Inhibition of PKCα Activation in Human Bone and Soft Tissue Sarcoma Cells by the Selective PKC Inhibitor PKC412

TERUYA KAWAMOTO[1], TOSHIHIRO AKISUE[1], KENTA KISHIMOTO[1], HITOMI HARA[1], MASAYA IMABORI[1], TAKUYA FUJIMOTO[1], MASAHIRO KUROSAKA[1], TOSHIAKI HITORA[2], YOJI KAWAGUCHI[2] and TETSUJI YAMAMOTO[2]

[1]*Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine Chuo-ku, Kobe, Hyogo;*
[2]*Department of Orthopaedic Surgery, Kagawa University School of Medicine Miki-cho, Kida-gun, Kagawa, Japan*

Abstract. *Background: PKC412, formerly CGP41251, N-benzoylstaurosporine, was initially developed as a selective protein kinase C (PKC) inhibitor, and it preferentially inhibits conventional PKC family members. In this study, the expression of PKCα was examined in human osteosarcoma and MFH cell lines, and the inhibitory effect of PKC412 on the proliferation of the cell lines was evaluated. Materials and Methods: Three human osteosarcoma cell lines (KTHOS, MG63 and KHOS) and four human MFH cell lines (TNMY1, GBS-1, Nara-F and Nara-H) were used. The expression of PKCα and phosphorylated PKCα were analyzed using both Western blotting analysis and immunocytochemical analysis. The effect of PKC412 on cell proliferation was evaluated using the MTS assay technique. Results: PKC412 inhibited cell proliferation of all seven cell lines in a dose- and time-dependent manner. Both Western blotting analysis and immunocytochemical analysis revealed that not only PKCα but also phosphorylated PKCα were expressed in all cell lines incubated with the culture medium without any stimuli. PKC412 suppressed phosphorylation of PKCα in all cell lines at a concentration of 1 μM. Conclusion: The inhibition of cell proliferation of the human osteosarcoma and MFH cell lines by PKC412 might be due to reduced PKCα activity. This suggests PKC412 might be a potent chemotherapeutic agent for human sarcomas.*

Osteosarcoma (OS) is the most common type of malignant bone tumor, accounting for 30% of primary bone malignancies. Malignant fibrous histiocytoma (MFH) is the most common high-grade soft tissue sarcoma in late adult life. Advances in treatment of both OS and MFH have led to multidisciplinary treatment including surgery, chemotherapy, and radiation therapy, resulting in great improvements in quality of life for patients with sarcomas. However, the present chemotherapeutic protocols for sarcomas are not as effective as in other malignancies, thus the prognosis of patients with sarcomas can sometimes be poor due to local recurrence and metastases (1, 2).

Molecular targeting drugs have been developed for other human malignancies including cancer and hematopoietic malignancies in recent years. These drugs have selective inhibitory effects on factors relating to tumor proliferation, migration and metastasis, including growth factor receptors and intracellular signaling factors. It has been previously proposed that overexpression and unusual activation of the growth factors and intracellular signaling factors may be related to tumor proliferation, migration and metastasis in OS (3) and MFH (4-8) as well as other malignancies, including small cell lung cancer (SCLC) (9), gastrointestinal stromal tumor (GIST) (10) and pancreatic cancer (11).

Protein kinase C (PKC) is a family of serine- and threonine-specific protein kinases consisting of three subfamilies: conventional (or classical), novel and atypical subspecies. PKC family members are involved in the process of dysregulation in cancer cells and dysregulated PKC activity has led to carcinogenesis and tumor progression in a variety of human malignancies (12), including rhabdomyosarcoma (13), OS (14) and fibrosarcoma (15). PKCα, one of the conventional PKC subfamily members, is widely expressed in tissues and dysregulation of PKCα has been reported in many malignant tumor cell lines (16, 17).

PKC412, formerly CGP41251, *N*-benzoylstaurosporine, was initially developed as a selective PKC inhibitor (18, 19). It preferentially inhibits the conventional PKC subfamily members (PKCα, PKCβ and PKCγ), also inhibiting the receptor tyrosine kinases, including fms-related tyrosine kinase 3 (FLT3), platelet-derived growth factor receptor (PDGFR), the receptor for the stem cell factor (c-kit), and

Correspondence to: Toshihiro Akisue, MD, Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650-0017, Japan. Tel: +81783825985, Fax: +81783516944, e-mail: akisue@med.kobe-u.ac.jp

Key Words: PKC412, PKCα, molecular targeting therapy, sarcoma, osteosarcoma, malignant fibrous histiocytoma (MFH).

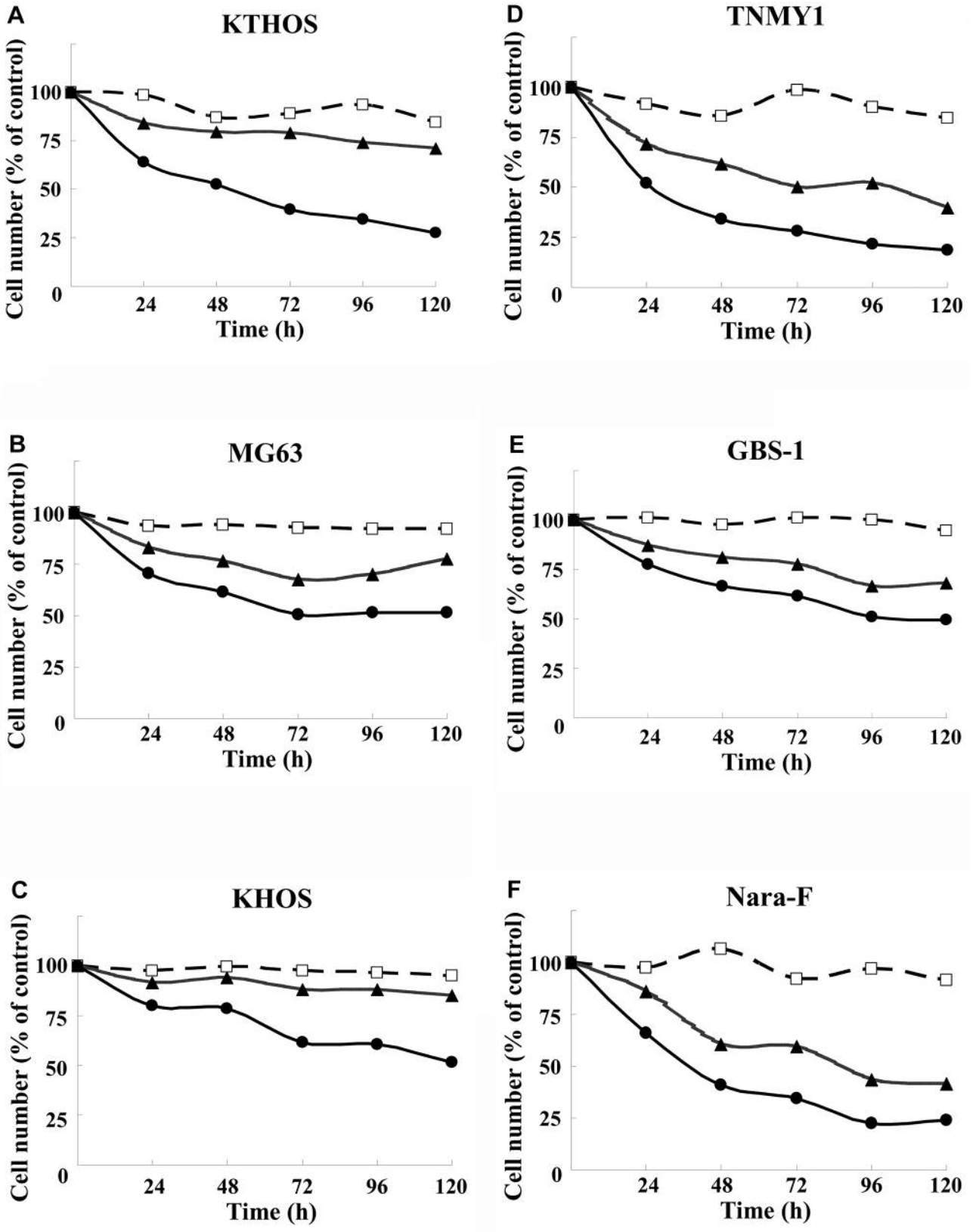

Figure 1. *continued*

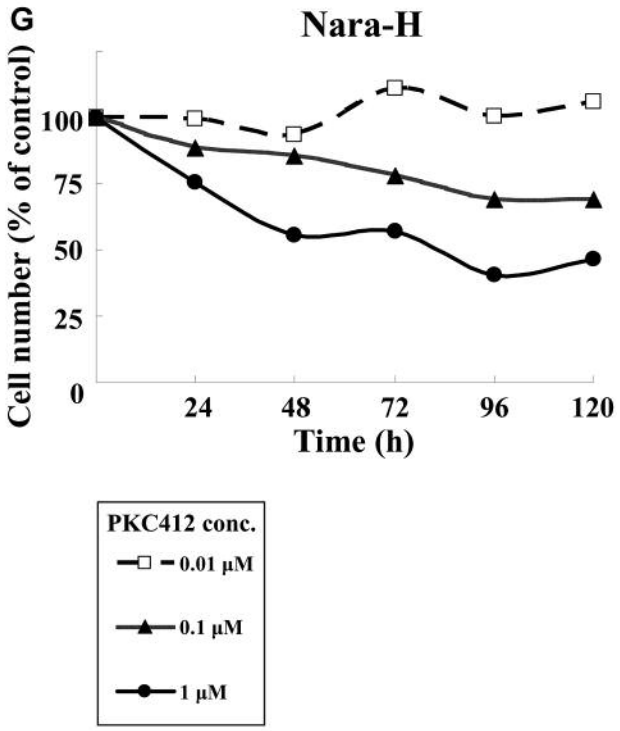

Figure 1. *Dose- and time-dependent inhibitory effects of PKC412 on cell proliferation of all seven cell lines, A, KTHOS; B, MG63; C, KHOS; D, TNMY1; E, GBS-1; F, Nara-F and G, Nara-H. PKC412 significantly inhibited cell proliferation of KTHOS, TNMY1, Nara-F, and Nara-H at a concentration (conc.) of 1 μM (●). PKC412 at 0.1 μM (▲) inhibited the cell proliferation of TNMY1 and Nara-F to less than 50% of control cell viability after 96 h of culture. The inhibitory effects on cell proliferation by PKC412 in MG63, KHOS and GBS-1 were less than those in KTHOS, TNMY1, Nara-F, and Nara-H. PKC412 inhibited cell proliferation to less than 50% of control cell viability at a concentration of 1 μM.*

the kinase insert domain receptor (KDR; vascular endotherial growth factor receptor (VEGFR) 2). Clinical trials of PKC412 have been studied in acute myelomonocytic leukemia (AML) targeting FLT3, which is activated by mutations in about 30% of AML. In addition, *in vitro* and *in vivo* studies have demonstrated that PKC412 also inhibits cell proliferation of human malignancies, including NSCLC (20, 21), ovarian cancer (22), multiple myeloma (MM) (23), prostate cancer (24) and malignant melanoma (25). However, the effect of PKC412 on human sarcomas has not been studied either experimentally or clinically. We therefore hypothesize that conventional PKCα will contribute to the tumor growth of human sarcomas and further that PKC412 has an inhibitory effect on the growth of human sarcomas. We examined the expression of PKCα in human OS and MFH cell lines and the inhibitory effects of PKC412 on proliferation of cell lines *in vitro*.

Materials and Methods

Cell lines. Three human OS cell lines (KTHOS, MG63 and KHOS) and four human MFH cell lines (TNMY1, GBS-1, Nara-F and Nara-H) were used in this study. KTHOS and TNMY1 were previously established in our laboratory (7, 26). GBS-1 was provided by Dr. H. Kanda (Department of Pathology, The Cancer Institute of the Japanese Foundation for Cancer Research, Tokyo, Japan) (27). Nara-F and Nara-H were purchased from ScienStuff Co., Nara, Japan (28). All cell lines were grown in culture medium consisting of Dulbecco's modified Eagle's medium (DMEM) with glutamine (Sigma-Aldrich Co., St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich) and penicillin/streptomycin solution (Sigma-Aldrich). The cell lines were routinely maintained at 37ïC in a humidified 5% CO_2 atmosphere.

Reagents. PKC412 was donated by Novartis Pharrma AG (Basel, Switzerland). Stock solution was prepared in dimethylsulfoxide (DMSO) and diluted to the required concentrations in the culture medium before use.

Inhibitory effect of PKC412 on cell proliferation of human sarcoma cell lines. Cell proliferation was determined by MTS assay with CellTiter 96® AQueous One Solution Reagent (Promega, Madison, WI, USA). Cells were trypsinized and seeded at a density of approximately $5x10^3$ cells/well in 96-well cell culture plates in 100 μl culture medium with 10% FBS. After 48 hours (h), the medium was refreshed with 1% FBS containing PKC412 at the indicated concentrations (0, 0.01, 0.1 and 1 μM). After 24, 48, 72, 96, and 120 h, the medium was removed and washed with phosphate-buffered saline (PBS), then refreshed with fresh medium containing MTS reagent (100 μl medium without FBS plus 20 μl MTS reagent/well). The optical density was measured at 490 nm using an automatic microplate reader, SpectraMax Plus384 (Molecular Devices, Sunnyvale, CA, USA) after 2 h of further incubation at 37ïC in a humidified atmosphere of 5% CO_2. The percentage viability of each well was calculated. At least three independent cultures were performed for each study.

Immunocytochemical analysis. Cells were trypsinized and seeded at a density of approximately $15x10^3$ cells/well in 4-well cell chamber slides in 500 μl culture medium with 10% FBS. After 48 h, cells were treated for 1 h with 1% FBS containing PKC412 at the indicated concentrations (0, 0.1 and 1 μM), and then fixed for 30 minutes with 4% paraformaldehyde (Sigma) in PBS. Endogenous peroxidase was blocked using 3% H_2O_2. Slides were incubated with a 1:100 dilution of primary monoclonal mouse anti-PKCα (clone M4) (Upstate Biotechnology, Lake Placid, NY, USA) and primary polyclonal rabbit anti-phospho-PKCα (p-PKCα) (Ser657) (Upstate Biotechnology) overnight at 4ïC. Slides were rinsed three times with PBS and incubated with secondary antibody with Histofine simple stain MAX-PO(M) (NICHIREI BIOSCIENCES, Tokyo, Japan) for primary mouse antibody and Histofine simple stain MAX-PO(R) (NICHIREI BIOSCIENCES) for primary rabbit antibody for 30 minutes at room temperature, and then, stained with DAB chromogen (Simple stain DAB; NICHIREI BIOSCIENCES).

Western blotting analysis. Cells were trypsinized and seeded at a density of approximately $1x10^6$ cells/well in 60 mm cell culture dishes in 3 ml culture medium with 10% FBS. After 48 h, cells were treated for 1 h with 1% FBS containing PKC412 at the indicated

concentrations (0 and 1 μM). Whole cell lysates were collected for protein content and were separated by SDS polyacrylamide gel electrophoresis under reducing conditions. Gels were electrophoretically transferred to PVDF membranes and then membranes were blocked for 1 h and incubated with a 1:500 dilution of primary monoclonal mouse anti-PKCα (clone M4) (Upstate Biotechnology) and primary polyclonal rabbit anti-phospho-PKCα (p-PKCα) (Ser657) (Upstate Biotechnology) overnight at 4ïC. Dilutions (1:10000) of anti-mouse or anti-rabbit IgG, HRP-linked whole antibody (GE Healthcare Bio-Sciences, Piscataway, NJ, USA) were used as secondary antibodies. Bound antibodies were detected using the ECL Plus Western blotting detection system (GE Healthcare Bio-Sciences).

Statistical analysis. The data were analyzed statistically using ANOVA with Fisher's PLSD *post hoc* test. A value of $p<0.05$ was regarded as statistically significant.

Results

Inhibitory effect of PKC412 on cell proliferation of human sarcoma cell lines. PKC412 showed a dose- and time-dependent inhibitory effect on all seven cell lines. PKC412 significantly inhibited cell proliferation of KTHOS, TNMY1, Nara-F and Nara-H at a concentration of 1 μM to less than 50% of control cell viability. In paticular, 0.1 μM PKC412 inhibited cell proliferation of TNMY1 and Nara-F to less than 50% of control cell viability after 96 h of culture. The inhibitory effect on cell proliferation by PKC412 in MG63, KHOS and GBS-1 was lower than that in the other four cell lines (KTHOS, TNMY1, Nara-F and Nara-H). The inhibition rate of cell proliferation by PKC412 was less than 50% in MG63, KHOS, and GBS-1 at a concentration of 1 μM (Figure 1 A-G).

Immunocytochemical analysis. Both PKCα and phosphorylated-PKCα (p-PKCα) were detected immunocytochemically in all cell lines incubated with the culture medium without any stimuli. Expression of p-PKCα was suppressed to some extent in all cell lines incubated for 1 h with the medium containing 1 μM PKC412 (Figure 2).

Western blotting analysis. Western blotting revealed that not only PKCα, but also p-PKCα, was expressed in all human sarcoma cell lines incubated with the culture medium without any stimuli. Expression of p-PKCα was suppressed in all cell lines incubated for 1 h with the medium containing 1 μM PKC412 (Figure 3).

Discussion

Bone and Soft tissue sarcomas, especially high-grade sarcomas such as MFH, are clinically aggressive and have a high metastatic behavior to various organs. Although many chemotherapeutic protocols including doxorubicin, cisplatin and ifosfamide are used for human sarcomas, protocols for sarcomas have been ineffective and the prognosis of patients can be extremely poor due to local recurrence and metastases (1, 2).

Recently, molecular targeting drugs have been developed for human cancer and hematopoietic malignancies. Previous clinical studies for molecular targeting drugs have also shown remarkable antitumoral effects on human malignancies. Molecular targeting drugs have selective inhibitory effects on factors related to tumor proliferation, migration, and metastasis. It was previously proposed that overexpression and unusual activation of these factors occurs in human malignancies including OS (3) and MFH (4) as well as in SCLC (9), GIST (10) and pancreatic cancer (11). We also reported the expression of growth factors and their receptors in sarcomas in both *in vitro* and *in vivo* studies (5-8). Several studies have demonstrated the antitumoral effects of molecular targeting drugs on human sarcoma cells *in vitro* and *in vivo* (3, 10, 11, 29). In our previous studies, STI571 inhibited cell proliferation of human MFH cell lines that expressed PDGFRs and/or c-kit in a dose- and time-dependent mannar *in vitro* (30) and STI571 also inhibited tumorigenicity of MFH cells *in vivo* (31). This suggests the antitumoral effects of molecular targeting drugs might be expected to improve the chemotherapy of human sarcomas.

PKC is a family of serine- and threonine-specific protein kinases consisting of at least 12 subspecies. These subspecies are categorized into three subfamilies based on their second messenger requirements: conventional (or classical; α, β and γ), novel (δ, ε, η and θ), and atypical (μ, ξ and ι). PKC family members are involved in various processes in cancer cells, including growth factor-mediated signaling, cell cycling and topoisomerase activity. Dysregulated PKC activity has led to carcinogenesis and tumor progression in a variety of human malignancies (12), including rhabdomyosarcoma (13), OS (14) and fibrosarcoma (15). PKCα, one of the conventional PKC subfamily members, is widely expressed in tissues and involved in the regulation, transduction and signaling leading to cell proliferation and differentiation. Dysregulation and overexpression of PKCα has been found in many malignant tumor cell lines (16, 17). Dysregulation and overexpression of PKCα are thought to be linked to invasion, proliferation, drug resistance and genetic instability. The introduction of oncogenic *Ras* to keratinocytes has been shown to up-regulate PKCα (32) and p53 signaling has been linked to regulation of PKCα expression (33). PKCα activity might be associated with multi-drug resistance since activated PKCα increases P-glycoprotein stability and expression (34). Therefore, PKCα has been recognized as an inducer of cell proliferation and suppressor of apoptosis, suggesting that PKCα could be a reasonable target for molecular targeting therapy (35).

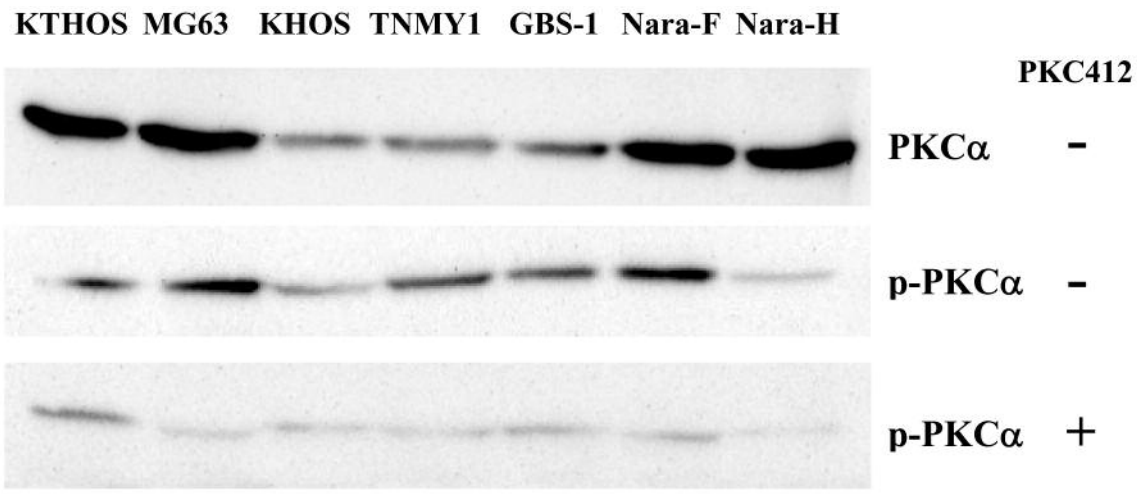

Figure 2. *p-PKCα was detected immunohistochemically in all cell lines. Expression of p-PKCα was suppressed in all cell lines treated for 1 h with the medium containing 1 μM PKC412.*

PKC412, formerly CGP41251, *N*-benzoylstaurosporine, is a novel molecular targeting drug. It was initially developed as a selective protein kinase C (PKC) inhibitor. It preferentially inhibits the conventional PKC subfamilies including PKCα. PKC412 has antitumoral properties in *in vitro* and *in vivo* cancer models (20-25). Clinical trials of PKC412 targeting FLT3 in AML have been studied.

In this study, we demonstrated that PKCα is consistently expressed in human OS and MFH cell lines, and that PKC412 inhibits the cell proliferation of these cell lines *in vitro*. It was also revealed that PKCα is consistently activated in human OS and MFH cell lines and that PKC412 was able to suppress PKCα activation in these cell lines. Several studies have reported that PKCα is dysregulated and overexpressed in various human malignancies (16, 17) and that PKC412 has antitumoral effects on cell proliferation (20-25).

Ikegami *et al.* examined the antitumoral effect of PKC412 on two kinds of human NSCLC cell lines (adenocarcinoma A549 and squamous cell carcinoma NCI-H520). They reported that 0.5 or 1.0 μM PKC412 inhibited the cell proliferation of these cell lines *in vitro* and that 200 mg/kg PKC412 suppressed tumor growth in nude mice xenografts (21). Sharkey *et al.* reported that PKC412 induced JNK-dependent apoptosis in both the human multiple myeloma (MM) cell line and primary MM cells, and that the apoptotic effect of PKC412 was enhanced by NFκB inhibition (23). Nakamura *et al.* demonstrated that PKC412 inhibited the invasiveness of melanoma cells *in vitro* and also showed the anti-metastatic effect *in vivo* using a spontaneous metastatic mouse model with B16-BL6 melanoma cells (25). In the recent studies, the anti-proliferative or the anti-metastatic effects of PKC412 have been shown in human sarcoma cell lines at concentrations from 0.5 to 50 μM. In completed phase I clinical trials of PKC412 (36-38), Propper *et al.* reported that mean plasma concentrations after administration of PKC412 ranging from 12.5 to 300 mg/day were in the range 0.3 to 7 μM, and thus 150 mg/day is suitable for a phase II trial (36). In the present study, we demonstrated that PKC412 inhibited the cell proliferation of human sarcoma cell lines at a concentration of 1 μM *in vitro*. However, for the clinical application of PKC412, it is necessary to evaluate the antitumoral effect of PKC412 on human bone and soft tissue sarcoma cells *in vivo*.

In conclusion, the current study demonstrated dose- and time-dependent inhibitory effects of PKC412 on cell proliferation of human OS and MFH cell lines *in vitro*, in which PKCα, a target of PKC412, is consistently activated. PKC412 can be a potent chemotherapeutic agent for human sarcomas, although the selection of targets is important in molecular targeting therapy. Further studies are needed to explore the precise molecular mechanisms of the inhibitory effect of PKC412 on the cell proliferation in human sarcoma cells.

References

1 Papai Z, Bodoky G, Szanto J, Poller I, Rahoty P, Eckhardt S, Lang I and Szendroi M: The efficacy of a combination of etoposide, ifosfamide, and cisplatin in the treatment of patients with soft tissue sarcoma. Cancer *89*: 177-180, 2000.

2 Spira AI and Ettinger DS: The use of chemotherapy in soft-tissue sarcomas. Oncologist *7*: 348-359, 2002.

3 McGary EC, Weber K, Mills L, Doucet M, Lewis V, Lev DC, Fidler IJ and Bar-Eli M: Inhibition of platelet-derived growth factor-mediated proliferation of osteosarcoma cells by the novel tyrosine kinase inhibitor STI571. Clin Cancer Res *8*: 3584-3591, 2002.

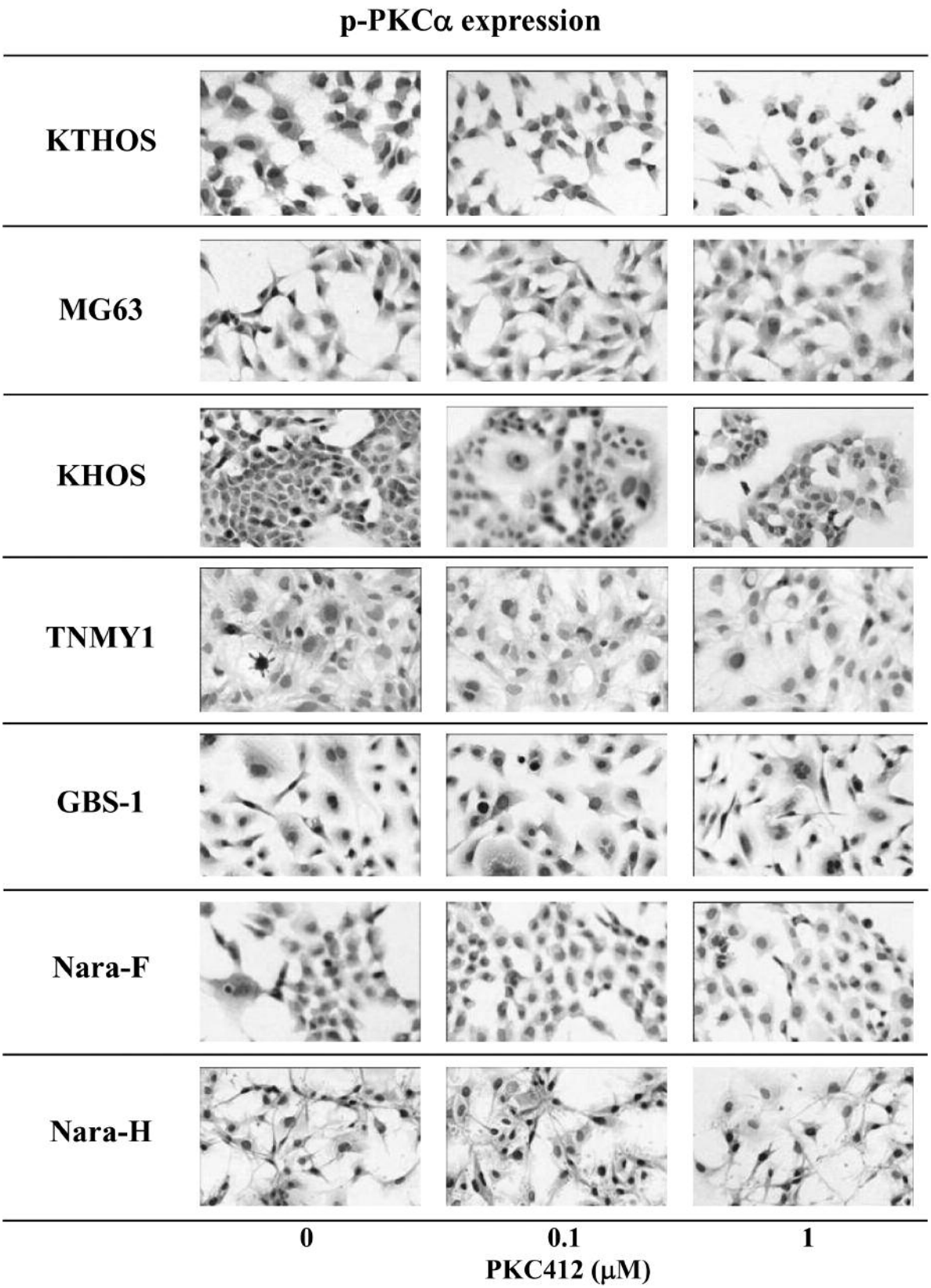

Figure 3. *Western blotting analysis. Both PKCα and p-PKCα were expressed in all human sarcoma cell lines without any stimuli, suggesting that PKCα was consistently activated in all seven cell lines. Expression of p-PKCα in all seven cell lines was suppressed at one hour after treatment with 1 μM PKC412.*

4 Abdiu A, Walz TM, Nishikawa BK, Wingren S, Larsson SE and Wasteson A: Human malignant fibrous histiocytomas *in vitro*: growth characteristics and their association with expression of mRNA for platelet-derived growth factor, transforming growth factor-alpha and their receptors. Eur J Cancer *34*: 2094-2100, 1998.

5 Yamamoto T, Akisue T, Marui T, Fujita I, Matsumoto K, Kawamoto T, Hitora T, Nakatani T, Nagira K and Kurosaka M: Immunohistochemical analysis of platelet-derived growth factor and its receptors in soft tissue malignant fibrous histiocytoma. Anticancer Res *23*: 4325-4328, 2003.

6 Nakatani T, Marui T, Yamamoto T, Hitora T, Akisue T, Kawamoto T, Nagira K, Fujita I, Matsumoto K, Yoshiya S and Kurosaka M: Expression of stem cell factor and c-kit in human malignant fibrous histiocytoma cell line (TNMY1). Anticancer Res *23*: 2329-2333, 2003.

7 Hitora T, Yamamoto T, Akisue T, Marui T, Nakatani T, Kawamoto T, Nagira K, Yoshiya S and Kurosaka M: Establishment and characterization of a KIT-positive and stem cell factor-producing cell line, KTHOS, derived from human osteosarcoma. Pathol Int *55*: 41-47, 2005.

8 Hara H, Akisue T, Fujimoto T, Imabori M, Kawamoto T, Kuroda R, Fujioka H, Yamamoto T, Doita M and Kurosaka M; Expression of VEGF and its receptors and angiogenesis in bone and soft tissue tumors. Anticancer Res *26*: 4307-4311, 2006.

9 Hibi K, Takahashi T, Sekido Y, Ueda R, Hida T, Ariyoshi Y, Takagi H and Takahashi T: Coexpression of the stem cell factor and the c-kit genes in small-cell lung cancer. Oncogene *6*: 2291-2296, 1991.

10 Bauer S, Corless CL, Heinrich MC, Dirsch O, Antoch G, Kanja J, Seeber S and Schutte J: Response to imatinib mesylate of a gastrointestinal stromal tumor with very low expression of KIT. Cancer Chemother Pharmacol *51*: 261-265, 2003.

11 Hwang RF, Yokoi K, Bucana CD, Tsan R, Killion JJ, Evans DB and Fidler IJ: Inhibition of platelet-derived growth factor receptor phosphorylation by STI571 (Gleevec) reduces growth and metastasis of human pancreatic carcinoma in an orthotopic nude mouse model. Clin Cancer Res *9*: 6534-6544, 2003.

12 Koivunen J, Aaltonen V and Peltonen J: Protein kinase C (PKC) family in cancer progression. Cancer Lett *235*: 1-10, 2006.

13 Hanania N, Lezenes JR and Castagna M: Tumorigenicity-associated expression of protein kinase C isoforms in rhabdomyosarcoma-derived cells. FEBS Lett *303*: 15-18, 1992.

14 Geng WD, Boskovic G, Fultz ME, Li C, Niles RM, Ohno S and Wright GL: Regulation of expression and activity of four PKC isozymes in confluent and mechanically stimulated UMR-108 osteoblastic cells. J Cell Physiol *189*: 216-228, 2001.

15 Keller HU, Hunziker IP, Sordat B, Niggli V and Sroka J: Protein kinase C isoforms involved in regulation of cell shape and locomotion of human fibrosarcoma HT1080 cells. Int J Cancer *88*: 195-203, 2000.

16 Carey I, Williams CL, Ways DK and Noti JD: Overexpression of protein kinase C-alpha in MCF-7 breast cancer cells results in differential regulation and expression of alphavbeta3 and alphavbeta5. Int J Oncol *15*: 127-136, 1999.

17 Stewart JR and O'Brian CA: Protein kinase C-alpha mediates epidermal growth factor receptor transactivation in human prostate cancer cells. Mol Cancer Ther *4*: 726-732, 2005.

18 Fabbro D, Buchdunger E, Wood J, Mestan J, Hofmann F, Ferrari S, Mett H, O'Reilly T and Meyer T: Inhibitors of protein kinases: CGP 41251, a protein kinase inhibitor with potential as an anticancer agent. Pharmacol Ther *82*: 293-301, 1999.

19 Fabbro D, Ruetz S, Bodis S, Pruschy M, Csermak K, Man A, Campochiaro P, Wood J, O'Reilly T and Meyer T: PKC412-a protein kinase inhibitor with a broad therapeutic potential. Anticancer Drug Des *15*: 17-28, 2000.

20 Hemstrom TH, Joseph B, Schulte G, Lewensohn R and Zhivotovsky B: PKC 412 sensitizes U1810 non-small cell lung cancer cells to DNA damage. Exp Cell Res *305*: 200-213, 2005.

21 Ikegami Y, Yano S and Nakao K: Antitumor effect of CGP41251, a new selective protein kinase C inhibitor, on human non-small cell lung cancer cells. Jpn J Pharmacol *70*: 65-72, 1996.

22 Kim M, Park IY, Lim J, Kim Y, Han KT, Chung WH and Han K: Antitumor and normal cell protective effect of PKC412 in the athymic mouse model of ovarian cancer. Ann Clin Lab Sci *36*: 455-460, 2006.

23 Sharkey J, Khong T and Spencer A: PKC412 demonstrates JNK-dependent activity against human multiple myeloma cells. Blood *109*: 1712-1719, 2007.

24 Shih A, Zhang S, Cao HJ, Boswell S, Wu YH, Tang HY, Lennartz MR, Davis FB, Davis PJ and Lin HY: Inhibitory effect of epidermal growth factor on resveratrol-induced apoptosis in prostate cancer cells is mediated by protein kinase C-alpha. Mol Cancer Ther *3*: 1355-1364, 2004.

25 Nakamura K, Yoshikawa N, Yamaguchi Y, Kagota S, Shinozuka K and Kunitomo M: Effect of PKC412, an inhibitor of protein kinase C, on spontaneous metastatic model mice. Anticancer Res *23*: 1395-1399, 2003.

26 Nakatani T, Marui T, Yamamoto T, Kurosaka M, Akisue T and Matsumoto K: Establishment and characterization of cell line TNMY1 derived from human malignant fibrous histiocytoma. Pathol Int *51*: 595-602, 2001.

27 Fang Z, Mukai H, Nomura K, Shinomiya K, Matsumoto S, Kawaguchi N, Kitagawa T and Kanda H: Establishment and characterization of a cell line from a malignant fibrous histiocytoma of bone developing in a patient with multiple fibrous dysplasia. J Cancer Res Clin Oncol *128*: 45-49, 2002.

28 Kiyozuka Y, Nakagawa H, Uemura Y, Senzaki H, Yamamoto A, Noguchi T, Mizuta H, Nakanishi K, Nakano S and Tsubura A: Novel cell lines established from a human myxoid malignant fibrous histiocytoma arising in the uterus. Cancer Genet Cytogenet *127*: 7-15, 2001.

29 Sjoblom T, Shimizu A, O'Brien KP, Pietras K, Dal Cin P, Buchdunger E, Dumanski JP, Ostman A and Heldin CH; Growth inhibition of dermatofibrosarcoma protuberans tumors by the platelet-derived growth factor receptor antagonist STI571 through induction of apoptosis. Cancer Res *61*: 5778-5783, 2001.

30 Kawamoto T, Akisue T, Marui T, Nakatani T, Hitora T, Fujita I, Kurosaka M and Yamamoto T: Inhibitory effect of STI571 on cell proliferation of human malignant fibrous histiocytoma cell lines. Anticancer Res *24*: 2675-2679, 2004.

31 Irsan I, Akisue T, Hara H, Fujimoto T, Imabori M, Doita M, Kuroda R, Fujioka H, Kawamoto T, Yamamoto T and Kurosaka M: Imatinib mesylate inhibits tumorigenicity of malignant fibrous histiocytoma cells *in vivo*. Anticancer Res *27*: 423-429, 2007.

32 Dlugosz AA, Cheng C, Williams EK, Dharia AG, Denning MF and Yuspa SH: Alterations in murine keratinocyte differentiation induced by activated rasHa genes are mediated by protein kinase C-alpha. Cancer Res *54*: 6413-6420, 1994.

33 Zhan M, Yu D, Liu J, Glazer RI, Hannay J and Pollock RE: Transcriptional repression of protein kinase Calpha *via* Sp1 by wild-type p53 is involved in inhibition of multidrug resistance 1 P-glycoprotein phosphorylation. J Biol Chem *280*: 4825-4833, 2005.

34 Gill PK, Gescher A and Gant TW: Regulation of *MDR1* promoter activity in human breast carcinoma cells by protein kinase C isozymes alpha and theta. Eur J Biochem *268*: 4151-4157, 2001.

35 Hofmann J; Modulation of protein kinase C in antitumor treatment. Rev Physiol Biochem Pharmacol *142*: 1-96, 2001.

36 Propper DJ, McDonald AC, Man A, Thavasu P, Balkwill F, Braybrooke JP, Caponigro F, Graf P, Dutreix C, Blackie R, Kaye SB, Ganesan TS, Talbot DC, Harris AL and Twelves C: Phase I and pharmacokinetic study of PKC412, an inhibitor of protein kinase C. J Clin Oncol *19*: 1485-1492, 2001.

37 Eder JP Jr, Garcia-Carbonero R, Clark JW, Supko JG, Puchalski TA, Ryan DP, Deluca P, Wozniak A, Campbell A, Rothermel J and LoRusso P: A phase I trial of daily oral 4'-N-benzoyl-staurosporine in combination with protracted continuous infusion 5-fluorouracil in patients with advanced solid malignancies. Invest New Drugs *22*: 139-150, 2004.

38 Monnerat C, Henriksson R, Le Chevalier T, Novello S, Berthaud P, Faivre S and Raymond E: Phase I study of PKC412 (N-benzoyl-staurosporine), a novel oral protein kinase C inhibitor, combined with gemcitabine and cisplatin in patients with non-small-cell lung cancer. Ann Oncol *15*: 316-323, 2004.

Received October 17, 2007
Revised December 4, 2007
Accepted January 7, 2008