INHIBITORY EFFECT OF PKC412 ON CELL PROLIFERATION OF HUMAN BONE AND SOFT TISSUE SARCOMA CELL LINES

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INTRODUCTION
Among the bone and soft tissue sarcomas, malignant fibrous histiocytoma (MFH) is one of the most common high-grade sarcomas in bone and soft tissue. The prognosis of the disease is poor due to its chemoresistance. PKC412 is a protein kinase inhibitor that was initially developed as a protein kinase C (PKC) inhibitor. PKC412 also selectively inhibits some receptor tyrosine kinases: the platelet-derived growth factor receptor (PDGFR), the receptor for the stem cell factor (c-kit) and the kinase insert domain receptor (KDR; vascular endothelial growth factor receptor 2). We examined the expression of PDGFR, c-kit and KDR in human bone and soft tissue sarcoma cell lines, and the effect of PKC412 on the cell proliferation.

MATERIALS AND METHODS
Cell Lines. 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 in this study. KTHOS and TNMY1 were previously established in our laboratory. GBS-1 was kindly provided by Dr. H. Kanda (Department of Pathology, The Cancer Institute of the Japanese Foundation for Cancer Research, Tokyo, Japan). Nara-F and Nara-H were purchased from ScienStuff Co., Nara, Japan. All cell lines were grown in culture medium consisting of minimum essential medium eagle (Sigma-Aldrich Co., St. Louis, MO) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich Co.), penicillin G (100 U/ml), streptomycin (100 µg/ml), and L-glutamine (2 mM). The cell lines were routinely maintained at 37°C in a humidified 5% CO2 atmosphere.

RT-PCR of receptor tyrosine kinases. Total RNAs were eluted by selective binding to a silica-gel-based membrane using an RNeasy Mini Kit® (QIAGEN Inc., Valencia, CA). Reverse transcription of RNA into cDNA was performed by incubating 1µl of RNA in a reaction mixture containing 0.5µg/ml of oligo (dT) primer, Reverse Transcriptase 10xBuffer, 10mM dNTP Mix, and AMV Reverse Transcriptase (Promega Corporation, Madison, WI). Polymerase chain reaction (PCR) was performed using a Perkin-Elmer DNA thermal cycler (Perkin-Elmer, Norwalk, CT). Receptor tyrosine kinases (PDGFRβ, c-kit and KDR) and GAPDH were examined by reverse transcription (RT-) PCR. After PCR amplification, 8-µl aliquots of the PCR products were electrophoresed in a 2.5% agarose gel, followed by ethidium bromide dye.

The Effect of PKC412 on the cell proliferation of human osteosarcoma and MFH cell lines. The cell proliferation was assayed using the MTS assay technique. Cells were trypsinized and seeded at a density of approximately 5000 cells/well in 96-well cell culture plates in 100-µl culture medium with 10% FBS. After 48 h, the medium was refreshed with 1% FBS containing PKC412 in the indicated concentrations (0, 0.01, 0.1 and 1 µM; 0 µM as control). After 24, 48, 72, 96, and 120 h, the medium was removed and washed with phosphate buffered saline, 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 after 2 h of further incubation at 37°C in a humidified atmosphere of 5% CO2. The percent viability of each well was calculated. At least three independent cultures were performed for each study. The percent viability of each well was calculated. The data were analyzed statistically using ANOVA with Fisher's PLSD post hoc test.

RESULTS
Expression of Receptor tyrosine kinases. The PDGFRβ and c-kit mRNAs were expressed in all osteosarcoma cell lines and three MFH cell lines except Nara-H. The KDR mRNA was expressed in KTHOS, TNMY1, Nara-F and Nara-H, but was absent in MG63, KHOS and GBS-1 (Fig. 1).

The Effect of PKC412. PKC412 significantly inhibited the cell proliferation of KTHOS, TNMY1 and Nara-F in a dose- and time-dependent manner at concentrations of 1µM or more. 0.1µM PKC412 inhibited the cell proliferation of KTHOS and TNMY1 at the percent viability of 50% or less. The cell proliferation inhibition by PKC412 in KHOS and Nara-H was lower than in that KTHOS, TNMY1 and Nara-F. In KHOS, MG63 and GBS-1, PKC412 did not show the cell proliferation inhibition of 50% or more in a dose- and time-dependent manner at concentrations of 1µM (Fig. 2).

DISCUSSION
PKC412 had a dose- and time-dependent inhibitory effect on the cell proliferation of KTHOS, TNMY1 and Nara-F, which express all receptor tyrosine kinases (PDGFRβ, c-kit and KDR) are the targets of PKC412. The cell proliferation of KHOS, MG63 and GBS-1 without KDR expression was not inhibited 50% or more in a dose- and time-dependent manner at concentrations of 1µM by PKC412. Therefore, KDR expression and its activation might be very important for the cell proliferation of these cell lines. These results suggest that PKC412 may be an inhibitor of receptor tyrosine kinases in human osteosarcoma and MFH cells and decreases tumor growth and that PKC412 can be a potent chemotherapeutic agent for human bone and soft tissue sarcomas. PKC412 is a potent competitive inhibitor of ATP binding to conventional PKC subtypes, so further studies are needed to explore the precise molecular mechanisms for the inhibitory effect of PKC412 on cell proliferation in human osteosarcoma and MFH cells.

REFERENCES