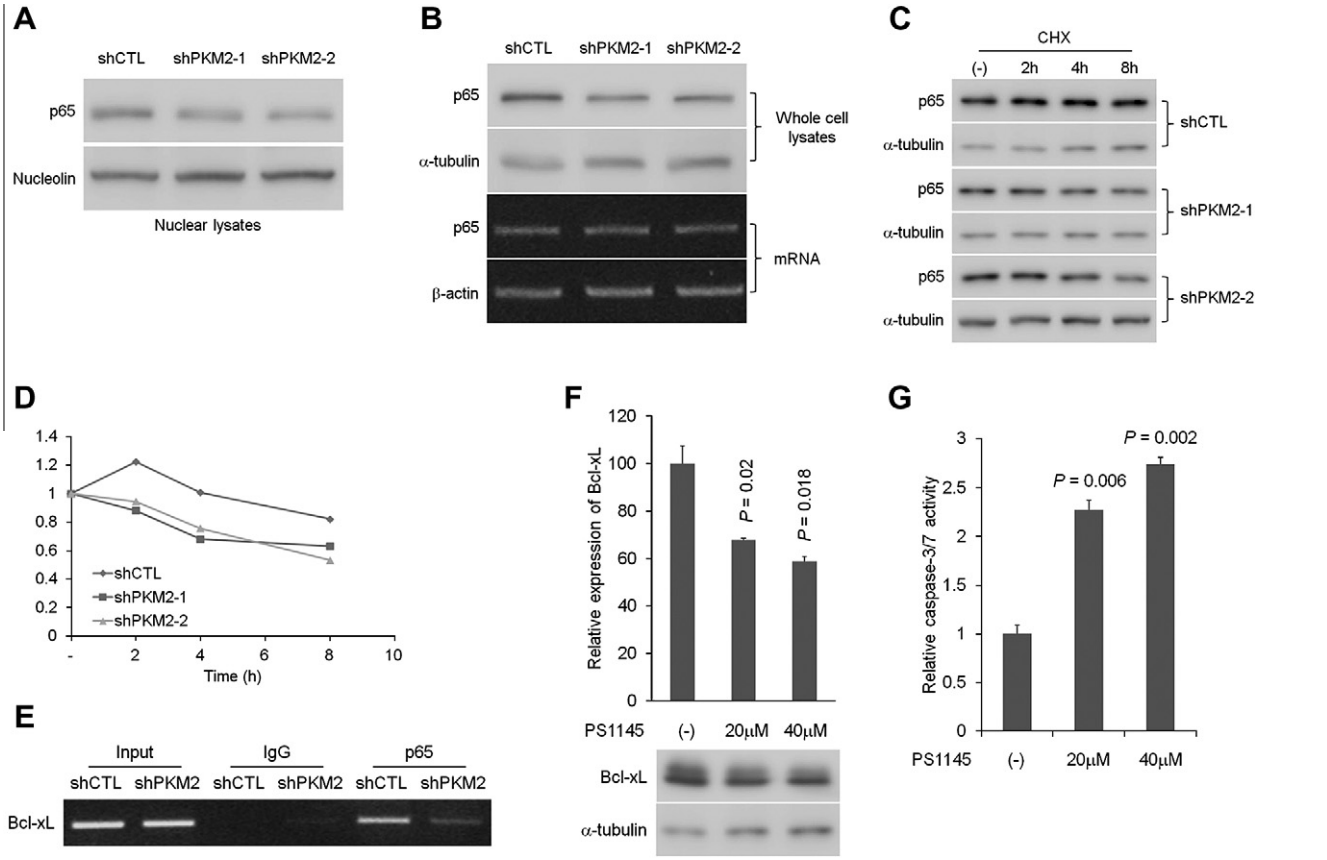

Fig. 4. Effect of PKM2 knockdown on promoter binding activity of p65. (A) Nuclear lysates were prepared from AGS cells transduced with PKM2 shRNAs or control shRNA and subjected to immunoblot analysis with anti-p65 or anti-actin antibodies. (B) Whole cell lysates were prepared from AGS cells and analyzed using immunoblotting with anti-p65 or anti-tubulin antibodies (*top*). Total RNA samples were also analyzed by RT-PCR using p65- or beta-actin-specific primers (*bottom*). (C) AGS cells were treated with 100 μg/ml of cycloheximide in serum-free medium, and whole cell lysates were harvested at the indicated time points, followed by anti-p65 immunoblotting. (D) Quantitative results of panel c by the IMAGE J software. The α-tubulin-normalized p65 protein levels at 0 h were defined as 1 for each panel. (E) ChIP assays were performed on chromatin derived from PKM2 shRNA (shPKM2)- or control shRNA (shCTL)-transduced AGS cells. (F) AGS cells were treated with 20 or 40 μM PS1145 for 72 h. Bcl-xL expression was analyzed by qRT-PCR (*top*) and western blotting (*bottom*). (G) A plot of caspase-3/7 activity normalized to protein concentration in AGS cells treated with 20 or 40 μM PS1145 for 72 h.

protein stability and activate HIF-1α-inducible gene expression [26], indicating that glycolysis is an important part in regulating protein stability. However, Lu et al. excluded the possibility that pyruvate inhibits the proteasomal system [26]. At present, the mechanism involved remains obscure and is a subject for further study.

In summary, we showed that PKM2 expression in tumor tissues was higher than adjacent normal tissues, and high levels of PKM2 expression associated with poor clinical outcome among GC patients. We suggest that one of the reasons why PKM2 expression associates with poor prognosis may be PKM2-mediated regulation of Bcl-xL expression. Moreover, PKM2-mediated stabilization of p65 may be one of important mechanisms in regulating the expression of Bcl-xL gene at transcriptional level. We propose that PKM2/NF-κB/Bcl-xL may be a potential cascade in cancer progression.

Acknowledgments

This work was supported by grants from the Basic Science Research Program through the National Research Foundation of Korea (NRF2010-0008143) funded by the Ministry of Education, Science and Technology (MOEST), from the National R & D Program for Cancer Control, the Ministry for Health and Welfare, the Republic of Korea (1020360), from the KRIBB-Pfizer Collaboration Research Program (IGE1641012), and from the KRIBB Research Initiative.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.05.063.

References

[1] S. Mazurek, Pyruvate kinase type M2: a key regulator of the metabolic budget system in tumor cells, Int. J. Biochem. Cell Biol. 43 (2010) 969–980.
[2] S. Mazurek, E. Eigenbrodt, The tumor metabolome, Anticancer Res. 23 (2003) 1149–1154.
[3] S. Mazurek, H. Grimm, C.B. Boschek, P. Vaupel, E. Eigenbrodt, Pyruvate kinase type M2: a crossroad in the tumor metabolome, Br. J. Nutr. 87 (Suppl 1) (2002) S23–29.
[4] E. Eigenbrodt, D. Basenau, S. Holthusen, S. Mazurek, G. Fischer, Quantification of tumor type M2 pyruvate kinase (Tu M2-PK) in human carcinomas, Anticancer Res. 17 (1997) 3153–3156.
[5] H.R. Christofk, M.G. Vander Heiden, M.H. Harris, A. Ramanathan, R.E. Gerszten, R. Wei, M.D. Fleming, S.L. Schreiber, L.C. Cantley, The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth, Nature 452 (2008) 230–233.
[6] B. Zhang, J.Y. Chen, D.D. Chen, G.B. Wang, P. Shen, Tumor type M2 pyruvate kinase expression in gastric cancer, colorectal cancer and controls, World J. Gastroenterol. 10 (2004) 1643–1646.
[7] M.S. Hayden, S. Ghosh, Signaling to NF-kappaB, Genes Dev. 18 (2004) 2195–2224.
[8] A.S. Baldwin, Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappaB, J. Clin. Invest. 107 (2001) 241–246.
[9] M. Karin, Y. Cao, F.R. Greten, Z.W. Li, NF-kappaB in cancer: from innocent bystander to major culprit, Nat. Rev. Cancer 2 (2002) 301–310.
[10] O.H. Kwon, J.L. Park, M. Kim, J.H. Kim, H.C. Lee, H.J. Kim, S.M. Noh, K.S. Song, H.S. Yoo, S.G. Paik, S.Y. Kim, Y.S. Kim, Aberrant up-regulation of LAMB3 and

LAMC2 by promoter demethylation in gastric cancer, Biochem. Biophys. Res. Commun. 406 (2011) 539–545.
[11] G. Shin, T.W. Kang, S. Yang, S.J. Baek, Y.S. Jeong, S.Y. Kim, GENT: gene expression database of normal and tumor tissues, Cancer Inform. 10 (2011) 149–157.
[12] B. Kefas, L. Comeau, N. Erdle, E. Montgomery, S. Amos, B. Purow, Pyruvate kinase M2 is a target of the tumor-suppressive microRNA-326 and regulates the survival of glioma cells, Neuro. Oncol. 12 (2010) 1102–1112.
[13] O.H. Kwon, S.J. Park, T.W. Kang, M. Kim, J.H. Kim, S.M. Noh, K.S. Song, H.S. Yoo, Y. Wang, D. Pocalyko, S.G. Paik, Y.H. Kim, S.Y. Kim, Y.S. Kim, Elevated fibroblast growth factor-inducible 14 expression promotes gastric cancer growth via nuclear factor-kappaB and is associated with poor patient outcome, Cancer Lett. 314 (2011) 73–81.
[14] K. Kawauchi, K. Araki, K. Tobiume, N. Tanaka, Loss of p53 enhances catalytic activity of IKKbeta through O-linked beta-N-acetyl glucosamine modification, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 3431–3436.
[15] F. Vegran, R. Boidot, C. Michiels, P. Sonveaux, O. Feron, Lactate influx through the endothelial cell monocarboxylate transporter MCT1 supports an NF-{kappa}B/IL-8 pathway that drives tumor angiogenesis, Cancer Res. 71 (2011) 2550–2560.
[16] B. Marinari, A. Costanzo, V. Marzano, E. Piccolella, L. Tuosto, CD28 delivers a unique signal leading to the selective recruitment of RelA and p52 NF-kappaB subunits on IL-8 and Bcl-xL gene promoters, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 6098–6103.
[17] R.J. DeBerardinis, J.J. Lum, G. Hatzivassiliou, C.B. Thompson, The biology of cancer: metabolic reprogramming fuels cell growth and proliferation, Cell Metab. 7 (2008) 11–20.
[18] Y. Zhao, B.J. Altman, J.L. Coloff, C.E. Herman, S.R. Jacobs, H.L. Wieman, J.A. Wofford, L.N. Dimascio, O. Ilkayeva, A. Kelekar, T. Reya, J.C. Rathmell, Glycogen synthase kinase 3alpha and 3beta mediate a glucose-sensitive antiapoptotic signaling pathway to stabilize Mcl-1, Mol. Cell. Biol. 27 (2007) 4328–4339.
[19] H.S. Shi, D. Li, J. Zhang, Y.S. Wang, L. Yang, H.L. Zhang, X.H. Wang, B. Mu, W. Wang, Y. Ma, F.C. Guo, Y.Q. Wei, Silencing of pkm2 increases the efficacy of docetaxel in human lung cancer xenografts in mice, Cancer Sci. 101 (2010) 1447–1453.
[20] R.O. Escarcega, S. Fuentes-Alexandro, M. Garcia-Carrasco, A. Gatica, A. Zamora, The transcription factor nuclear factor-kappa B and cancer, Clin. Oncol. (R Coll. Radiol.) 19 (2007) 154–161.
[21] A. Garg, B.B. Aggarwal, Nuclear transcription factor-kappaB as a target for cancer drug development, Leukemia 16 (2002) 1053–1068.
[22] T. Grimm, S. Schneider, E. Naschberger, J. Huber, E. Guenzi, A. Kieser, P. Reitmeir, T.F. Schulz, C.A. Morris, M. Sturzl, EBV latent membrane protein-1 protects B cells from apoptosis by inhibition of BAX, Blood 105 (2005) 3263–3269.
[23] M. Bentires-Alj, E. Dejardin, P. Viatour, C. Van Lint, B. Froesch, J.C. Reed, M.P. Merville, V. Bours, Inhibition of the NF-kappa B transcription factor increases Bax expression in cancer cell lines, Oncogene 20 (2001) 2805–2813.
[24] K. Nihira, Y. Ando, T. Yamaguchi, Y. Kagami, Y. Miki, K. Yoshida, Pim-1 controls NF-kappaB signalling by stabilizing RelA/p65, Cell Death Differ. 17 (2010) 689–698.
[25] T. Tanaka, M.J. Grusby, T. Kaisho, PDLIM2-mediated termination of transcription factor NF-kappaB activation by intranuclear sequestration and degradation of the p65 subunit, Nat. Immunol. 8 (2007) 584–591.
[26] H. Lu, R.A. Forbes, A. Verma, Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis, J. Biol. Chem. 277 (2002) 23111–23115.