Compound K attenuates stromal cell-derived growth factor 1 (SDF-1)-induced migration of C6 glioma cells

Hyuck Kim1, Hyo Sun Roh2, Jai Eun Kim3, Sun Dong Park4, Won Hwan Park1 and Jin-Young Moon5

1Department of Diagnostics, College of Korean Medicine, Dongguk University, Goyang 10326, Korea
2Department of Acupoint, College of Korean Medicine, Dongguk University, Dongguk-Ro 32, Goyang 10326, Korea
3Department of Pathology, College of Korean Medicine, Dongguk University, Goyang 10326, Korea
4Department of Prescription, College of Korean Medicine, Dongguk University, Goyang 10326, Korea

BACKGROUND/OBJECTIVES: Stromal cell-derived growth factor 1 (SDF-1), also known as chemokine ligand 12, and chemokine receptor type 4 are involved in cancer cell migration. Compound K (CK), a metabolite of propanaxadiol-type ginsenoside by gut microbiota, is reported to have therapeutic potential in cancer therapy. However, the inhibitory effect of CK on SDF-1 pathway-induced migration of glioma has not yet been established.

MATERIALS/METHODS: Cytotoxicity of CK in C6 glioma cells was determined using an EZ-Cytox cell viability assay kit. Cell migration was tested using the wound healing and Boyden chamber assay. Phosphorylation levels of protein kinase C (PKC)α and extracellular signal-regulated kinase (ERK) were measured by western blot assay, and matrix metalloproteinases (MMP) were measured by gelatin-zymography analysis.

RESULTS: CK significantly reduced the phosphorylation of PKCα and ERK1/2, expression of MMP9 and MMP2, and inhibited the migration of C6 glioma cells under SDF-1-stimulated conditions.

CONCLUSIONS: CK is a cell migration inhibitor that inhibits C6 glioma cell migration by regulating its downstream signaling molecules including PKCα, ERK1/2, and MMPs.

Keywords: Panax ginseng, compound K, SDF-1, anti-tumor, C6 glioma

INTRODUCTION

Glioma is acknowledged as an incurable brain tumor, which has high propensity for invasion and cell migration [1,2]. Cancer migration occurs in response to stimuli such as growth factors and cytokines [3,4]. In particular, stromal cell-derived growth factor 1 (SDF-1) controls the enhanced migration and proliferation of glioma [5], and its receptor, chemokine receptor type 4 (CXCR4), is highly expressed in various cancer cells including melanoma, colon cancer, breast cancer, ovarian cancer, and glioma [5-9]. Interaction between CXCR4 and SDF-1 is crucial to tumor growth and metastasis [10-11].

SDF-1 and CXCR4 propagate a down-stream signal, activating phospholipase C (PLC) followed by activation of protein kinase C (PKC), which plays a key role in malignant cancer progression. PKC activation is associated with various cellular functions, including proliferation, adhesion, malignant transformation, and migration [12,13]. On the other hand, extracellular signal-regulated kinase (ERK), a down-stream target of SDF-1/CXCR4, is also involved in cell migration and proliferation [14]. Cancer motility is promoted by the activated matrix metalloproteinases (MMPs) under the influence of phosphorylated kinases.

Panax ginseng C.A. Meyer has been in use as an oriental herbal medicine for more than 2000 years [15]. The major functional component, saponin, otherwise known as ginsenoside, was reported to exhibit pharmacological properties including anti-inflammatory, anti-diabetic, and anti-tumor [16-18]. While more than 30 ginseng saponins have been identified thus far, their chemical structure renders their absorption difficult, when taken orally [19,20]. CK, 20-O-β-(d-glucopyranosyl)-20(S)-protopanaxadiol (Fig. 1A) is a metabolite of ginseng saponin, produced by intestinal bacteria after oral administration of ginseng. It was reported to have therapeutic potential in cancer therapy [21-24]. Hence, the purpose of this work was to determine the anti-migration property of CK and define the mechanism of its action through the suppression of wound-healing in SDF-1-stimulated C6 glioma cells. However, the effect of CK on SDF-1-induced migration of glioma cells remains unclear. Our study is the first to report evidence for CK-mediated regulation of C6 glioma cell migration through down-regulation of CXCR4 via the PKC/ERK-mediated pathway.
Fig. 1. Effect of CK on viability of C6 glioma cells. (A) Chemical structure of compound K (CK), [20-O-β-(D-glucopyranosyl)-20(S)-protopanaxadiol]. (B) C6 glioma cells were incubated in the presence or absence of CK (0.03 μM to 10 μM) in serum-depleted medium for 24 h. Bar graph indicates the effect of CK on C6 glioma cell viability. The values were expressed as mean ± SE of three independent experiments. * P < 0.05 vs. untreated C6 glioma cells.

MATERIALS AND METHODS

Chemicals

CK was purchased from BioMechatronic (Seoul, Korea). Cell culture materials and EZ-cytox cell viability assay kit were purchased from Gibco BRL (Gaithersburg, MD, USA) and Daeil Lab service (Seoul, Korea), respectively. SDF-1 was obtained from PeproTech Inc. (Rocky Hill, NJ, USA). Diff Quick stain was purchased from Sigma (St. Louis, MO, USA). PKCα and phospho-PKCα antibodies were obtained from Cell Signaling Technology Inc. (Beverly, MA, USA), ERK1/2 and phospho-ERK1/2 antibodies were obtained from Santa Cruz (Santa Cruz, MA, USA). All other chemicals were of analytical grade.

Cell culture and cell viability assay

Rat C6 glioma cells (Korean Cell Line Bank, Seoul, Korea) were cultured in DMEM medium (containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin) at 37°C, humidified with 5% atmospheric CO2. Cell viability analyses were performed as previously reported [25]. Briefly, C6 glioma cells (5 × 10^3 cells/well) were seeded and incubated in a 96-well micro-plate for 24 h. The cells were replaced with serum-free medium and incubated for the next 24 h. Subsequently, the cells were treated with different concentrations of CK (0.03 μM to 10 μM dissolved in 0.05% DMSO and diluted in serum-free medium), followed by re-incubation for another 24 h. Cell viability was measured using an EZ-cytox cell viability assay kit. Absorbance was determined using ELISA at a wavelength of 450 nm. Each assay was performed in triplicate.

Scratch wound healing assay

Scratch wound healing analyses were performed as previously reported [25]. Briefly, C6 glioma cells were plated in 6-well plates (2 × 10^5 cells) and incubated in medium containing 10% FBS. After the cells were grown to 70% confluence, the medium was replaced with serum-free medium and incubated for 24 h. A wound scratch was made on the monolayer of cultured cells across the center of each well using a 200 μL pipette tip. The cells were washed twice with phosphate-buffered saline for removal of non-adherent cells and subsequently treated with or without CK or SDF-1 (100 ng/ml) for 24 h. An image of the cells that had migrated into the cell-free wound scratch area was observed under an inverted microscope (Nikon, Tokyo, Japan) and photographed. The migration distance was measured using Image J software.

Boyden chamber assay

Boyden chamber assay was performed using a 48-well chemotaxis chamber (Neuro-Probe, Gaithersburg, MD, USA) to confirm the inhibitory effect of CK on migration of C6 glioma cells. Scratch wound healing analyses were performed as previously reported [25]. Briefly, C6 glioma cells were suspended in serum-free medium at a density of 1 × 10^5 cells/ml, and 50 μl of the cell suspension was loaded in the upper chamber wells. Treatment with SDF-1 with or without CK at different concentrations was performed in the lower chamber with incubation at 37°C and 5% CO2 atmosphere for 90 min. Cells on the lower surface of the membrane were fixed and stained using Diff Quick solutions. After staining, the migrated cells were photographed and counted under a microscope (Nikon, Tokyo, Japan). The migrated cells were measured using Image J software.

Western blot analysis

Protein levels were analyzed by western blotting as previously reported [25]. Briefly, the proteins (20 μg) were loaded, separated, and transferred to polyvinylidene difluoride membranes in transfer buffer. After blocking overnight at 4°C in 5% skim milk (dissolved in Tris-buffered saline buffer), the membrane was treated with Tris-buffered saline containing 0.1% Tween 20 (TBS-T) and washed three times. The membrane was incubated with phopho-ERK1/2 and phospho-PKCα, ERK1/2 and PKCα antibodies at 1:1000 dilutions. The membranes were washed with TBS-T, followed by incubation with a 1:1000 dilution of secondary antibody conjugated to horseradish peroxidase. The proteins were analyzed by a chemiluminescent reaction (ECL plus kit, Amersham Pharmacia Biotech, Buckinghamshire, UK); the bands were detected using Hyperfilm ECL (Amersham Pharmacia Biotech, Buckinghamshire, UK). Image J Software was used for visualization and quantification of proteins.

Gelatin-zymography

Gelatin-zymography analyses were performed as previously reported [26]. Briefly, the supernatant medium obtained from
SDF-1 with or without CK treated C6 cells was re-suspended in a sample buffer and loaded without boiling in 10% polyacrylamide gel containing 0.25% (w/v) gelatin. The supernatants were loaded and separated on running-gel. After electrophoresis, the gel was soaked in 2.5% Triton X-100 at room temperature and then washed in ddH2O. The gel was incubated at 37°C for 24 h and placed in a tank containing incubation buffer (50 mM Tris HCl, 0.15 M NaCl, and 10 mM CaCl2, pH 7.8). To determine the activity of bands, clear bands were visualized using destaining solution.

**Statistical analysis**

The results were expressed as the mean ± standard error (SE) from at least three independent experiments. Statistical significance was analyzed using Student’s t-test for each paired experiment using GraphPad Prism 4.0 software (version 4.00 for Windows, San Diego, CA, USA) with significance defined at 0.05 ($P < 0.05$).

**RESULTS**

**CK is non-cytotoxic to C6 Glioma cells at a minimal dose**

Cytotoxicity of CK in C6 glioma cells was determined by quantitative assessment of cell viability using the EZ-cytox cell viability assay kit. As shown in Fig. 1B, CK had no cytotoxic effect until the dose of 1 μM. However, CK exhibited cytotoxicity at higher concentrations (3 μM and 10 μM) (Fig. 1B).

**CK reduced scratch wound-healing in SDF-1-stimulated C6 cells**

The scratch wound-healing assay was performed to assess the inhibitory effect of CK on SDF-1-induced migration of C6 glioma cells. Scratched C6 glioma cells were incubated in CK (0.1 μM to 1 μM) in serum-free medium for 1 h, followed by stimulation with SDF-1 (100 ng/ml) for the next 24 h. As shown in Fig. 2, compared to control condition, SDF-1 (SDF-1 alone) induced cell migration to approximately 215%. SDF-1-induced migration of C6 glioma cells was inhibited by 0.3 μM and 1 μM CK to 76% and 88% of SDF-1 alone control (Fig. 2).

**CK inhibits migration of SDF-1-stimulated C6 cells**

Next, the inhibitory effect of CK on SDF-1-induced migration of C6 cells was tested using the Boyden chamber assay. As shown in Fig. 3, SDF-1 significantly induced the cell migration to 226.3% compared to the untreated group. In contrast, CK treatment attenuated the migration of SDF-1-stimulated C6 cells at a minimum dosage of 0.3 μM and the maximum inhibitory...
Fig. 4. Effect of CK on phosphorylation of PKCα and ERK1/2 in SDF-1-stimulated C6 glioma cells. C6 glioma cells were pretreated with CK (0.1 µM - 1 µM), followed by stimulation with SDF-1 (100 ng/ml) for an additional 24 h. (A) Expression levels of phospho-PKCα (P-PKCα), PKCα (T-PKCα), phospho-ERK1/2 (P-ERK1/2), and ERK1/2 (T-ERK1/2) were analyzed by western blotting. (B-C) The intensity of phosphorylation of PKCα and ERK1/2 was quantified by densitometry. The values were expressed as mean ± SE of three independent experiments. *P<0.05 vs. SDF-1 alone.

Fig. 5. The effect of CK on MMPs in SDF-1-stimulated C6 glioma cells. C6 glioma cells were incubated in serum-depleted medium for 24 h. Cells were pre-treated with CK (0.03 µM to 1 µM) for 1 h, followed by treatment with SDF-1 for 23 h. These supernatants were analyzed by gelatin-zymography analysis. (A) Expression of MMP9 and MMP2 in zymography gel. (B-C) The graph indicates the quantification of MMP2 and MMP9 by densitometry. The values are expressed as mean ± SE of three independent experiments. *P<0.05 vs. SDF-1 alone.

In the current study, we examined the anti-migratory effect of CK. Scratch wound-healing assay, Boyden chamber assay, western blot, and gelatin-zymography assay were performed in SDF-1-stimulated C6 glioma cells. Because gliomas have a tendency of rapid migration and invasion of the surrounding tissues, surgery and radio-irradiation methods are not viable options [27,28]. The severity of cancer is often aggravated by metastasis [2]. Therefore, targeting cancer migration is an anti-cancer strategy for glioma [25]. Our study demonstrated that SDF-1-induced migration of glioma was suppressed by CK. In oriental medicine, ginseng, derived from the root of P. ginseng Meyer, was used for centuries to cure both acute and malignant diseases. Ginsenoside, commonly known as saponin, is the major active component of this herbal medicine [19,20]. Although several reports have demonstrated that ginseng has a therapeutic effect on cancer, oral administration of this herb renders it difficult to absorb by the body [20]. Therefore, development of functional food is an alternative pharmacological approach [25]. Alternatively, a particular ginsenoside metabolite of ginseng, compound K (CK), was reported to be easily absorbed and metabolized by the human body [29].
metabolite of saponin produced during fermentation might be used in development of functional foods. Hence, based on its inhibitory effect of glioma migration, we suggest CK as a potential candidate for use in development of functional foods.

Numerous studies have demonstrated the interaction of chemokine SDF-1 to its receptor CXCR4 [30]. SDF-1 acts as the critical signaling factor, activating various cellular responses in tumor cells including angiogenesis, invasion, and migration [31-33]. Rapid transmission of these cellular signals in the brain is a key trait of glioma, potentiating malignant progression of brain tumor [1,26,27]. CK was reported to suppress cell growth in several types of cancer cells [23]. However, its pharmacological activity in the receptor signaling pathway is still unclear. In the current study, CK (1 μM) significantly inhibited SDF-1-induced C6 glioma migration in wound healing assay and Boyden chamber assay. Our data demonstrate that CK is an effective inhibitor of C6 glioma cell migration and has the potential for treatment of early stage or prevention of malignant progression of glioma.

Cancer cell migration and proliferation were mediated by activation of SDF-1/CXCR4 downstream signaling molecules, PKC and mitogen-activated protein kinase (MAPK) [33]. Stimulation with SDF-1 results in activation of PLC and formation of diacylglycerol (DAG); these pathways are known to activate PKC signaling [31]. Seven different isoforms of PKC, α, β1, β2, γ, δ, ε, η, θ, λ, and μ are present in cells and were implicated in diverse cellular functions [34]. Among members of the PKC superfamily, phosphorylation of PKCα regulates robust cell migration and malignant progression of glioma [35]. CXCR4 and SDF-1 exhibit a signal transduction mechanism, not only via PKCα phosphorylation but also via ERK1/2 phosphorylation [35]. ERK activity was reported to regulate migration and proliferation of diverse types of cancer cells, including glioma [32,36]. Our results indicate that 1 μM CK inhibits glioma cell migration through reduced phosphorylation of PKCα and ERK1/2. These results suggest that CK acts as a CXCR4 antagonist and inhibits SDF-1 induced C6 glioma cell migration by inhibiting CXCR4 receptor-mediated activation of PKCα and ERK1/2.

Increased expression of extracellular endopeptidases such as MMPs was observed in various cancers [25]. MMP 2 and 9 are involved in cell migration and proliferation, particularly in cancer [37]. Interaction between chemokine and MMPs induces the development of cancer. Thus, the design of this study involved down-regulation of both cell migration and MMPs as a therapeutic strategy for glioma. Our results indicate that CK (1 μM) significantly inhibited the expression of MMP2 and MMP9 in SDF-1-stimulated C6 glioma cells.

In conclusion, CK regulates SDF-1-stimulated C6 glioma migration. CK (1 μM) inhibited the migration of SDF-1-stimulated C6 glioma cells as demonstrated by the scratch wound-healing assay and Boyden chamber assay. CK treatment resulted in down-regulated phosphorylation of PKCα and ERK1/2, and expression of MMP2 and MMP9 in SDF-1-stimulated C6 glioma cells. These data suggest that CK inhibits SDF-1-stimulated C6 glioma cell migration by down-regulating the activation of PKCα and ERK1/2. The crucial observation of our study is that CK can alter the downstream signaling of the CXCR4/CXCR12 pathway in C6 glioma cells. Further studies are required to determine the CK-derived mechanism to the blood brain barrier as their structure means transcellular diffusion will be limited. The cellular mechanisms of CK derived to brain via blood brain barrier remain to be elucidated.

REFERENCES

1. Amberger VR, Hensel T, Ogata N, Schwab ME. Spreading and migration of human glioma and rat C6 cells on central nervous system myelin in vitro is correlated with tumor malignancy and involves a metalloproteolytic activity. Cancer Res 1998;58:149-58.
2. Chintala SK, Rao JK. Invasion of human glioma: role of extracellular matrix proteins. Front Biosci 1996;1:d324-39.
3. Stock AM, Hahn SA, Troost G, Niggemann B, Zänker KS, Entschladen F. Induction of pancreatic cancer cell migration by an autocrine epidermal growth factor receptor activation. Exp Cell Res 2014;326:307-14.
4. Lippitz BE. Cytokine patterns in patients with cancer: a systematic review. Lancet Oncol 2013;14:e218-28.
5. Bajetto A, Barbieri F, Dorcaratto A, Barbero S, Daga A, Porcile C, Ravetti JL, Zona G, Spaziante R, Corte G, Schettini G, Florio T. Expression of CXC chemokine receptors 1-5 and their ligands in human glioma tissues: role of CXCR4 and SDF1 in glioma cell proliferation and migration. Neurochem Int 2006;49:423-32.
6. Di Cesare S, Marshall JC, Fernandes BF, Logan P, Antecka E, Filho VB, Burnier MN Jr. In vitro characterization and inhibition of the CXCR4/CXCL12 chemokine axis in human uveal melanoma cell lines. Cancer Cell Int 2007;7:17.
7. Heckmann D, Maier P, Laufs S, Wenz F, Zeller WJ, Friehauff S, Allgayer H. CXCR4 expression and treatment with SDF-1α or Plerixafor modulate proliferation and chemosensitivity of colon cancer cells. Transl Oncol 2013;6:124-32.
8. Luker KE, Lewin SA, Mihalko LA, Schmidt BT, Winkler JS, Coggins NL, Thomas DG, Luker GD. Scavenging of CXCL12 by CXCR7 promotes tumor growth and metastasis of CXCR4-positive breast cancer cells. Oncogene 2012;31:4750-8.
9. Ray P, Lewin SA, Mihalko LA, Schmidt BT, Luker KE, Luker GD. Noninvasive imaging reveals inhibition of ovarian cancer by targeting CXCL12-CXCR4. Neoplasia 2011;13:1152-61.
10. Raman D, Baugher PJ, Thu YM, Richmond A. Role of chemokines in tumor growth. Cancer Lett 2007;256:137-65.
11. Iwasa S, Yanagawa T, Fan J, Katoh R. Expression of CXCR4 and its ligand SDF-1 in intestinal-type gastric cancer is associated with lymph node and liver metastasis. Anticancer Res 2009;29:4751-8.
12. Lahn MM, Sundell KL, Paterson BM. The role of protein kinase C-alpha in malignancies of the nervous system and implications for the clinical development of the specific PKC-alpha inhibitor aproncaren (Review). Oncol Rep 2004;11:515-22.
13. Kolvenbus J, Aaltonen V, Peltonen J. Protein kinase C (PKC) family in cancer progression. Cancer Lett 2006;235:1-10.
14. Troppeimair J, Bruder JT, Munoz H, Lloyd PA, Kyriakis J, Banerjee P, Avruch J, Rapp UR. Mitogen-activated protein kinase/extracellular signal-regulated protein kinase activation by oncogenes, serum, and 12-O-tetradecanoylphorbol-13-acetate requires Raf and is necessary for transformation. J Biol Chem 1994;269:7030-5.
15. Lee DC, Lau AS. Effects of Panax ginseng on tumor necrosis factor-α-mediated inflammation: a mini-review. Molecules 2011;16:2802-16.
16. Paul S, Shin HS, Kang SC. Inhibition of inflammations and...
macrophage activation by ginsenoside-Re isolated from Korean ginseng (Panax ginseng C.A. Meyer). Food Chem Toxicol 2012;50: 1354-5.

17. Vukan V, Sung MK, Sievenpiper JL, Stavro PM, Jenkins AL, Di Buono M, Lee KS, Leiter LA, Nam KY, Amason JT, Choi M, Naeem A. Korean red ginseng (Panax ginseng) improves glucose and insulin regulation in well-controlled, type 2 diabetes: results of a randomized, double-blind, placebo-controlled study of efficacy and safety. Nutr Metab Cardiovasc Dis 2008;18:46-56.

18. Hofseth LJ, Wargovich MJ. Inflammation, cancer, and targets of ginseng. J Nutr 2007;137 Suppl:183S-185S.

19. Yuan CS, Wang CZ, Wicks SM, Qi LW. Chemical and pharmacological studies of saponins with a focus on American ginseng. J Ginseng Res 2010;34:160-7.

20. Qi LW, Wang CZ, Du GJ, Zhang ZY, Calway T, Yuan CS. Metabolism of ginseng and its interactions with drugs. Curr Drug Metab 2011;12:818-22.

21. Chen Y, Xu Y, Zhu Y, Li X. Anti-cancer effects of ginsenoside compound K on pediatric acute myeloid leukemia cells. Cancer Cell Int 2013;13:24.

22. Wang CZ, Kim KE, Du GJ, Qi LW, Wen XD, Li P, Bauer BA, Bissonnette MB, Musch MW, Chang EB, Yuan CS. Ultra-performance liquid chromatography and time-of-flight mass spectrometry analysis of ginsenoside metabolites in human plasma. Am J Chin Med 2011;39: 1161-71.

23. Song G, Guo S, Wang W, Hu C, Mao Y, Zhang B, Zhang H, Hu T. Intestinal metabolite compound K of ginseng saponin potently attenuates metastatic growth of hepatocellular carcinoma by augmenting apoptosis via a Bid-mediated mitochondrial pathway. J Agric Food Chem 2010;58:12753-60.

24. Chae S, Kang KA, Chang WY, Kim MJ, Lee SJ, Lee YS, Kim HS, Kim DH, Hyun JW. Effect of compound K, a metabolite of ginseng saponin, combined with gamma-ray radiation in human lung cancer cells in vitro and in vivo. J Agric Food Chem 2009;57:5777-82.

25. Lee KP, Choi NH, Kim JT, Park IS. The effect of yacon (Samilanthus sonchifolius) ethanol extract on cell proliferation and migration of C6 glioma cells stimulated with fetal bovine serum. Nutr Res Pract 2015;9:256-61.

26. Kobayashi T, Hattori S, Shinkai H. Matrix metalloproteinases-2 and -9 are secreted from human fibroblasts. Acta Derm Venereol 2003;83:105-7.

27. Chinot OL, Macdonald DR, Abrey LE, Zahlmann G, Kerloëguen Y, Cloughesy TF. Response assessment criteria for glioblastoma: practical adaptation and implementation in clinical trials of antiangiogenic therapy. Curr Neurol Neurosci Rep 2013;13:347.

28. Hess KR, Broglio KR, Bondy ML. Adult glioma incidence trends in the United States, 1977-2000. Cancer 2004;101:2293-9.

29. Paek IB, Moon Y, Kim J, Ji HY, Kim SA, Sohn DH, Kim JB, Lee HS. Pharmacokinetics of a ginseng saponin metabolite compound K in rats. Biopharm Drug Dispos 2006;27:39-45.

30. Valenzuela-Fernández A, Planchenault T, Balex F, Staropoli I, Le-Barillec K, Leduc D, Delaunay T, Lazarini F, Virelizier JL, Chignard M, Picard D, Arenzana-Seisdedos F. Leukocyte elastase negatively regulates Stromal cell-derived factor-1 (SDF-1)/CXCR4 binding and functions by amino-terminal processing of SDF-1 and CXCR4. J Biol Chem 2002;277:15677-89.

31. Burger JA, Kipps TJ. CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. Blood 2006;107:1761-7.

32. Hattermann K, Mentein R. An infernal trio: the chemokine CXCL12 and its receptors CXCR4 and CXCR7 in tumor biology. Ann Anat 2013;195:103-10.

33. Mukherjee D, Zhao J. The role of chemokine receptor CXCR4 in breast cancer metastasis. Am J Cancer Res 2013;3:46-57.

34. Ehtesham M, Min E, Issar NM, Kasl RA, Khan IS, Thompson RC. The role of the CXCR4 cell surface chemokine receptor in glioma biology. J Neurooncol 2013;113:153-62.

35. do Carmo A, Patricio I, Cruz MT, Carvalheiro H, Oliveira CR, Lopes MC. CXCL12/CXCR4 promotes motility and proliferation of glioma cells. Cancer Biol Ther 2010;9:56-65.

36. Jung SH, Woo MS, Kim SY, Kim WK, Hyun JW, Kim EJ, Kim DH, Kim HS. Ginseng saponin metabolite suppresses phorbol ester-induced matrix metalloproteinase-9 expression through inhibition of activator protein-1 and mitogen-activated protein kinase signaling pathways in human astroglia cells. Int J Cancer 2006;118:490-7.

37. Fang W, Li H, Kong L, Niu G, Gao Q, Zhou K, Zheng J, Wu B. Role of matrix metalloproteinases (MMPs) in tumor invasion and metastasis: serial studies on MMPs and TIMPs. Beijing Da Xue Xue Bao 2003;35:441-3.