Type II cGMP-dependent protein kinase inhibits ERK/JNK-mediated activation of transcription factors in gastric cancer cells

JIANRONG SANG, YONGCHANG CHEN, LU JIANG, YAN TAO, YAN WU, YING WANG, YUEYING LI, TING LAN and GENBAO SHAO

School of Medical Science and Laboratory Medicine, Jiangsu University, Jiangsu 212013, P.R. China

Received May 5, 2012; Accepted August 13, 2012

DOI: 10.3892/mmr.2012.1050

Abstract. A previous study has shown that type II cGMP-dependent protein kinase (PKG II) inhibits the proliferation of gastric cancer cells through blocking EGF-triggered MAPK/ERK signal transduction, indicating that the kinase may be a potential anticancer factor. In the present study, the role of PKG II in the EGF-induced activation of transcription factors in the MAPK/ERK signal transduction pathway was investigated. BGC-823 human gastric cancer cells were infected with adenoviral constructs encoding the cDNA of PKG II (pAd-PKG II) to increase the expression of PKG II and treated with 8-pCPT-cGMP to activate the enzyme. Using luciferase reporter assays, it was revealed that PKG II markedly suppressed the EGF-induced transcriptional activities of AP-1 and Elk1. Consistent with the inhibitory effect of PKG II on AP-1 activity, the expression levels of c-Jun and c-Fos, components of AP-1, were also inhibited. Co-immunoprecipitation analysis demonstrated that EGF treatment increased the AP-1 content through inducing the formation of p-c-Jun-c-Jun homodimers and p-c-Jun-c-Fos heterodimers. However, this combination was efficiently blocked by activated PKG II. While pretreatments with MAPK inhibitors suppressed the EGF-induced transcriptional activities of AP-1 and Elk1, PKG II prevented the EGF-induced phosphorylation/activation of ERK and JNK, but not the phosphorylation of p38MAPK induced by EGF. These data suggest that PKG II inhibits the EGF-triggered proliferation of gastric cancer cells through suppressing ERK-/JNK-, but not p38MAPK, -mediated AP-1 and Elk1 transactivation.

Correspondence to: Dr Jianrong Sang, School of Medical Science and Laboratory Medicine, Jiangsu University, 301 XueFu Road, Zhenjiang, Jiangsu 212013, P.R. China
E-mail: sangjianrong@yahoo.com.cn

Key words: type II cGMP-dependent protein kinase, ERK/JNK, transcription factors, gastric cancer cells

Introduction

cGMP-dependent protein kinase (PKG) is a serine/threonine kinase. Mammalian cells have two PKG genes, which encode the cytosolic PKG I and membrane-bound PKG II (1,2). PKG I controls multiple physiological functions, including proliferation, apoptosis, migration and the differentiation of several cell types (3,4), and has been identified as a tumor suppressor (5). A growing body of evidence indicates that PKG II plays a role in inhibiting tumor cell proliferation (6-8). However, more evidence is required to identify PKG II as a tumor suppression factor. Previously, it has been demonstrated that PKG II has a suppressive effect on EGF-induced cell proliferation and cell cycle progression through inhibiting the MAPK/ERK-mediated signal transduction pathway (8,9). The current study provides direct evidence linking increased PKG activity to the inhibition of EGF-induced activation of the transcription factors AP-1 and Elk1 in gastric cancer cells.

AP-1 is a transcription factor and has been shown to play a critical role in promoting carcinogenesis (10). AP-1 proteins consist of homodimers of Jun family members (c-Jun, JunB, JunD) or heterodimers of members of the Jun and Fos families (c-Fos, FosB, Fra-1 and Fra-2) (11,12). It is well established that the modulation of AP-1 activity is critical in the control of cell proliferation and apoptosis (12-14). Several studies have shown that AP-1 is activated by three MAPK pathways (ERK, JNK and p38MAPK) in a selective manner and serves as a common integrator of MAPK signaling to specific target gene expression (12,15). However, the exact mechanism of EGF treatment on AP-1 activation and the relative roles of various MAPKs in these processes are diverse. Whether activated PKG II also modulates the members of AP-1 family expression in response to the stimulation of EGF in gastric cancer cells is unclear.

The transcription factor Elk1 is activated by a variety of extracellular signals via the MAPK phosphorylation cascades (16). Activated Elk1 binds to the serum response element of a variety of genes that regulate cell growth, including c-Fos (17,18). The molecular mechanism that results in Elk1 playing dominant roles in regulating proliferation in gastric cancer has not yet been elucidated.

We have previously demonstrated a potential role of PKG II in regulating cancer cell proliferation, which is likely to be

associated with the activities of the MAPK-mediated pathway; however, the downstream steps involved in this process remain to be determined. The current study investigated the effect of PKG II on the EGF-induced activation of AP-1 and Elk1 and the possible association between the effect and the activities of MAPKs, including ERK, JNK and p38MAPK, in order to provide further evidence for indentifying PKG II as a cancer inhibitory factor.

Materials and methods

Cell lines and reagents. The human gastric cancer cell line BGC-823 was provided by the Institute of Cell Biology (Shanghai, China). Adenoviral vectors encoding the cDNA β-galactosidase (pAd-LacZ) and PKG II (pAd-PKG II) were gifts from Dr Gerry Boss and Dr Renate Pilz, University of California, San Diego, CA, USA. Dulbecco's modified Eagle's medium (DMEM) and newborn calf serum (NBCS) were purchased from Gibco (Grand Island, NY, USA). Antibodies against JNK, phospho-JNK (Thr183/Tyr185), p-c-Jun (Ser73) and p-c-Jun (Ser63) were obtained from Bioworld Technology (St. Louis Park, MN, USA). Antibodies against Lamin A, β-actin, c-Fos and c-Jun were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Antibodies against phospho-Elk1 (Ser383), p38MAPK, phospho-p38MAPK, ERK and phospho-ERK were from Cell Signaling Technology (Danvers, MA, USA). The horseradish peroxidase (HRP)-conjugated secondary antibodies were from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). The cellular permeable cGMP analog 8-pCPT-cGMP was from Calbiochem (San Diego, CA, USA). PD98059, SP600125, SB203580 and EGF were from Sigma (St. Louis, MO, USA). Electrochemiluminescence (ECL) reagents were from Millipore (Billerica, MA, USA). All other reagents used were of analytical grade.

Cell culture and infection with adenoviral vectors. BGC-823 cells were cultured in DMEM supplied with 10% NBCS and maintained at 37˚C in a humidified incubator with 95% air and 5% CO_2. The medium was changed every two days and the cells were subcultured at confluence. On the day prior to infection with adenovirus, cells were freshly planted at 70-80% confluence and infection was performed as reported previously (21). All experiments were approved by the ethics committee of Jiangsu University.

Luciferase reporter experiments. BGC-823 cells were subcultured in 24-well plates in triplicate 24 h prior to transfection. pAP-1-luciferase or pElk1-luciferase was co-transfected with β-galactosidase reporter plasmids (as an internal control) using Lipofectamine™ 2000 in an antibiotic-free medium. Following 18 h incubation, the cells were treated as designated. The cells were then lysed in lysis buffer (25 mM Tris-phosphate, pH 7.8, 2 mM EDTA, 1% Triton X-100 and 10% glycerol) and reporter gene activity was determined with the Promega luciferase assay system using LUMI-ONE luminometer. β-galactosidase activity was measured using the Promega β-galactosidase enzyme assay system and used for normalization of transfection efficiency.

Nuclear protein preparation. According to the method of Chen *et al* (19), cells growing on 100-mm plates were harvested in HEM buffer (10 mM HEPES pH 7.5, 2 mM EDTA, 1 mM $MgCl_2$) and homogenized with an ultrasonic homogenizer. The homogenate was centrifuged at 500 x g at 4˚C for 5 min to obtain the nuclei of the cells. Preheated SDS-PAGE loading buffer was added to the pellet and boiled for 5 min to obtain nuclear proteins.

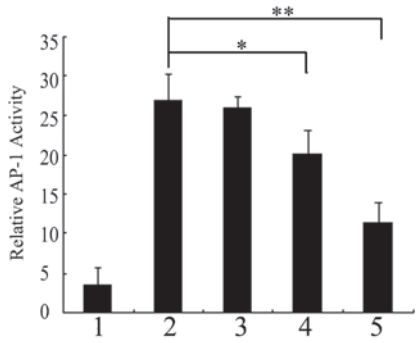

Figure 1. PKG II blocks EGF-induced AP-1 transcriptional activity. BGC-823 cells were transiently transfected with pAP-1-Luc and β-galactosidase plasmids. Cells were infected with either pAd-LacZ or pAd-PKG II 8 h after transfection, serum-starved for 12 h, stimulated with 8-pCPT-cGMP for 1 h and then treated with EGF (100 ng/ml) for 5 min (1, LacZ; 2, LacZ+EGF; 3, PKG II+EGF; 4, PKG II+100 μM cGMP+EGF; 5, PKG II+250 μM cGMP+EGF). Cell extracts were prepared with luciferase reporter lysis buffer and analyzed for luciferase activity. The relative values of AP-1 luciferase activity to β-galactosidase are shown as the mean ± SE of three independent experiments (*P<0.05 and **P<0.01 vs. pAd-LacZ+EGF). PKG II, type II cGMP-dependent protein kinase.

Western blotting. Proteins from whole-cell and nuclear extracts were separated by 10% SDS-PAGE. The primary antibodies were incubated overnight at 4˚C and the corresponding secondary antibodies were incubated for 1 h at RT, with three washes following each incubation. ECL reagents were used to show the positive bands on the membrane.

Co-immunoprecipitation. The binding between c-Fos and c-Jun was detected by co-immunoprecipitation. The cells were lysed with RIPA buffer (50 mM Tris-HCl pH 7.4, 1% Triton X-100, 1 mM EDTA, 1 mM leupeptin, 1 mM phenylmethylsulfonyl fluoride, 10 mM NaF, 1 mM Na_3VO_4). Antibodies against c-Fos and c-Jun and isotype-matched IgG were used for immunoprecipitation and immunoblotting assay.

Statistical analysis. The data are shown as the mean ± standard error (SE). Statistical significance was performed using ANOVA with SPSS statistical software. P<0.05 was considered to indicate a statistically significant difference.

Results

PKG II inhibits EGF-induced AP-1 transcriptional activity. The AP-1 transcription factor is a key regulatory molecule that plays a central role in the control of cell proliferation and transformation by converting MAPK signals into the expression of specific target genes (20). In order to test the effect of PKG II on AP-1 transcriptional activity, BGC-823 cells were transiently transfected with a reporter plasmid containing a luciferase gene driven by a minimal human collagenase gene

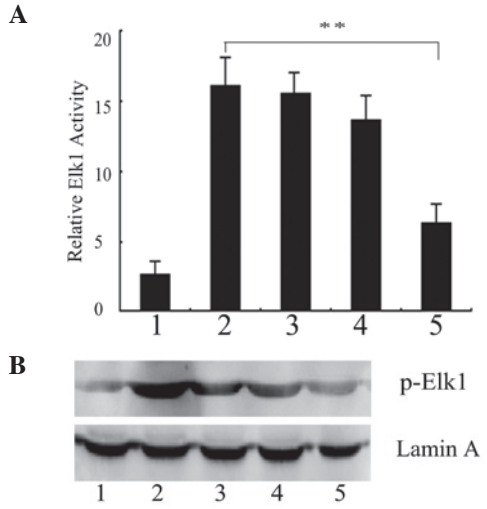

Figure 2. PKG II inhibits the EGF-induced transcriptional activity and phosphorylation of Elk1. BGC-823 cells were transiently transfected with pElk1-Luc and β-galactosidase plasmids. Cells were infected with either pAd-LacZ or pAd-PKG II 8 h following transfection, serum-starved for 12 h, stimulated with 8-pCPT-cGMP for 1 h and then treated with EGF (100 ng/ml) for 5 min (lane 1, LacZ; lane 2, LacZ+EGF; lane 3, PKG II+EGF; lane 4, PKG II+100 μM cGMP+EGF; lane 5, PKG II+250 μM cGMP+EGF). (A) Cell extracts were prepared with luciferase reporter lysis buffer and analyzed for luciferase activity. (B) Non-transfected cells were treated as described above, the cells were lysed and the nuclear proteins were obtained. Western blotting with the indicated antibody was used to analyze the amount of phospho-Elk1 (Ser383) in the nuclei of BGC-823 cells. (A) The relative values of Elk1 luciferase activity to β-galactosidase are shown as the mean ± SE of three independent experiments (*P<0.05 and **P<0.01 vs. pAd-LacZ+EGF). (B) Representative results from three independent experiments are shown. PKG II, type II cGMP-dependent protein kinase.

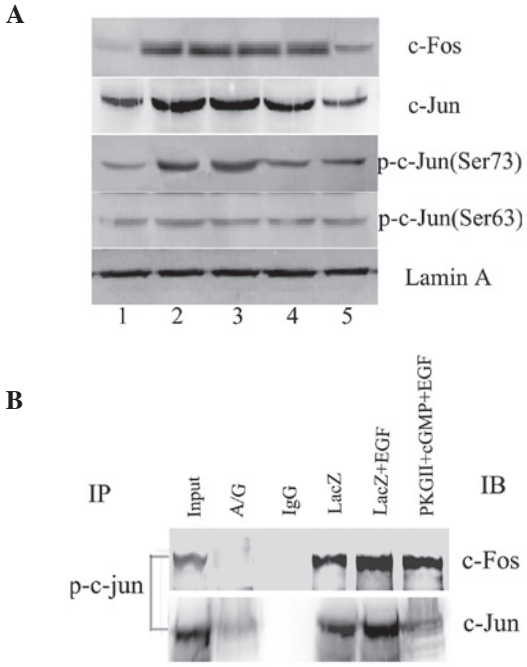

Figure 3. PKG II inhibits the expressions of c-Jun and c-Fos and blocks the combination between c-Jun, c-Fos and p-c-Jun (Ser73). BGC-823 cells were infected with either pAd-LacZ or pAd-PKG II, serum-starved for 12 h, stimulated with 8-pCPT-cGMP for 12 h and treated with EGF (100 ng/ml) for 12 h (lane 1, LacZ; lane 2, LacZ+EGF; lane 3, PKG II+EGF; lane 4, PKG II+100 μM cGMP+EGF; lane 5, PKG II+250 μM cGMP+EGF). (A) Cells were lysed and the nuclear proteins were obtained. Western blotting with the indicated antibodies was used to analyze the protein levels of c-Jun, c-Fos and p-c-Jun (Ser73). (B) Cell nuclear lysates were subjected to IP with an antibody against p-c-Jun followed by IB with antibodies against c-Jun and c-Fos. Representative results from three independent experiments are shown. IP, immunoprecipitation; IB, immunoblotting. PKG II, type II cGMP-dependent protein kinase.

promoter that contains a single AP-1 site. To normalize transfection efficiency, a plasmid containing a β-galactosidase gene was co-transfected as an internal control. As shown in Fig. 1, preinfecting pAd-LacZ or pAd-PKG II cells with EGF for 5 min resulted in a 7.8-fold increase in AP-1 transcriptional activity. The level of AP-1 luciferase activity in pAd-PKG II-infected cells stimulated with cGMP decreased as compared with those infected with pAd-LacZ along with EGF, indicating that activated PKG II efficiently inhibits EGF-induced AP-1 transcriptional activity in BGC-823 cells.

PKG II blocks the EGF-induced transcriptional activity of Elk1. ERK is known to be activated by stress stimuli and one of the downstream targets of the ERK pathway is the transcription factor Elk1. EGF increased the pElk1-luc activity almost 6-fold compared with the cells infected with pAd-LacZ alone. However, in the cells infected with pAd-PKG II and stimulated with 8-pCPT-cGMP, the increase was only ~2.3-fold by EGF treatment, suggesting that PKG II markedly reduces the EGF-induced Elk1 transcriptional activity. The EGF-induced Elk1 luciferase activity decreased gradually with the increasing concentrations of cGMP, suggesting that the high expression and activity of PKG II results in the dose-dependent reduction of the Elk1 activation induced by EGF (Fig. 2A). Nuclear lysates were prepared and the phosphorylation of Elk1 was detected by western blot analysis. Fig. 2B shows that EGF markedly induced the Ser383 phosphorylation of Elk1, but such activation was blocked by activated PKG II.

PKG II inhibits the expression of c-Jun and c-Fos, which are the predominant components of the EGF-induced AP-1 complex. In order to investigate whether PKG II altered the protein levels of AP-1 family members in BGC-823 cells, nuclear lysates from pAd-PKG II-infected cells treated with various concentrations of 8-pCPT-cGMP were subjected to western blot analysis. Low levels of c-Jun and c-Fos proteins were detected in the cells infected with pAd-LacZ. EGF treatment induced a substantial increase in the nuclear protein levels of c-Jun and c-Fos (Fig. 3A). In the cells infected with pAd-PKG II and stimulated with cGMP, the increased levels of EGF-induced c-Jun and c-Fos were significantly inhibited. These data clearly reveal that c-Fos and c-Jun were components of the EGF-induced AP-1 complex and that the expression of c-Jun was more sensitive and more susceptible to EGF and PKG II than that of c-Fos. c-Jun transcriptional activation, which is necessary for tumor development, is regulated by a variety of post-translational modifications. The results shown in Fig. 3A indicate that EGF markedly enhanced the Ser73 phosphorylation of c-Jun and activated PKG II almost completely blocked the increase in the phosphorylation of c-Jun induced by EGF, without affecting the level of Ser63 phosphorylation of c-Jun. These results imply that PKG II inhibited the phosphorylation of c-Jun at Ser73, which may impair the formation of a heterodimer of the JNK substrate c-Jun and c-Fos to form the

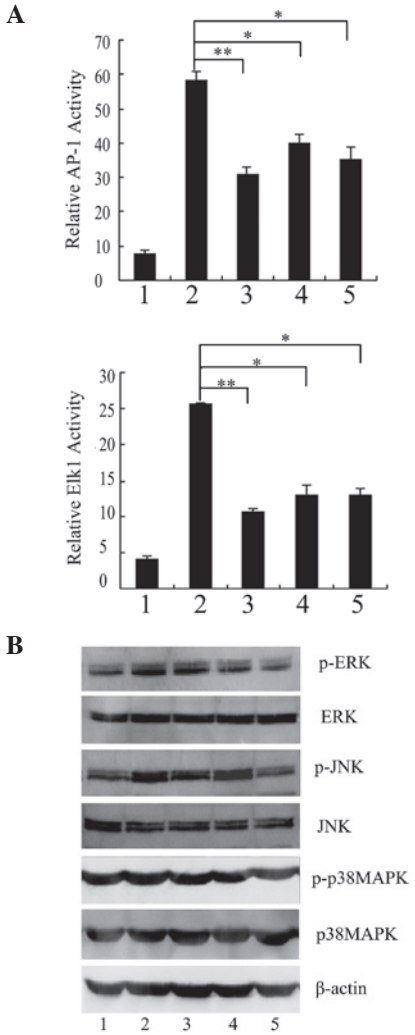

Figure 4. The inhibitory effect of PKG II on AP-1 and Elk1 transactivation is dependent on the PKG II-inhibited phosphorylation of JNK and ERK in BGC-823 cells exposed to EGF treatment. (A) BGC-823 cells were transiently transfected with pAP-1-Luc/pElk1-Luc and β-galactosidase plasmids. Cells were infected with pAd-LacZ 8 h following transfection, serum-starved for 12 h, pretreated with PD98059 (10 μM), SP600125 (10 μM) or SB203580 (10 μM) for 2 h and then treated with EGF (100 ng/ml) for 5 min. 1, LacZ; 2, LacZ+EGF; 3, LacZ+PD98059+EGF; 4, LacZ+SP600125+EGF; 5, LacZ+SB203580+EGF. Cell extracts were prepared with luciferase reporter lysis buffer and analyzed for luciferase activity. (B) BGC-823 cells were infected with either pAd-LacZ or pAd-PKG II, serum-starved for 12 h, stimulated with 8-pCPT-cGMP for 1 h and treated with EGF (100 ng/ml) for 5 min (lane 1, LacZ; lane 2-LacZ+EGF; lane 3, PKGII+EGF; lane 4, PKG II+100 μM cGMP+EGF; lane 5, PKG II+250 μM cGMP+EGF). Cell lysates were analyzed by western blotting with anti-JNK/phospho-JNK, anti-ERK/phospho-ERK and anti-p38MAPK/phospho-p38MAPK. (A) The relative values of AP-1/Elk1 luciferase activity to β-galactosidase are shown as the mean ± SE of three independent experiments (*P<0.05 and **P<0.01 vs. pAd-LacZ+EGF). (B) Representative results from three independent experiments are shown. IP, immunoprecipitation; IB, immunoblotting. PKG II, type II cGMP-dependent protein kinase.

AP-1 complex. To test this hypothesis and further determine the pattern of the activated-AP-1 complex induced by EGF in BGC-823 cells, co-immunoprecipitation experiments were performed. As shown in Fig. 3B, co-immunoprecipitation performed with anti-p-c-Jun antibody revealed the coprecipitation with c-Fos and c-Jun from nuclear extracts of BGC-823 cells. These results confirm that the EGF-activated AP-1 complex was constituted by the p-c-Jun-c-Jun homodimers and p-c-Jun-c-Fos heterodimers and the high expression and activity of PKG II restrained the combination.

Blocking of EGF-induced activation of AP-1 and Elk1 by PKG II was not associated with the inhibition of activation of p38MAPK. The activation of AP-1 was regulated by MAPKs, including ERK, p38MAPK and JNK, in various cell types. To elucidate the correlation between MAPKs and AP-1/Elk1 transactivation in BGC-823 cells, the effects of MAPK inhibitors on the transcriptional activities of AP-1 and Elk1 in response to EGF was investigated. Pretreatments with PD98059, SP600125 and SB203580 (inhibitors of ERK, JNK and p38MAPK, respectively) completely blocked the increases in the luciferase activities of AP-1 and Elk1 induced by EGF (Fig. 4A), but these pretreatments alone did not affect the activities of AP-1 and Elk1 in the cells infected with pAd-LacZ alone (data not shown). These results suggest that ERK, JNK and p38MAPK are necessary for EGF-induced AP-1 and Elk1 transactivation in BGC-823 cells.

To test the effect of PKG II on these signaling cascades, the phosphorylation and activation of ERK, JNK and p38MAPK were examined. As shown in Fig. 4B, the high level of expression and activity of PKG II markedly inhibited the EGF-induced phosphorylation of ERK and JNK in a concentration-dependent manner, but had no effect on the phosphorylation of p38MAPK. Activated PKG II hardly affected the total protein level of ERK, JNK and p38MAPK. Combining with the results of Fig. 4A, these data suggest that the inhibition of ERK and JNK phosphorylation by activated PKG II is one of the underlying mechanisms involved in its suppressive effect on EGF-induced AP-1 and Elk1 transactivation.

Discussion

AP-1 complexes induce cellular proliferation and transformation and may also promote differentiation and trigger apoptosis, depending upon their composition (21). Thus, the effect of AP-1 activation on cell proliferation may be temporally and spatially restricted. c-Jun and c-Fos are typical immediate early genes induced by a wide variety of growth factors and trigger downstream events relevant to cell cycle progression and proliferation (10). In the present study, PKG II suppressed the EGF-induced AP-1 activation through inhibiting the expression of c-Jun and c-Fos, which have positive effects on cell proliferation (22,23). In accordance with these results, it has previously been confirmed that increasing PKG II activity suppresses the proliferation of BGC-823 cells by inducing G0/G1 phase arrest (9). The inhibitory pattern of c-Jun expression by cGMP in a dose-dependent manner was extremely similar to that of AP-1 transcriptional activity, suggesting that the inhibition of c-Jun expression may be correlated with AP-1 transcriptional activity inhibition. Therefore, the expression of the c-Jun protein may be crucial for the activity of AP-1-regulated genes. Since c-Jun activity is directly modulated at the protein level via regulatory phosphorylation occurring on Ser63, Ser73 and Thr91 within the transactivation domain (24), the results clearly suggest that the inhibitory effect of PKG II on AP-1 transcriptional activity acts by suppressing the Ser73-phosphorylated-c-Jun. Furthermore, it was revealed that activated AP-1 consisted of p-c-Jun-c-Jun homodimers

and p-c-Jun-c-Fos heterodimers following EGF treatment, but this induction was blocked in cells infected with pAd-PKG II and following 8-pCPT-cGMP exposure. The present study used only antibodies against c-Jun and c-Fos to detect the formation of AP-1, therefore, other dimeric forms of the AP-1 transcription factor involved in regulating the AP-1 activity in BGC-823 cells cannot be excluded from this process.

It is well established that the activation of JNK, p38MAPK and/or ERK induces cancer proliferation through the AP-1 signaling pathway. Data in this study revealed that all three MAPK inhibitors blocked the activation of AP-1 and Elk1, suggesting that the JNK and p38MAPK pathways are also involved in EGF-induced transcriptional activities of AP-1 and Elk1. However, western blot analysis revealed that activated PKG II had a selective inhibitory effect on ERK/JNK activation but not p38MAPK activation. Therefore, it is believed that PKG II inhibits EGF-induced AP-1 and Elk1 transactivation in gastric cancer cells through suppressing ERK and JNK signaling pathways.

It has been reported that the critical step in the EGF/ERK signaling cascade is the change in cellular localization of phosphorylated ERK from the cytoplasm to the nucleus, which results in the activation of the transcription factor of Elk1 (25). The phosphorylation at Ser383 and Ser389 of Elk1 is critical for its transcriptional activity and is accomplished by all three types of MAPKs (26,27). A recent study has shown that PKG II significantly inhibited the EGF-induced nuclear translocation of phospho-ERK (8). This study demonstrated that activated-PKG II suppressed the EGF-induced Ser383-phosphorylated-Elk1, a crucial downstream molecule of ERK, in the nuclei of BGC-823 cancer cells.

In summary, we have shown that PKG II inhibits the EGF-stimulated ERK/JNK-dependent activation of AP-1 and Elk1 in gastric cancer cells. Since AP-1/Elk1-associated transcriptional activity is crucial in mediating gastric cancer cell proliferation and growth, it is further confirmed that PKG II inhibited the proliferation of gastric cancer cells through blocking the signal transduction of MAPK (ERK/JNK)-mediated pathways. This is likely to provide direct evidence for the therapeutic value of PKG II as a cancer suppression factor.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (nos. 81001100, 31040002 and 31100974) and by the Specialized Research Fund for Senior Personnel Program of Jiangsu University (no. 08JDG033). We thank Dr Gerry Boss and Dr Renate Pilz, University of California, San Diego, CA, USA, for the kind gifts of adenoviral constructs.

References

1. Orstavik S, Natarajan V, Taskén K, Jahnsen T and Sandberg M: Characterization of the human gene encoding the type I alpha and type I beta cGMP-dependent protein kinase (PRKG1). Genomics 42: 311-318, 1997.
2. Orstavik S, Solberg R, Taskén K, Nordahl M, Altherr MR, Hansson V, Jahnsen T and Sandberg M: Molecular cloning, cDNA structure and chromosomal localization of the human type II cGMP-dependent protein kinase. Biochem Biophys Res Commun 220: 759-765, 1996.
3. Feil R, Hofmann F and Kleppisch T: Function of cGMP-dependent protein kinases in the nervous system. Rev Neurosci 16: 23-41, 2005.
4. Pilz RB and Broderick KE: Role of cyclic GMP in gene regulation. Front Biosci 10: 1239-1268, 2005.
5. Hou Y, Gupta N, Schoenlein P, Wong E, Martindale R, Ganapathy V and Browning D: An anti-tumor role for cGMP-dependent protein kinase. Cancer Lett 240: 60-68, 2006.
6. Cook AL and Haynes JM: Protein kinase G II-mediated proliferative effects in human cultured prostatic stromal cells. Cell Signal 16: 253-261, 2004.
7. Cook AL and Haynes JM: Phosphorylation of the PKG substrate, vasodilator-stimulated phosphoprotein (VASP), in human cultured prostatic stromal cells. Nitric Oxide 16: 10-17, 2007.
8. Wu Y, Chen YC, Qu R, Lan T and Sang JR: Type II cGMP-dependent protein kinase inhibits EGF-triggered signal transduction of the MAPK/ERK-mediated pathway in gastric cancer cells. Oncol Rep 27: 553-558, 2012.
9. Chen YC, Ren F, Sang JR, Tao Y and Xu WR: Type II cGMP-dependent protein kinase inhibits proliferation of the gastric cancer cell line BGC-823. Mol Med Rep 3: 361-366, 2010.
10. Angel P and Karin M: The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochim Biophys Acta 1072: 129-157, 1991.
11. Chiariello M, Marinissen MJ and Gutkind JS: Multiple mitogen-activated protein kinase signaling pathways connect the cot oncoprotein to the c-jun promoter and to cellular transformation. Mol Cell Biol 20: 1747-1758, 2000.
12. Shaulian E and Karin M: AP-1 in cell proliferation and survival. Oncogene 20: 2390-2400, 2001.
13. Shaulian E and Karin M: AP-1 as a regulator of cell life and death. Nat Cell Biol 4: E131-E136, 2002.
14. Eferl R and Wagner EF: AP-1: a double-edged sword in tumorigenesis. Nat Rev Cancer 3: 859-868, 2003.
15. Whitmarsh AJ, Shore P, Sharrocks AD and Davis RJ: Integration of MAP kinase signal transduction pathways at the serum response element. Science 269: 403-407, 1995.
16. Buchwalter G, Gross C and Wasylyk B: Ets ternary complex transcription factors. Gene 324: 1-14, 2004
17. Hao D, Gao P, Liu P, Zhao J, Wang Y, Yang W, Lu Y, Shi T and Zhang X: AC3-33, a novel secretory protein, inhibits Elk1 transcriptional activity via ERK pathway. Mol Biol Rep 38: 1375-1382, 2011.
18. Ely HA, Mellon PL and Coss D: GnRH induces the c-Fos gene via phosphorylation of SRF by the calcium/calmodulin kinase II pathway. Mol Endocrinol 25: 669-680, 2011.
19. Chen JC, Zhuang S, Nguyen TH, Boss GR and Pilz RB: Oncogenic Ras leads to Rho activation by activating the mitogen-activated protein kinase pathway and decreasing Rho-GTPase-activating protein activity. J Biol Chem 278: 2807-2818, 2003.
20. Ono K and Han J: The p38 signal transduction pathway: activation and function. Cell Signal 12: 1-13, 2000.
21. Milde-Langosch K: The Fos family of transcription factors and their role in tumourigenesis. Eur J Cancer 41: 2449-2461, 2005.
22. Brown JR, Nigh E, Lee RJ, Ye H, Thompson MA, Saudou F, Pestell RG and Greenberg ME: Fos family members induce cell cycle entry by activating cyclin D1. Mol Cell Biol 18: 5609-5619, 1998.
23. Szabowski A, Maas-Szabowski N, Andrecht S, Kolbus A, Schorpp-Kistner M, Fusenig NE and Angel P: c-Jun and JunB antagonistically control cytokine-regulated mesenchymal-epidermal interaction in skin. Cell 103: 745-755, 2000.
24. Karin M: The regulation of AP-1 activity by mitogen-activated protein kinases. J Biol Chem 270: 16483-16486, 1995.
25. Gille H, Kortenjann M, Thomae O, Moomaw C, Slaughter C, Cobb MH and Shaw PE: ERK phosphorylation potentiates Elk-1-mediated ternary complex formation and transactivation. EMBO J 14: 951-962, 1995.
26. Ricote M, García-Tuñón I, Fraile B, Fernández C, Aller P, Paniagua R and Royuela M: p38 MAPK protects against TNF-alpha-provoked apoptosis in LNCaP prostatic cancer cells. Apoptosis 11: 1969-1975, 2006.
27. Vikman P, Ansar S and Edvinsson L: Transcriptional regulation of inflammatory and extracellular matrix-regulating genes in cerebral arteries following experimental subarachnoid hemorrhage in rats. Laboratory investigation. J Neurosurg 107: 1015-1022, 2007.