Aurora kinase A induces migration and invasion by inducing epithelial-to-mesenchymal transition in colon cancer cells

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INTRODUCTION

Aurora kinase A is a family of serine/threonine kinases intimately associated with mitotic progression and the development of human cancers. Studies have shown that aurora kinases are important for the protein kinase C (PKC)-induced invasion of colon cancer cells. Recent studies have shown that aurora kinase A promotes distant metastasis by inducing epithelial-to-mesenchymal transition (EMT) in colon cancer cells. However, the role of aurora kinase A in colon cancer metastasis remains unclear. In this study, we investigated the effects of aurora kinase A on PKC-induced cell invasion, migration, and EMT in human SW480 colon cancer cells. Treatment with 12-O-tetradecanoylphorbol-13-acetate (TPA) changed the expression levels of EMT markers, increasing α-SMA, vimentin, and MMP-9 expression and decreasing E-cadherin expression, with changes in cell morphology. TPA treatment induced EMT in a PKC-dependent manner. Moreover, the inhibition of aurora kinase A by siRNAs and inhibitors (reversine and VX-680) suppressed TPA-induced cell invasion, migration, and EMT in SW480 human colon cells. Inhibition of aurora kinase A blocked TPA-induced vimentin and MMP-9 expression, and decreased E-cadherin expression. Furthermore, the knockdown of aurora kinase A decreased the transcriptional activity of NF-κB and AP-1 in PKC-stimulated SW480 cells. These findings indicate that aurora kinase A induces migration and invasion by inducing EMT in SW480 colon cancer cells. To the best of our knowledge, this is the first study that showed aurora kinase A is a key molecule in PKC-induced metastasis in colon cancer cells. [BMB Reports 2022; 55(2): 87-91]

Keywords: Aurora kinase A, Colon cancer, Epithelia-to-mesenchymal transition, Matrix metalloproteinase-9, Protein kinase C

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Aurora kinase induces EMT in colon cancer cells
On-Yu Hong, et al.

88
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Reports
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by siRNAs and aurora kinase A inhibitors inhibited TPA-induced cell invasion, migration, and EMT in SW480 human colon cells. These results indicate that aurora kinase A induces migration and invasion by inducing EMT in SW480 colon cancer cells.

RESULTS

PKC treatment induced EMT in SW480 cells
SW480 colon cells were derived from a primary tumor and had no metastatic properties. To determine whether TPA induces EMT in colon cancer cells, we treated SW480 cells with TPA. Treatment of cells with TPA induced changes in EMT markers, such as increased α-SMA, vimentin, MMP-9 expression and decreased E-cadherin with changes in cell morphology (Fig. 1A, B). We then confirmed the migration and invasion potential of cells treated with TPA, and observed that cells developed strong migration and invasive ability after 24 h of TPA treatment (Fig. 1C). Next, to investigate whether the induction of EMT by TPA is PKC-dependent, cells were treated with GF 109203X, a PKC inhibitor, 1 h before TPA treatment. Pretreatment with GF 109203X blocked TPA-induced EMT (Fig. 1C, D). These results confirmed that TPA induced EMT in a PKC-dependent manner.

Aurora kinase A is involved in PKC-induced EMT in SW480 cells
We explored whether the expression of aurora kinase A was altered in SW480 cells after exposure to TPA. TPA treatment increased aurora kinase A expression in SW480 cells (Fig. 2A). Therefore, the role of aurora kinase A in PKC-induced EMT was examined in SW480 cells. The protein expression of aurora kinase A was determined to confirm that it was silenced by siRNA treatment (Fig. 2B). Next, we examined the alteration of EMT markers induced by aurora kinase A expression. The inhibition of aurora kinase A using siRNA and specific inhibitors (reversine and VX-680) suppressed the alteration in expressions of vimentin, MMP-9, and E-cadherin (Fig. 2C, D). Therefore, these results suggest that aurora kinase A plays a major role in PKC-induced EMT in colon cells.

Aurora kinase A regulates PKC-induced cell invasion and migration in SW480 cells
To investigate the role of aurora kinase A in PKC-induced cell invasion and migration of SW480 cells, we performed in vitro Matrigel invasion and scratch migration assays. The inhibition of aurora kinase A using siRNA suppressed TPA-induced invasion and migration (Fig. 3A). Additionally, we checked the effect of aurora kinase inhibitors such as reversine and VX680 in cell invasion. We observed that both agents inhibited TPA-stimulated cell invasion (Fig. 3B). These results suggest that the silencing of aurora kinase A suppressed PKC-induced invasion and migration in colon cells.
Aurora kinase A might control NF-κB with aurora kinase inhibitors (Fig. 4C). These results indicate that aurora kinase A regulates PKC-induced NF-κB/AP-1 activation in SW480 cells.

NF-κB and AP-1 are major transcription factors that regulate EMT (17, 18). As shown in Fig. 4, TPA increased p65 (NF-κB subunit) expression and levels of phosphorylated c-Jun (AP-1 subunit) in the nucleus. To elucidate whether aurora kinase A expression was involved in NF-κB/AP-1 activation, suppression of aurora kinase A in SW480 cells by treatment with siRNA or inhibitors was performed. Treatment was done after exposing the cells to TPA for 3 h, and then the total nuclear protein was examined by extract transcription factor activity. Inhibition of aurora kinase A suppressed TPA-induced expression of p65 and p-c-Jun in the nucleus (Fig. 4A, B). Indeed, TPA-induced NF-κB DNA-binding activity was partially blocked by treatment with aurora kinase inhibitors (Fig. 4C). These results indicate that aurora kinase A might control NF-κB and AP-1 in colon cells.

**DISCUSSION**

Aurora kinase plays an important role in maintaining chromosome stability as mitotic regulators. Moreover, recent data have shown that aurora kinase A activates EMT (19), and the stabilization and accumulation of aurora kinases, which drives the transition of breast cells from an epithelial to a highly invasive mesenchymal phenotype (20). Aurora kinase expression in patients with CRC liver metastases is associated with poor prognosis (21, 22). These findings strongly indicate that aurora kinases are negative regulators of the highly invasive mesenchymal phenotype in cancer cells. Our study suggests that aurora kinase A induces migration and invasion by inducing EMT in SW480 cells. This study shows that aurora kinase A is key to PKC-induced metastasis and is a novel therapeutic molecule in SW480 colon cancer cells.

EMT is a stage of phenotypic alteration in cancer cells, in which cells undergo morphological transformation. During EMT, cells lose their epithelial feature and get a mesenchymal phenotype. Phenotypic changes are thought to be derived from a shift in the expression between epithelial (E-cadherin) and mesenchymal (Vimentin, Snail, and Twist-1) factors (8, 9). EMT markers have been a major potential target for the prevention and treatment of various human cancers (19, 22). As a result, the transition from an epithelial to a mesenchymal phenotype in cancer cells increases their capability to invade and migrate to surrounding tissues.

MMP-9 plays a major role in metastasis (23, 24). In addition, the induction of invasiveness and EMT has been associated with the activation of NF-κB and AP-1 in cancer cells (25-27), suggesting that MMP-9 expression leads to EMT in cancer. However, the signaling systems that lead to EMT in colon cancer cells remain unclear. A previous study found that the aurora kinases regulates TPA-induced MMP-9 expression in breast cancer cells (15). This suggests that aurora kinases is also associated with PKC-induced MMP-9 expression in colon cancer cells.

In this study, we found that the silencing of aurora kinases A by their respective siRNAs and inhibitors blocked TPA-induced cell invasion, migration, MMP-9 expression, and EMT in SW480 cells (Figs. 2 and 3). These results show that aurora kinase stimulates migration and invasion by inducing EMT and MMP-9 expression in SW480 cells. Furthermore, the knockdown of aurora kinase A reduced the transcriptional activity of NF-κB and AP-1 in PKC-mediated SW480 cells (Fig. 4). Our results indicate that aurora kinase plays a role in PKC-induced MMP-9 expression through the NF-κB and AP-1 signaling pathways in SW480 cells. Supporting this hypothesis, another study indicated that aurora kinase is an important target in cancer through the inhibition of the EMT pathway (19). These results suggest that
aurora kinase is a key regulator of EMT in SW480 colon cancer metastasis.

In conclusion, our study shows that MMP-9 expression and the metastasis of colon cancer cells may be modulated via an aurora kinase A-induced EMT signaling mechanism. Our data showed that aurora kinase A might be a target for the prevention and treatment of colon cancer.

MATERIALS AND METHODS

Cell culture and materials
SW480 cells were purchased from the Korean Cell Line Bank. The cells were cultured in high-glucose DMEM containing 10% bovine serum albumin and 1% antibiotics (10,000 U/ml penicillin and 10,000 μg/ml streptomycin) at 37°C in an incubator with 5% CO₂, TPA, DMSO, and β-actin antibodies were obtained from Sigma-Aldrich (St. Louis, MO, USA). GF 109203X and reversine were obtained from Calbiochem (St. Louis, MO, USA). VX-680 was purchased from Selleck (Houston, TX, USA). Primary antibodies against α-SMA, vimentin, MMP-9, E-cadherin, p65, and PCNA were purchased from Santa Cruz (CA, USA), and aurora kinase A (AURKA) and p-c-Jun were obtained from Cell Signaling Technology (Beverly, MA, USA).

Western blotting
SW480 cell extracts were isolated using M-PER Mammalian Protein Extraction Reagent (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with a protease inhibitor (Millipore, Billerica, MA, USA). Twenty micrograms of protein was separated using 7-12% SDS-PAGE and transferred onto PVDF membranes (GE Healthcare Life Sciences, Buckinghamshire, UK). Membranes were incubated with the corresponding primary antibodies at 4°C for overnight and HRP-conjugated secondary antibodies were incubated with the corresponding primary antibodies at 4°C for 1 h. HRP was detected using an imaging analyzer (LAS 1000; FujiFilm Corporation, Japan).

Immunofluorescence analysis
Cell fixation was carried out with 4% formalin for 30 min at room temperature, and cell permeability was measured using 0.2% Triton-X100 in PBS for 20 min. Next, the cells were blocked with 2% bovine serum albumin in PBS to prevent nonspecific antibody binding. The cells were then incubated with anti-E-cadherin and anti-vimentin antibodies, followed by Alexa Fluor-400 conjugated secondary antibodies (Molecular Probes, Eugene, OR, USA). Confocal images were obtained using a Carl Zeiss laser confocal microscope (Model no. LSM 510 META).

Matrigel invasion assay
Invasion assays were conducted using a Transwell insert chamber (8 μm pore size) coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). SW480 cells were added to the upper chamber, and a chemoattractant was added to the bottom well. The wells were filled with a conditioned medium containing TPA for 24 h. After incubation, the invading cells were fixed, stained with toluidine blue, and photographed (40× magnification). Three independent experiments were conducted.

Scratch migration assay
A scratch migration assay was performed to compare the migratory abilities of normal and aurora kinase A (AURKA)-knockdown SW480 cells. All cells were cultured to confluency, scratched, photographed at 0 h, and then photographed after treatment with TPA for 24 h using a light microscope at 40× magnification. All experiments were performed in triplicate.

Electrophoretic mobility shifting assay
Nuclear proteins were extracted using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce Biotechnology, Rockford, IL, USA). Extraction was performed according to the manufacturer's protocol. Oligonucleotides containing the κ-chain (κB, 5′-CCGGTTAACAGAGGGGGCTTTCCGAG-3′) binding site were synthesized and used as probes for the gel retardation assays. The NF-κB oligonucleotide was labeled with [α-32P]dCTP using a Rediprime II DNA Labeling System (Amersham Life Science, Buckinghamshire, UK). In competition assays, a 100-fold excess of cold κB oligonucleotide was added. The DNA-protein complexes were analyzed via electrophoresis on a 4% polyacrylamide gel. After electrophoresis, the gel was dried and examined using autoradiography.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

REFERENCES

1. Privette LM and Petty EM (2008) CHFR: a novel mitotic checkpoint protein and regulator of tumorigenesis. Transl Oncol 1, 57-64
2. Carvajal RD, Tse A and Schwartz GK (2006) Aurora kinases: novel therapy targets in cancers. Oncotarget 1, 57-64
3. Yoo J, Minter-Dykhouse K, Malureanu L et al (2005) Chfr is required for tumor suppression and Aurora A regulation. Nat Genet 37, 401-406
4. Tang A, Gao K, Chu L, Zhang R, Yang J and Zheng J (2017) Aurora kinases: novel therapy targets in cancers. Oncotarget 8, 23937-23954
5. Du R, Huang C, Liu K, Li X and Dong Z (2021) Targeting AURKA in cancer: molecular mechanisms and opportunities for cancer therapy. Mol Cancer 20, 13
6. Jemal A, Center MM, DeSantis C and Ward EM (2010) Global patterns of cancer incidence and mortality rates and trends. Cancer Epidemiol Biomarkers Prev 19, 1893-1907
7. Siegel R, Naishadham D and Jemal A (2013) Cancer statistics, 2013. CA Cancer J Clin 63, 11-30
8. Mathias RA, Gopal SK and Simpson RJ (2013) Contribution of cells undergoing epithelial-mesenchymal transition to the tumour microenvironment. J Proteomics 78, 545-557
9. Thiery JP and Sleeman JP (2006) Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 7, 131-142
10. Batlle E, Sancho E, Franci C et al (2000) The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. Nat Cell Biol 2, 84-89
11. Usman S, Waseem NH, Nguyen TKN et al (2021) Vimentin is at the heart of epithelial mesenchymal transition (emt) mediated metastasis. Cancers (Basel) 13, 4985
12. Parekh DB, Ziegler W and Parker PJ (2000) Multiple pathways control protein kinase C phosphorylation. EMBO J 19, 496-503
13. Tarafdar A and Michie AM (2014) Protein kinase C in cellular transformation: a valid target for therapy? Biochem Soc Trans 42, 1556-1562
14. Masur K, Lang K, Niggemann B, Zanker KS and Entschladen F (2001) High PKC alpha and low E-cadherin expression contribute to high migratory activity of colon carcinoma cells. Mol Biol Cell 12, 1973-1982
15. Noh EM, Lee VR, Hong OY, Jung SH, Youn HJ and Kim JS (2015) Aurora kinases are essential for PKC-induced invasion and matrix metalloproteinase-9 expression in MCF-7 breast cancer cells. Oncol Rep 34, 803-810
16. Serova M, Ghoul A, Benhadji KA et al (2006) Preclinical and clinical development of novel agents that target the protein kinase C family. Semin Oncol 33, 466-478
17. Julien S, Puig I, Caretti E et al (2007) Activation of NF-kappaB by Akt upregulates Snail expression and induces epithelium mesenchyme transition. Oncogene 26, 7445-7456
18. Nam EH, Lee Y, Moon B, Lee JW and Kim S (2015) Twist1 and AP-1 cooperatively upregulate integrin alpha5 expression to induce invasion and the epithelial-mesenchymal transition. Carcinogenesis 36, 327-337
19. D'Assoro AB, Haddad T and Galanis E (2015) Aurora-A kinase as a promising therapeutic target in cancer. Front Oncol 5, 295
20. D'Assoro, AB, Liu T, Quatraro C et al (2014) The mitotic kinase Aurora-A promotes distant metastases by inducing epithelial-to-mesenchymal transition in ERalpha(+) breast cancer cells. Oncogene 33, 599-610
21. Goos JA, Coupe VM, Diosdado B et al (2013) Aurora kinase A (AURKA) expression in colorectal cancer liver metastasis is associated with poor prognosis. Br J Cancer 109, 2445-2452
22. Steeghs N, Eskenis FA, Gelderblom H et al (2009) Phase I pharmacokinetic and pharmacodynamic study of the aurora kinase inhibitor danusertib in patients with advanced or metastatic solid tumors. J Clin Oncol 27, 5094-5101
23. Bauvois B (2012) New facets of matrix metalloproteinases MMP-2 and MMP-9 as cell surface transducers: outside-in signaling and relationship to tumor progression. Biochim Biophys Acta 1825, 29-36
24. Stetler-Stevenson WG (1999) Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention. J Clin Invest 103, 1237-1241
25. Nomura A, Majumder K, Giri B et al (2016) Inhibition of NF-kappa B pathway leads to deregulation of epithelial-mesenchymal transition and neural invasion in pancreatic cancer. Lab Invest 96, 1268-1278
26. Julien S, Puig I, Caretti E et al (2007) Activation of NF-kappaB by Akt upregulates Snail expression and induces epithelium mesenchyme transition. Oncogene 26, 7445-7456
27. Bakiri L, Macho-Maschler S, Cusic I (2013) Fra-1/AP-1 induces EMT in mammary epithelial cells by modulating Zeb1/2 and TGFβ expression. Cell Death Differ 22, 336-350