Aurora kinases are essential for PKC-induced invasion and matrix metalloproteinase-9 expression in MCF-7 breast cancer cells

EUN-MI NOH[1*], YOUNG-RAE LEE[2*], ON-YU HONG[1], SUNG HOO JUNG[3], HYUN JO YOUN[3] and JONG-SUK KIM[1]

[1]Department of Biochemistry and Institute for Medical Sciences, Chonbuk National University Medical School, Jeonju 560-182; [2]Department of Oral Biochemistry and Institute of Biomaterials-Implant, School of Dentistry, Wonkwang University, Iksan 570-749; [3]Department of Surgery and Research Institute of Clinical Medicine, Chonbuk National University Medical School, Jeonju 560-182, Republic of Korea

Received February 3, 2015; Accepted May 18, 2015

DOI: 10.3892/or.2015.4027

Abstract. The Aurora kinase family of serine/threonine kinases are known to be crucial for cell cycle control. Aurora kinases are considered a target of anticancer drugs. However, few studies have assessed the effect of Aurora kinases in breast cancer. In the present study, to determine whether Aurora kinases play a role in oncogenic actions of protein kinase C (PKC), we investigated the effect of Aurora kinases on PKC-induced invasion and MMP-9 expression using breast cancer cells. Treatment of MCF-7 cells with 12-O-tetradecanoylphorbol-13-acetate (TPA) induced the upregulation and phosphorylation of Aurora kinases via the MAPK signaling pathway. Moreover, the inhibition of Aurora kinases by their siRNAs and inhibitors suppressed TPA-induced cell invasion and expression of MMP-9 by inhibiting the activation of NF-κB/AP-1, major transcription factors for MMP-9 expression in MCF-7 cells. These results suggested that Aurora kinases mediate PKC-MAPK signal to NF-κB/AP-1 with increasing MMP-9 expression and invasion of MCF-7 cells. To the best of our knowledge, this is the first study to show that Aurora kinases are key molecules in PKC-induced invasion in breast cancer cells.

Correspondence to: Professor Hyun Jo Youn, Department of Surgery and Research Institute of Clinical Medicine, Chonbuk National University Medical School, Jeonju 560-182, Republic of Korea
E-mail: yhj0903@jbnu.ac.kr

Professor Jong-Suk Kim, Department of Biochemistry and Institute for Medical Sciences, Chonbuk National University Medical School, Jeonju 560-182, Republic of Korea
E-mail: jsukim@jbnu.ac.kr

*Contributed equally

Abbreviations: PKC, protein kinase c; TPA, 12-O-tetradecanoylphorbol-13-acetate; MMP-9, matrix metalloproteinase-9; NF-κB, nuclear factor-κB; AP-1, activator protein-1; MAPKs, mitogen activator protein kinases

Key words: Aurora kinases, protein kinase C, cancer cell invasion, matrix metalloproteinase-9

Introduction

Protein kinase C (PKC) is a major signaling enzymes that regulates a variety of cell processes including proliferation, apoptosis, cell survival and migration (1-3). In addition, several oncogenes, such as *RAS, FOS, MYC* and *PKC* cooperate during transformation, indicating the involvement of PKC in tumorigenesis (4-6). Therefore, PKC has been the subject of extensive studies as a molecular target for the treatment of various types of cancer. However, the cofactors involved in the PKC-mediated tumorigenesis are not clearly defined.

The Aurora kinase family of serine/threonine kinases has been known to be crucial for cell cycle control (7,8). Mammalian genomes contain three genes encoding Aurora kinases: Aurora kinases A, B and C (9). Dysregulated expression of Aurora kinases is thought to promote oncogenesis, since an increased expression of Aurora kinases A and B has been observed in numerous tumor cells (10-12). Overexpression of Aurora kinase A is sufficient to induce colony formation in cultured cells and tumors in nude mice, indicating that Aurora kinase A is important for tumor formation and progression (13,14). However, few studies have demonstrated the role and regulation of Aurora kinases in carcinogenesis.

Matrix metalloproteinases (MMPs) are key regulators of many physiologic and pathologic cell processes such as wound healing and cancer metastasis. In cancer invasion, MMPs directly play a pivotal role in the migration of cancer cells (15). MMPs are now considered to be promising targets for cancer therapy, and clinical trials have begun with a large number of synthetic and natural MMP inhibitors (16). Among MMPs, gelatinases A (MMP-2) and B (MMP-9) are involved in tumor invasion and metastasis (17,18). Although MMP-2 is constitutively expressed in tissues (19), the induction of MMP-9

was reported to be important for human cancer cell invasion, particularly breast cancer cells (20-22). Therefore, the specific inhibition of MMP-9 expression has been suggested to be a reasonable pharmacological strategy for reducing the invasive potential of tumor cells (23-27). MMP-9 can be upregulated by several different growth factors and inflammatory cytokines (28,29). Moreover, phorbol esters, the PKC activators, have been identified as tumor-promoting agents but also strong activators of MMPs (30), indicating that PKC is primarily involved in the expression of MMPs in cancer.

In the present study, we first examined whether PKC regulates Aurora kinases in MCF-7 breast cancer cells. Subsequently, we investigated whether the inhibition of Aurora kinases blocks PKC-induced invasion and MMP-9 expression of MCF-7 breast cancer cells. The results showed that Aurora kinase is activated by PKC and is essential for MMP-9 expression and invasion of MCF-7 breast cancer cells.

Materials and methods

Cells and reagents. MCF-7 was purchased from ATCC (Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics at 37˚C in a 5% CO_2 incubator. Reversine and Aurora kinase inhibitor were obtained from Calbiochem (St. Louis, MO, USA). VX-680 was purchased from Selleck (Houston, TX, USA). 12-O-tetradecanoylphorbol-13-acetate (TPA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethylsulfoxide (DMSO) and anti-β-actin antibodies were obtained from Sigma (St. Louis, MO, USA). Primary antibodies against Aurora kinase A and B, phosphorylated (p)-Aurora kinase A, p-Aurora kinase B, p-IκBα, p-c-Jun, p38, p-p38, JNK, p-JNK, ERK and p-ERK were purchased from the Cell Signaling Technology (Beverly, MA, USA). Antibodies against MMP-9, IκBα, p65, PCNA and horseradish peroxidase (HRP)-conjugated IgG were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). DMEM, FBS and phosphate-buffered saline (PBS) were obtained from Gibco-BRL (Gaithersburg, ME, USA). Ninety-six-well plates, 6-well plates, 6 and 10 cm dishes were purchased from SPL Life Sciences (Pocheon, Gyeonggi, Korea). Matrigel was purchased from BD Biosciences (Bedford, MA, USA).

Determination of cell viability. A cell viability assay was performed using an MTT assay. Briefly, the cells were seeded at a density of $3x10^4$ cells/well and allowed to attach to the plate surface. After 24 h, the cells were treated with Aurora kinase inhibitors. After incubation for an additional 24 h, the cells were washed with PBS, treated with MTT (0.5 mg/ml PBS) and incubated at 37˚C for 30 min. Formazan crystals were dissolved with DMSO (100 µl/well) and detected at 570 nm using a microplate reader (Model 3550; Bio-Rad, Richmond, CA, USA).

Western blot analysis. The cells were lysed with an ice-cold M-PER® Mammalian Protein Extraction reagent (Pierce Biotechnology, Rockford, IL, USA). The protein concentration of each lysate was determined using the Bradford assay (31). Samples (20 µg) were resolved by electrophoresis on 10% acrylamide gel, and transferred electrophoretically to Hybond™-PVDF membranes. The membranes were blocked with 5% bovine serum albumin or 5% skim milk and subsequently incubated overnight with primary antibodies (1 µg/ml), followed by incubation with secondary antibodies (HRP-conjugated anti-IgG). ECL reagents (GE Healthcare) were used as substrates for the detection of peroxidase.

Gelatin zymography assay. The procedure for a gelatin zymography assay was performed as previously described (32).

Quantitative PCR assay. Total RNA was extracted from cells using a FastPure™ RNA kit (Takara, Shiga, Japan). cDNA was synthesized from 1 µg total RNA using a PrimeScript™ RT reagent kit (Takara). The mRNA levels were determined by quantitative PCR using the ABI PRISM 7900 sequence detection system and SYBR-Green (Applied Biosystems, Foster City, CA, USA). Aurora kinase A and B, and 18S primers were purchased from SABiosciences (Qiagen Inc., Valencia, CA, USA). The primers used were: MMP-9 (NM 004994) sense, CCTGGAGACCTGAGAACCAATCT and antisense, CCA CCCGAGTGTAACCATAGC; and GAPDH (NM 002046) sense, ATGGAAATCCCATCACCATCTT and antisense, CGCCCCACTTGATTTTGG. To control for variation in mRNA concentration, the results were normalized to the housekeeping gene, *GAPDH* or 18S. Relative quantification was performed using the comparative Ct method according to the manufacturer's instructions.

Electrophoretic mobility shift assay (EMSA). Cells were washed twice, scraped into 1.5 ml of ice-cold PBS (pH 7.5) and pelleted at 3,000 rpm for 3 min. Cytosol and nuclear extracts were prepared from cells using the NE-PER nuclear and cytoplasmic extraction reagents (Pierce Biotechnology). Activation of AP-1 and NF-κB was analyzed by a gel mobility shift assay using nuclear extracts. Oligonucleotides containing the κ-chain (κB, 5'-CCGGTTAACAGAGGGGGCTTTCCGAG-3') and AP-1 (5'-CGCTTGATGAGTCAGCCGGAA-3') binding site were synthesized and used as probes for the gel retardation assays. Complementary strands were annealed and labeled with [α-^{32}P]dCTP. Labeled oligonucleotides (10,000 cpm), 10 µg of nuclear extracts and binding buffer [10 mM Tris-HCl, pH 7.6, 500 mM KCl, 10 mM EDTA, 50% glycerol, 100 ng poly(dI•dC), 1 mM DTT] were then incubated for 30 min at room temperature in a final volume of 20 µl. Reaction mixtures were analyzed by electrophoresis on 4% polyacrylamide gels in a 0.5X Tris-borate buffer (pH 8.3). The gels were dried and examined by autoradiography. Specific binding was demonstrated through competition with a 50-fold excess of cold κB or AP-1 oligonucleotide.

Invasion assay. The invasion assay was carried out in 24-well chambers (8-µm pore size) coated with Matrigel diluted in DMEM (52 µl/cm^2 of growth surface) according to the manufacturer's instructions (BD Biosciences). Matrigel coating was re-hydrated in 0.5 ml DMEM for 30 min immediately prior to experiments. Cells ($2x10^5$) were added to the upper chamber and chemoattractant was added to the bottom well. Conditioned medium (0.5 ml) was added to the lower compartment of the invasion chamber. After 24 h incubation, the cells on the upper

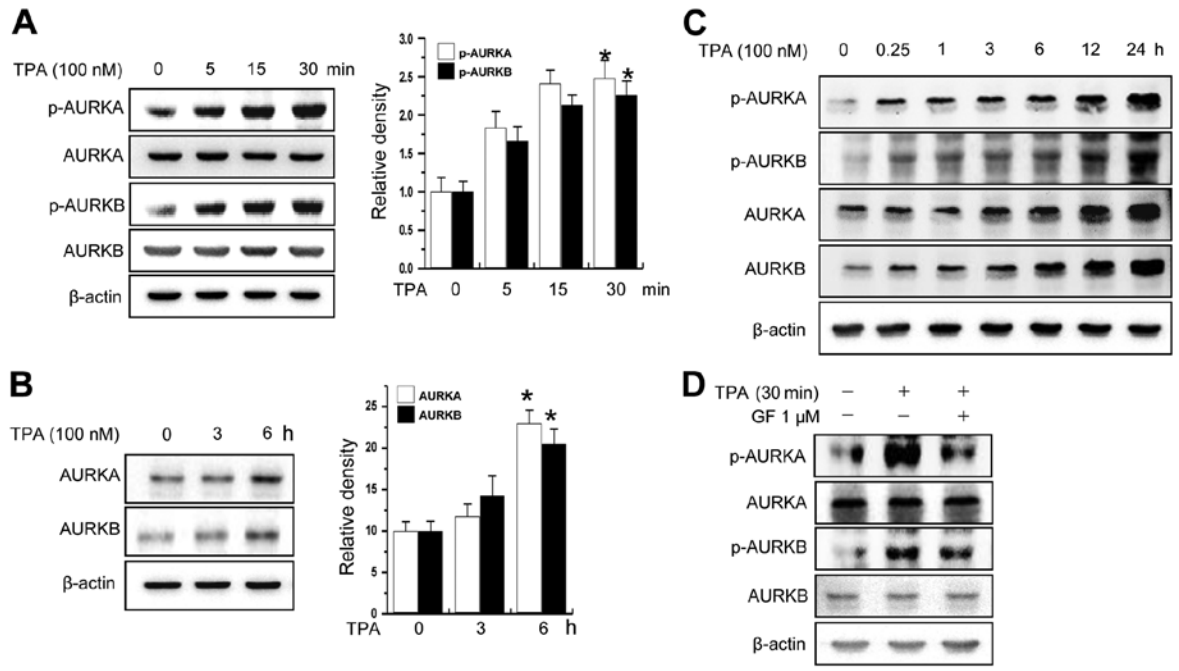

Figure 1. TPA induces the phosphorylation of Aurora kinase A and B in MCF-7 breast cancer cells. Phosphorylation and expression of aurora kinases were assessed by western blotting. (A-C) Monolayer MCF-7 cells were treated with TPA for the indicated time periods. (D) Cells were treated with PKC inhibitor (GF109203X) for 1 h prior to treatment with 100 nM TPA. Increased levels of Aurora kinases were quantified by densitometry. *P<0.01 vs. untreated control. Data are presented as means ± SE of three independent experiments. TPA, 12-O-tetradecanoylphorbol-13-acetate.

side of the chamber were removed using cotton swabs, and migrated cells were fixed and stained with toluidine blue solution. Invading cells were counted in five random areas of the membrane with the assistance of a light microscope.

Migration assay. The migration assay was carried out in 24-well chambers (8-μm pore size). Cells ($2x10^5$) were added to the upper chamber and chemoattractant was added to the bottom well. Conditioned medium (0.5 ml) was added to the lower compartment of the chamber. After a 24-h incubation, the cells on the upper side of the chamber were removed using cotton swabs, and migrated cells were fixed and stained with toluidine blue solution. Invading cells were counted in five random areas of the membrane with the assistance of a light microscope.

RNA interference. Aurora kinase-specific small interfering RNA (siRNA) and negative control siRNA were purchased from Bioneer (Daejeon, Korea). Transfection of MCF-7 cells was performed according to the manufacturer's instructions (Amaxa GmbH, Cologne, Germany). Briefly, cells ($3x10^5$) were collected and re-suspended in 100 μl of MCF-7 nucleofector solution. The cell suspensions were mixed with 100 pmol siRNA, placed in a sterile electroporation cuvette, and subjected to program P-020 with the Nucleofector II (Amaxa GmbH).

Preparation of the lentivirus. miR RNAi Aurora kinase A and B lentiviral vectors were produced using the BLOCK-iT™ Pol II miR RNAi Expression system protocol (Invitrogen, Carlsbad, CA, USA). Single-stranded DNA oligos (Aurora kinase A and B) were created by the BLOCK-iT™ RNAi Express system (Invitrogen). As a control, LacZ Oligo sequences were provided by the RNAi expression system kit. Lentivirus was produced by the ViraPower™ Lentiviral Expression systems protocol (Invitrogen).

Statistical analysis. Data are presented as means ± SE from three individual experiments performed in triplicate replicates. Statistical data analysis was performed using one-way ANOVA program and Student's t-test. Differences with $p<0.05$ were considered to indicate a statistically significant result.

Results

Activation of PKC increases the phosphorylation of Aurora kinase A and B in MCF-7 cells. Previous studies have shown that PKC strongly induces invasion in MCF-7 cells (33,34). To examine whether PKC activates Aurora kinases or regulates the expression of these kinases, we determined levels of phosphorylated Aurora kinases (p-AURKs) and total protein levels of the kinases using MCF-7 cells as a model system. As shown in Fig. 1A and B, treatment of cells with TPA, a PKC activator, increased the levels of total protein and phosphorylation of AURKA and AURKB in a time-dependent manner. The phosphorylation of AURKA and AURKB was constantly increased for 24 h (Fig. 1C). Furthermore, we confirmed that AURKA and B activation is dependent on PKC using PKC inhibitor (Fig. 1D).

PKC-induced phosphorylation of Aurora kinase A and B is mediated by MAPK. To investigate the molecular basis of the TPA-induced activation of Aurora kinases, MCF-7 cells were pretreated with various pharmacological inhibitors of cell signaling pathways, including MAPK, NF-κB and phosphatidylinositol 3-kinase (PI3K). The TPA-induced

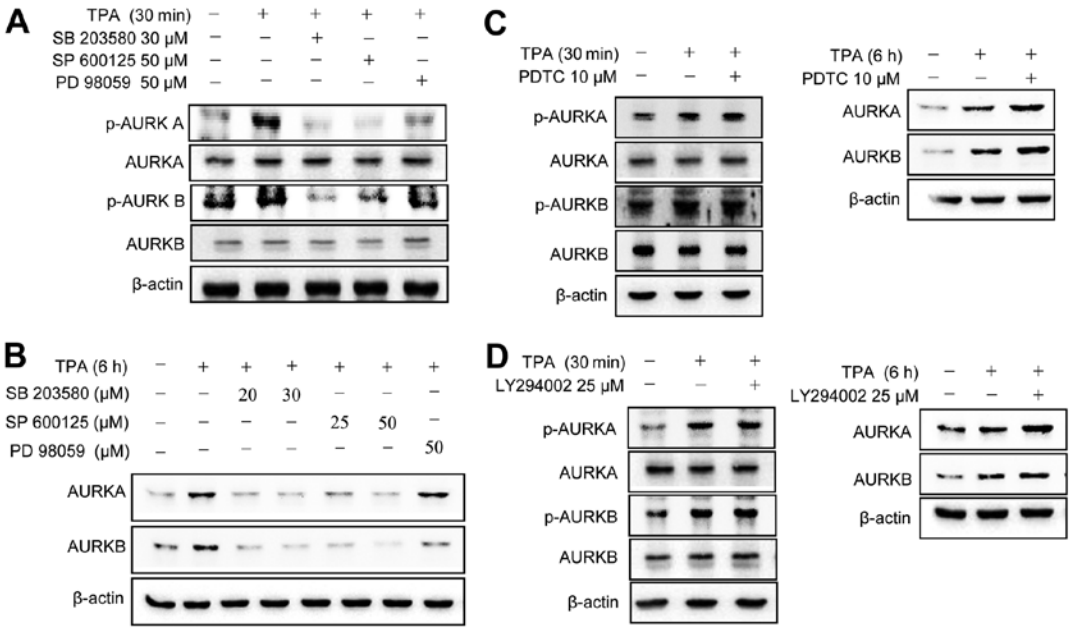

Figure 2. TPA upregulates Aurora kinases through MAPK signaling pathway in MCF-7 breast cancer cells. Cells were treated with (A and B) MAPK signaling inhibitors (p38 inhibitor, SB203580; JNK inhibitor, SP600125; ERK inhibitor, PD98059) or (C and D) NF-κB inhibitors (PDTC), PI3K inhibitor (LY294002) for 1 h priot to treatment with 100 nM TPA. Proteins in cell extracts were analyzed by western blotting. TPA, 12-O-tetradecanoylphorbol-13-acetate.

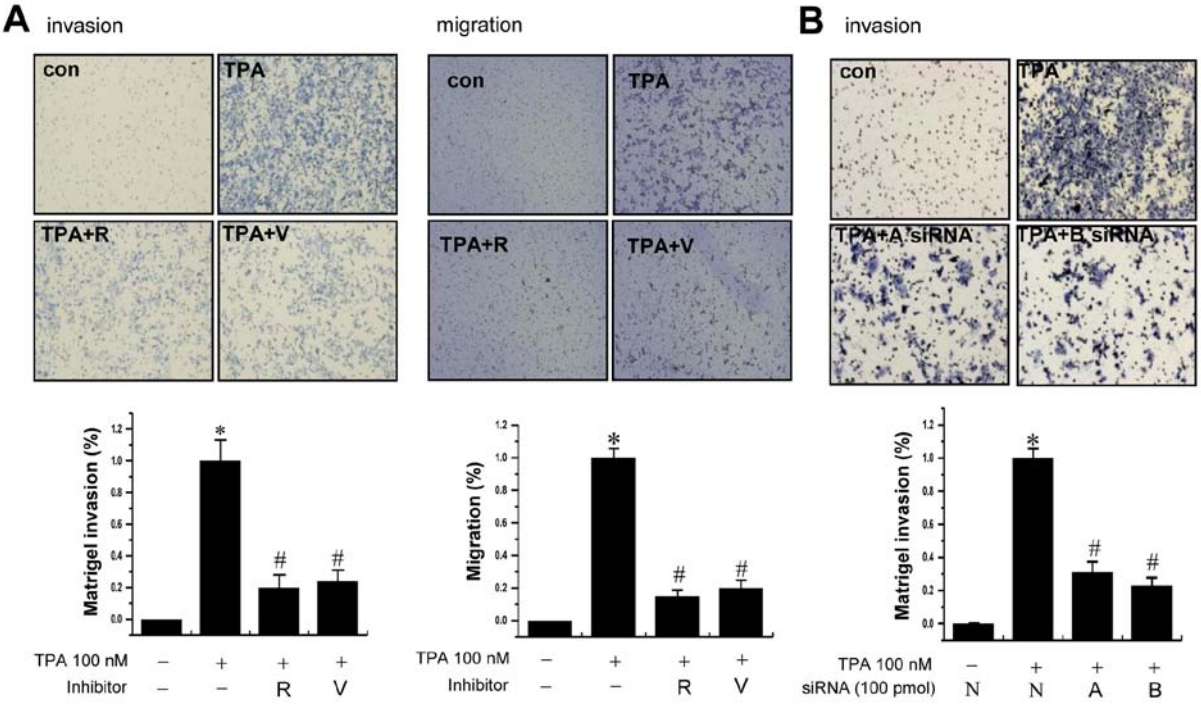

Figure 3. Suppression of Aurora kinases inhibited TPA-induced invasion/migration in MCF-7 breast cancer cells. (A) Cells of treated with pharmacological inhibitors of Aurora kinases [reversine (R), 5 μM; VX-680 (V) 5 μM] and (B) knockdown cells of Aurora kinases were analyzed by invasion and/or migration assay. Each value is the mean ± SE of three independent experiments. *P<0.01 vs. untreated control. #P<0.01 vs. TPA. TPA, 12-O-tetradecanoylphorbol-13-acetate.

phosphorylation (Aurora kinase A, Th288; Aurora kinase B, Th232) of Aurora kinases A and B was abrogated by MAPK inhibitors (Fig. 2A). TPA upregulated total protein levels of Aurora kinases were blocked by MAPK inhibitors (Fig. 2B). By contrast, NF-κB and PI3K inhibitors did not block the TPA-induced phosphorylation/upregulation of Aurora kinase A and B expression (Fig. 2C and D). These results indicated that TPA induces the phosphorylation of Aurora kinases through a MAPK-mediated pathway in MCF-7 cells.

Aurora kinases are involved in the PKC-induced invasion of MCF-7 cell. To examine whether AURKs are involved in the

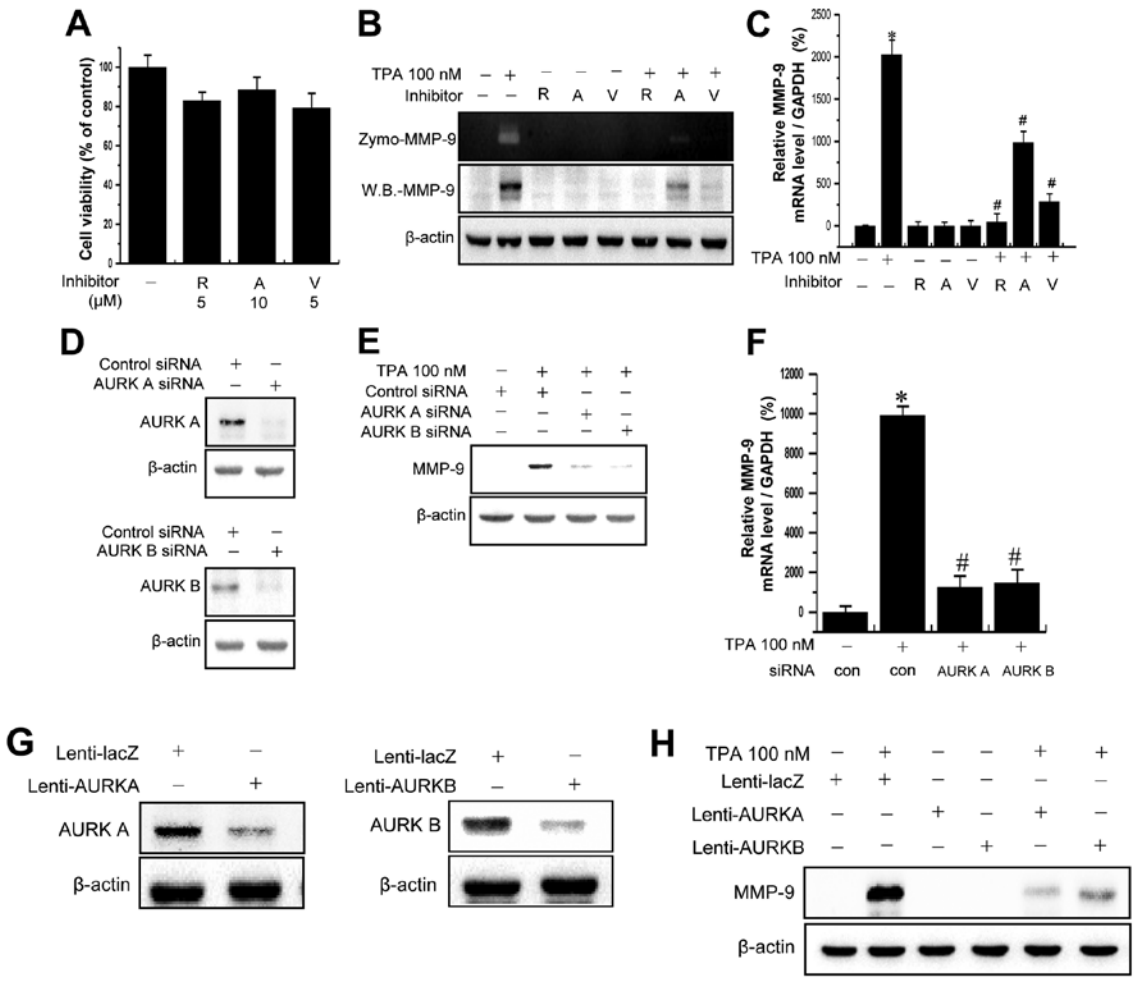

Figure 4. Suppressions of Aurora kinases prevent TPA-induced MMP-9 expression. (A) MCF-7 cells ($3x10^4$ cells/well) were cultured in 96-well plates until 70% confluence was reached. Aurora kinase inhibitors were added at the indicated concentrations for 24 h. [Reversine (R), 5 μM; Aurora kinases inhibitor II (A), 10 μM; VX-680 (V), 5 μM]. The cytotoxicity of each inhibitor was determined using an MTT assay. The optical density of the control cells was defined as 100%. Data are presented as means ± SE of three independent experiments. (B and E) Cell lysates subjected to western blot analysis. MMP-9 expression levels are relative to β-actin. Conditioned medium was prepared and used for MMP-9 gelatin zymography. (C and F) MMP-9 mRNA levels were analyzed by quantitative PCR, with GAPDH was used as an internal control. *P<0.01 vs. untreated control. $^\#$P<0.05 vs. TPA. Data are presented as means ± SE of three independent experiments. (D) MCF-7 cells were transfected with siRNAs and then cell lysates were analyzed by western blotting (G and H) Monolayer MCF-7 cells were infected with miR RNAi-LacZ and Aurora kinases lentivirus. Two days later, TPA treated in infected MCF-7 cells for 24 h. Proteins in cell lysates were immunoblotted with anti-MMP-9 and anti-β-actin. TPA, 12-O-tetradecanoylphorbol-13-acetate.

invasion of MCF-7 cells, we used Aurora kinase inhibitors (reversine and VX-680) and AURK A- and B-specific siRNAs. Treatment of cells with TPA significantly increased the invasion/migration of MCF-7 cells compared to that of control cells. The TPA-induced increase of cell invasion/migration was significantly reduced by pretreatment with the inhibitors of Aurora kinases (Fig. 3A). Supporting the results, transfection of cells with Aurora kinase A or B specific siRNA also reduced the TPA-induced invasion of cells (Fig. 3B).

Activation of Aurora kinases by TPA upregulates the expression of MMP-9. MMP-9 is a key enzyme for degrading type IV collagen, which is a major component of the basement membrane, therefore MMP-9 plays a critical role in cancer cell invasion. Treatment of MCF-7 cells with the Aurora kinase inhibitors did not lead to a significant change in cell viability, as measured by the MTT assay at the indicated concentrations and incubation lengths (Fig. 4A). To determine whether Aurora kinases are involved in TPA-induced MMP-9 expression, MCF-7 cells were pretreated with Aurora kinase inhibitors for 1 h and then additionally treated with TPA for 24 h. As shown in Fig. 4B, treatment of MCF-7 cells with TPA significantly increased MMP-9 expression and secretion. The TPA-induced upregulation of MMP-9 expression and secretion in MCF-7 cells was significantly suppressed by pretreatment with Aurora kinase inhibitors. RT-PCR analysis revealed that TPA increased the levels of MMP-9 mRNA in MCF-7 cells and that Aurora kinase inhibitors blocked the TPA-induced upregulation of MMP-9 mRNA expression (Fig. 4C). To confirm the result, we performed siRNA-mediated gene silencing of Aurora kinases in MCF-7 cells. As shown in Fig. 4D, transfection of MCF-7 cells with Aurora kinase A- and B-targeting siRNA markedly reduced the protein levels of Aurora kinases. The siRNA-mediated silencing of Aurora kinase A and B markedly decreased TPA-mediated increases in MMP-9 mRNA expression and protein levels (Fig. 4E and F). To determine the off-target effect, infected MCF-7 cells by miR RNAi-AURK A and B lentivirus were treated with TPA. The TPA-induced

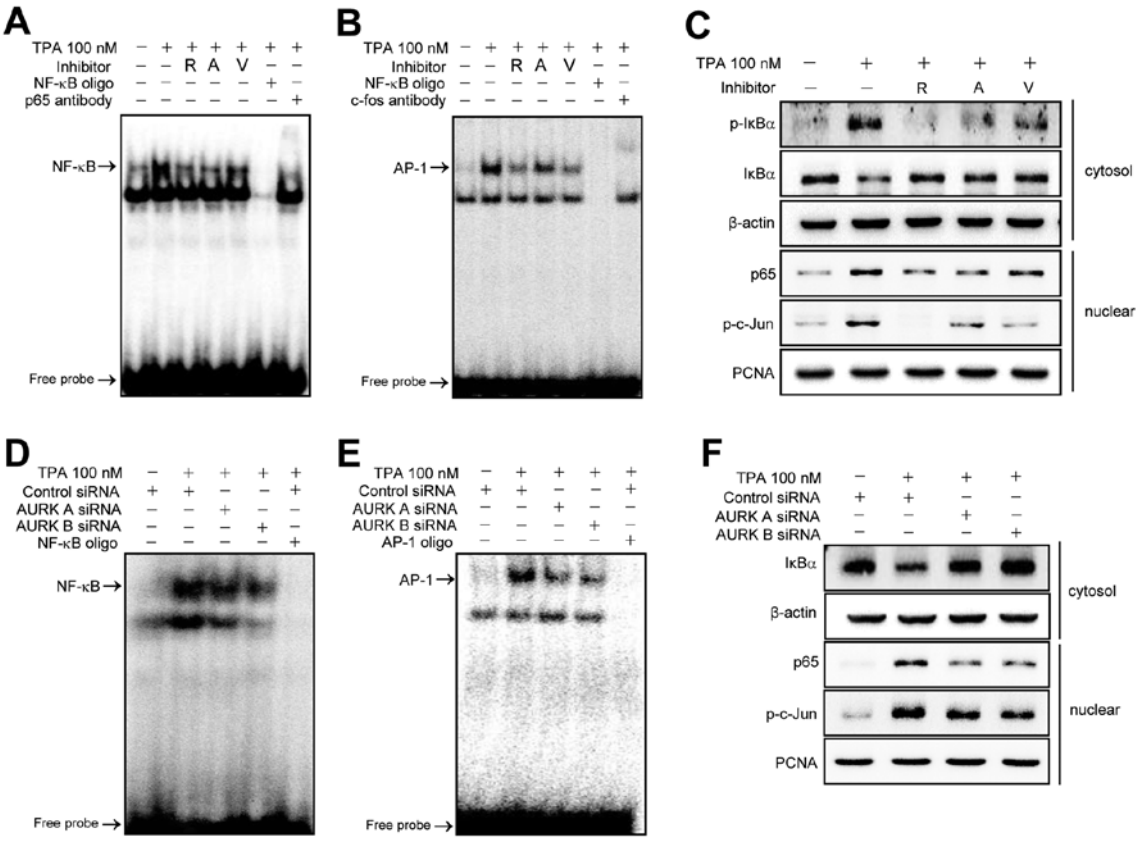

Figure 5. Inhibition of Aurora kinase blocks TPA-induced NF-κB and AP-1 activation. TPA was treated for 4 h in cells that were pre-treated with Aurora kinase inhibitors for 1 h or transfected with Aurora kinase-targeting siRNAs. EMSA results show DNA-binding activity of (A and D) NF-κB and (B and E) AP-1, respectively. (C and F) Western blotting of proteins from the cells analyzed using the indicated antibodies. TPA, 12-O-tetradecanoylphorbol-13-acetate; EMSA, electrophoretic mobility shift assay.

upregulation of MMP-9 expression in MCF-7 cells was significantly suppressed by miR RNAi-AURK A and B lentivirus (Fig. 4G and H). These results indicated that Aurora kinases serve as one of the underlying mechanisms for PKC-induced MMP-9 expression.

Suppression of Aurora kinases A and B inhibits the TPA-induced activation of NF-κB and AP-1 transcription factor. The MMP-9 promoter has been reported to contain NF-κB and AP-1 binding sites. These transcription factors are centrally involved in the induction of MMP-9 gene expression by TPA (27,32). Therefore, we examined whether the Aurora kinase-mediated upregulation of MMP-9 expression was nvolved NF-κB or AP-1. MCF-7 cells were pretreated with Aurora kinase inhibitors for 1 h and then treated with TPA (100 nM) for 3 h, and the nuclear extracts were analyzed by EMSA. As shown in Fig. 5A and B, NF-κB and AP-1 DNA binding activities were markedly increased by TPA. The TPA-induced increases of DNA binding were significantly blocked by Aurora kinase inhibitors. Additionally, the nuclear translocation of the p65 subunit of NF-κB and the phosphorylation status of c-Jun a subunit of AP-1 were analyzed by western blotting (Fig. 5C). Activation of NF-κB is known to be regulated by IκBα phosphorylation and degradation (33,35). Therefore, we examined the effect of Aurora kinase inhibitors on the TPA-induced phosphorylation and degradation of IκBα by western blotting. As shown in Fig. 5C Aurora kinase inhibitors suppressed the TPA-induced phosphorylation and degradation of IκBα. Moreover, in Aurora kinase A and B knockdown cells, NF-κB and AP-1 DNA binding activities, the nuclear translocation of p65 and degradation of IκBα and phosphorylation of c-Jun by TPA were significantly blocked (Fig. 5D-F, respectively).

Discussion

PKCs have been shown to be a transforming oncogene, and PKC-mediated oncogenic activity is linked to its ability to promote cell invasion (2). However, the mechanisms by which PKC signals cell invasion remain elusive. The present study has shown that TPA increases the phosphorylation of Aurora kinase A and B through MAPK, a major PKC downstream molecule. Our results also showed that AURKs are involved in the PKC-induced invasion of breast cancer cells, MMP-9 expression, and activation of NF-κB and AP-1. These findings suggest that Aurora kinases play central roles in cancer invasion.

Aurora kinase A has been shown to be overexpressed in a high proportion of invasive breast carcinomas, pre-invasive carcinomas and even proliferative benign breast disease (BBDs) (36-38), suggesting that Aurora kinase A has an important role in human mammary tumor metastasis. Aurora kinases A and B promote inappropriate cellular mobility (39,40), a characteristic of invasion and metastasis

of tumor cells. RNA interference targeting Aurora kinase A reduces the migration ability of human hepatic cancer cells, and Aurora kinase A has also been shown to enhance collagen-induced cell migration (41). However, the PKC-mediated expression of Aurora kinases has not been studied. Our results showed that PKC signaling upregulates the expression of Aurora kinases.

PKC regulates various cell functions, including signal transduction and gene expression (42). Thus, alterations in PKC signaling leads to malignant transformation and tumor progression. Overexpression of several PKCs has been reported in malignant breast tissue and breast cancer cell lines (43). As the major cell receptor for phorbol esters such as TPA, PKC acts as an important mediator for the transcriptional regulation of growth factor-responsive MMP genes (30). Notably, in the present study, the results reveal that activation of PKC with TPA increases phosphorylation of Aurora kinase A and B. TPA-induced phosphorylation (Aurora kinase A, Th288; Aurora kinase B, Th232) of Aurora kinases A and B were abrogated only by MAPK inhibitors. Supporting these observations, Oktay *et al* have shown that c-Jun N-terminal kinase, JNK functions upstream of Aurora kinase B (44). These findings suggest that PKC/MAPK signaling pathways activates Aurora kinases in MCF-7 cells.

Activation of PKCs has been shown to be correlated with the MMP-9 expression in breast cancer cells (20). MMP-9 is believed to be an important enzyme in tumor invasion due to its ability to degrade collagen (15,18). Numerous studies have shown that TPA stimulates the synthesis and secretion of MMP-9 in MCF-7 cells (45,46). In the present study, we found that inhibition of Aurora kinases strongly suppressed TPA-induced MMP-9 expression/secretion. The findings suggest that activation of Aurora kinases is necessary for the PKC-mediated induction of MMP-9 gene expression.

Nuclear transcriptional factors, AP-1 and NF-κB are involved in the upregulation of MMP-9 expression (47,48). AP-1 is a sequence-specific transcriptional factor composed of Jun, Fos and ATF family proteins, and is induced by multiple stimuli such as TPA (49). In the present study, suppression of Aurora kinases inhibited the TPA-mediated activation of NF-κB and AP-1. Recently, Briassouli *et al* have shown that Aurora kinase A regulates NF-κB signaling through IκBα phosphorylation (50). Collectively, these observations indicate that Aurora kinases play central roles in PKC-induced AP-1 and NF-κB activation, and MMP-9 expression.

In conclusion, the present study provides evidence that Aurora kinases A and B mediate PKC-MAPK signal to AP-1 and NF-κB with increasing MMP-9 expression and invasion of cancer cells. Tot the best of our knowledge, this is the first study showing that PKC regulates the activity of Aurora kinases, and provides insight into the molecular mechanism. Therefore, we suggest that Aurora kinases are key molecules in PKC-induced invasion in breast cancer cells.

Acknowledgements

The present study was supported by the Korea Breast Cancer Foundation, and the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (nos. 2011-0030716 and 2010-0012716).

References

1. Sumagin R, Robin AZ, Nusrat A and Parkos CA: Activation of PKCβII by PMA facilitates enhanced epithelial wound repair through increased cell spreading and migration. PLoS One 8: e55775, 2013.
2. Dumont JA and Bitonti AJ: Modulation of human melanoma cell metastasis and adhesion may involve integrin phosphorylation mediated through protein kinase C. Biochem Biophys Res Commun 204: 264-272, 1994.
3. Nakagawa Y: Artificial analogs of naturally occurring tumor promoters as biochemical tools and therapeutic leads. Biosci Biotechnol Biochem 76: 1262-1274, 2012.
4. Way D, Smith S, Sivendran S, Chie L, Kanovsky M, Brandt-Rauf PW, Chung DL, Michl J and Pincus MR: A protein kinase C inhibitor induces phenotypic reversion of ras-transformed pancreatic cancer cells and cooperatively blocks tumor cell proliferation with an anti-ras peptide. Cancer Chemother Pharmacol 49: 429-437, 2002.
5. Wu WS, Lin JK and Wu FY: Differential induction of c-fos and c-jun proto-oncogenes and AP-1 activity by tumor promoter 12-O-tetradecanoyl phorbol 13-acetate in cells at different stages of tumor promotion in vitro. Oncogene 7: 2287-2294, 1992.
6. Xie Z, Zeng X, Waldman T and Glazer RI: Transformation of mammary epithelial cells by 3-phosphoinositide-dependent protein kinase-1 activates beta-catenin and c-Myc, and down-regulates caveolin-1. Cancer Res 63: 5370-5375, 2003.
7. Bischoff JR and Plowman GD: The Aurora/Ipl1p kinase family: Regulators of chromosome segregation and cytokinesis. Trends Cell Biol 9: 454-459, 1999.
8. Chan F, Sun C, Perumal M, Nguyen QD, Bavetsias V, McDonald E, Martins V, Wilsher NE, Raynaud FI, Valenti M, *et al*: Mechanism of action of the Aurora kinase inhibitor CCT129202 and in vivo quantification of biological activity. Mol Cancer Ther 6: 3147-3157, 2007.
9. Nigg EA: Mitotic kinases as regulators of cell division and its checkpoints. Nat Rev Mol Cell Biol 2: 21-32, 2001.
10. Tatsuka M, Katayama H, Ota T, Tanaka T, Odashima S, Suzuki F and Terada Y: Multinuclearity and increased ploidy caused by overexpression of the aurora-and Ipl1-like midbody-associated protein mitotic kinase in human cancer cells. Cancer Res 58: 4811-4816, 1998.
11. Zhou H, Kuang J, Zhong L, Kuo WL, Gray JW, Sahin A, Brinkley BR and Sen S: Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. Nat Genet 20: 189-193, 1998.
12. Katayama H, Ota T, Jisaki F, Ueda Y, Tanaka T, Odashima S, Suzuki F, Terada Y and Tatsuka M: Mitotic kinase expression and colorectal cancer progression. J Natl Cancer Inst 91: 1160-1162, 1999.
13. Bischoff JR, Anderson L, Zhu Y, Mossie K, Ng L, Souza B, Schryver B, Flanagan P, Clairvoyant F, Ginther C, *et al*: A homologue of *Drosophila* aurora kinase is oncogenic and amplified in human colorectal cancers. EMBO J 17: 3052-3065, 1998.
14. Sen S, Zhou H and White RA: A putative serine/threonine kinase encoding gene *BTAK* on chromosome 20q13 is amplified and overexpressed in human breast cancer cell lines. Oncogene 14: 2195-2200, 1997.
15. Sato H and Seiki M: Regulatory mechanism of 92 kDa type IV collagenase gene expression which is associated with invasiveness of tumor cells. Oncogene 8: 395-405, 1993.
16. Mannello F, Tonti G and Papa S: Matrix metalloproteinase inhibitors as anticancer therapeutics. Curr Cancer Drug Targets 5: 285-298, 2005.
17. Chambers AF and Matrisian LM: Changing views of the role of matrix metalloproteinases in metastasis. J Natl Cancer Inst 89: 1260-1270, 1997.
18. Nabeshima K, Inoue T, Shimao Y and Sameshima T: Matrix metalloproteinases in tumor invasion: Role for cell migration. Pathol Int 52: 255-264, 2002.
19. Poulsom R, Hanby AM, Pignatelli M, Jeffery RE, Longcroft JM, Rogers L and Stamp GW: Expression of gelatinase A and TIMP-2 mRNAs in desmoplastic fibroblasts in both mammary carcinomas and basal cell carcinomas of the skin. J Clin Pathol 46: 429-436, 1993.
20. Liu JF, Crépin M, Liu JM, Barritault D and Ledoux D: FGF-2 and TPA induce matrix metalloproteinase-9 secretion in MCF-7 cells through PKC activation of the Ras/ERK pathway. Biochem Biophys Res Commun 293: 1174-1182, 2002.

21. Yao J, Xiong S, Klos K, Nguyen N, Grijalva R, Li P and Yu D: Multiple signaling pathways involved in activation of matrix metalloproteinase-9 (MMP-9) by heregulin-beta1 in human breast cancer cells. Oncogene 20: 8066-8074, 2001.
22. Nagai S, Nakamura M, Yanai K, Wada J, Akiyoshi T, Nakashima H, Ohuchida K, Sato N, Tanaka M and Katano M: *Gli1* contributes to the invasiveness of pancreatic cancer through matrix metalloproteinase-9 activation. Cancer Sci 99: 1377-1384, 2008.
23. Lee SO, Jeong YJ, Kim M, Kim CH and Lee IS: Suppression of PMA-induced tumor cell invasion by capillarisin via the inhibition of NF-kappaB-dependent MMP-9 expression. Biochem Biophys Res Commun 366: 1019-1024, 2008.
24. Kondraganti S, Mohanam S, Chintala SK, Kin Y, Jasti SL, Nirmala C, Lakka SS, Adachi Y, Kyritsis AP, Ali-Osman F, *et al*: Selective suppression of matrix metalloproteinase-9 in human glioblastoma cells by antisense gene transfer impairs glioblastoma cell invasion. Cancer Res 60: 6851-6855, 2000.
25. Thangapazham RL, Passi N and Maheshwari RK: Green tea polyphenol and epigallocatechin gallate induce apoptosis and inhibit invasion in human breast cancer cells. Cancer Biol Ther 6: 1938-1943, 2007.
26. Qi Q, Lu N, Wang XT, Gu HY, Yang Y, Liu W, Li C, You QD and Guo QL: Anti-invasive effect of gambogic acid in MDA-MB-231 human breast carcinoma cells. Biochem Cell Biol 86: 386-395, 2008.
27. Chung TW, Moon SK, Chang YC, Ko JH, Lee YC, Cho G, Kim SH, Kim JG and Kim CH: Novel and therapeutic effect of caffeic acid and caffeic acid phenyl ester on hepatocarcinoma cells: Complete regression of hepatoma growth and metastasis by dual mechanism. FASEB J 18: 1670-1681, 2004.
28. Fischoeder A, Meyborg H, Stibenz D, Fleck E, Graf K and Stawowy P: Insulin augments matrix metalloproteinase-9 expression in monocytes. Cardiovasc Res 73: 841-848, 2007.
29. Kondapaka SB, Fridman R and Reddy KB: Epidermal growth factor and amphiregulin up-regulate matrix metalloproteinase-9 (MMP-9) in human breast cancer cells. Int J Cancer 70: 722-726, 1997.
30. Mackay AR, Ballin M, Pelina MD, Farina AR, Nason AM, Hartzler JL and Thorgeirsson UP: Effect of phorbol ester and cytokines on matrix metalloproteinase and tissue inhibitor of metalloproteinase expression in tumor and normal cell lines. Invasion Metastasis 12: 168-184, 1992.
31. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248-254, 1976.
32. Noh EM, Chung EY, Youn HJ, Jung SH, Hur H, Lee YR and Kim JS: *Cis*-guggulsterone inhibits the IKK/NF-κB pathway, whereas *trans*-guggulsterone inhibits MAPK/AP-1 in MCF-7 breast cancer cells: Guggulsterone regulates MMP-9 expression in an isomer-specific manner. Int J Mol Med 31: 393-399, 2013.
33. Karin M: How NF-kappaB is activated: The role of the IkappaB kinase (IKK) complex. Oncogene 18: 6867-6874, 1999.
34. Krappmann D and Scheidereit C: A pervasive role of ubiquitin conjugation in activation and termination of IkappaB kinase pathways. EMBO Rep 6: 321-326, 2005.
35. Arenzana-Seisdedos F, Turpin P, Rodriguez M, Thomas D, Hay RT, Virelizier JL and Dargemont C: Nuclear localization of I kappa B alpha promotes active transport of NF-kappa B from the nucleus to the cytoplasm. J Cell Sci 110: 369-378, 1997.
36. Tanaka T, Kimura M, Matsunaga K, Fukada D, Mori H and Okano Y: Centrosomal kinase AIK1 is overexpressed in invasive ductal carcinoma of the breast. Cancer Res 59: 2041-2044, 1999.
37. Miyoshi Y, Iwao K, Egawa C and Noguchi S: Association of centrosomal kinase *STK15/BTAK* mRNA expression with chromosomal instability in human breast cancers. Int J Cancer 92: 370-373, 2001.
38. Goepfert TM, Adigun YE, Zhong L, Gay J, Medina D and Brinkley WR: Centrosome amplification and overexpression of aurora A are early events in rat mammary carcinogenesis. Cancer Res 62: 4115-4122, 2002.
39. Nguyen HG, Chinnappan D, Urano T and Ravid K: Mechanism of Aurora-B degradation and its dependency on intact KEN and A-boxes: Identification of an aneuploidy-promoting property. Mol Cell Biol 25: 4977-4992, 2005.
40. Wu JC, Chen TY, Yu CT, Tsai SJ, Hsu JM, Tang MJ, Chou CK, Lin WJ, Yuan CJ and Huang CY: Identification of V23RalA-Ser^{194} as a critical mediator for Aurora-A-induced cellular motility and transformation by small pool expression screening. J Biol Chem 280: 9013-9022, 2005.
41. Guan Z, Wang XR, Zhu XF, Huang XF, Xu J, Wang LH, Wan XB, Long ZJ, Liu JN, Feng GK, *et al*: Aurora-A, a negative prognostic marker, increases migration and decreases radiosensitivity in cancer cells. Cancer Res 67: 10436-10444, 2007.
42. Dekker LV and Parker PJ: Protein kinase C - a question of specificity. Trends Biochem Sci 19: 73-77, 1994.
43. Urtreger AJ, Kazanietz MG and Bal de Kier Joffé ED: Contribution of individual PKC isoforms to breast cancer progression. IUBMB Life 64: 18-26, 2012.
44. Oktay K, Buyuk E, Oktem O, Oktay M and Giancotti FG: The c-Jun N-terminal kinase JNK functions upstream of Aurora B to promote entry into mitosis. Cell Cycle 7: 533-541, 2008.
45. Woo MS, Jung SH, Kim SY, Hyun JW, Ko KH, Kim WK and Kim HS: Curcumin suppresses phorbol ester-induced matrix metalloproteinase-9 expression by inhibiting the PKC to MAPK signaling pathways in human astroglioma cells. Biochem Biophys Res Commun 335: 1017-1025, 2005.
46. Noh EM, Youn HJ, Jung SH, Han JH, Jeong YJ, Chung EY, Jung JY, Kim BS, Lee SH, Lee YR, *et al*: Cordycepin inhibits TPA-induced matrix metalloproteinase-9 expression by suppressing the MAPK/AP-1 pathway in MCF-7 human breast cancer cells. Int J Mol Med 25: 255-260, 2010.
47. Bond M, Fabunmi RP, Baker AH and Newby AC: Synergistic upregulation of metalloproteinase-9 by growth factors and inflammatory cytokines: An absolute requirement for transcription factor NF-kappa B. FEBS Lett 435: 29-34, 1998.
48. Takahra T, Smart DE, Oakley F and Mann DA: Induction of myofibroblast MMP-9 transcription in three-dimensional collagen I gel cultures: Regulation by NF-kappaB, AP-1 and Sp1. Int J Biochem Cell Biol 36: 353-363, 2004.
49. Lee SO, Jeong YJ, Im HG, Kim CH, Chang YC and Lee IS: Silibinin suppresses PMA-induced MMP-9 expression by blocking the AP-1 activation via MAPK signaling pathways in MCF-7 human breast carcinoma cells. Biochem Biophys Res Commun 354: 165-171, 2007.
50. Briassouli P, Chan F, Savage K, Reis-Filho JS and Linardopoulos S: Aurora-A regulation of nuclear factor-kappaB signaling by phosphorylation of IkappaBalpha. Cancer Res 67: 1689-1695, 2007.