ROLE OF PROTEIN KINASE C δ IN MUSCULOSKELETAL TUMORS

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INTRODUCTION
Protein kinase C delta (PKC δ), one of the isoforms of protein kinase C, has been shown to act as either positive or negative regulator of tumor progression [1], however its role in musculoskeletal tumors is still unknown. In this study, we investigated the expression of PKC δ in musculoskeletal tumors and the relationship between expression of PKC δ and malignancy. We also determined the role of PKC δ on cell proliferation of human malignant fibrous histiocytoma (MFH) in vitro.

MATERIALS AND METHODS
Human musculoskeletal tumor samples and cell lines: We used 41 human musculoskeletal tumor samples including 32 malignant and 9 benign tumors to analyze the mRNA expression of PKC δ. Seven human sarcoma cell lines including three osteosarcoma cell lines (KHOS, KTHOS, MG63) and four MFH cell lines (Nara F, Nara H, TNMY1, GBS-1) were also used for in vitro studies.

Real time PCR: We isolated total RNA from 41 human musculoskeletal tumor samples. PCR was performed using specific primers for PKC δ and β-actin. The values were normalized with those of β-actin and were relatively quantified.

Immunoblot analysis: Cell lysates were collected from 7 cell lines. Samples containing equal amounts of proteins were electrophoresed and transferred to blotting membrane. After blocking, membranes were incubated with primary antibody to PKCδ and were then incubated with secondary antibody. Binding proteins were detected using ECL reagent.

siRNA knockdown of PKC δ: Specific siRNA against PKC δ was used to silence PKC δ. Four human MFH cell lines were transfected with either PKC δ siRNA or non specific control siRNA by lipofection method. After transfection, both real time PCR and immunoblotting were performed to examine whether PKC δ siRNA decreased the expression of PKC δ.

Cell proliferation assay: To evaluate whether PKC δ siRNA affects cell proliferation of MFH cells, we performed cell proliferation assay. MFH cells transfected with either PKC δ siRNA or non specific control siRNA were seeded at a density of approximately 5000 cells/well in 96-well culture plates. At the indicated time, cell proliferation was assessed using WST-8 (Cell Counting Kit-8) assay.

Statistical analysis: Each experiment was performed at least three times independently. Statistical significance was evaluated using student’s t-test and all tests were considered significant at p<0.05.

RESULTS
Real time PCR analysis with human musculoskeletal tumor samples showed that mRNA expression of PKC δ in malignant tumors was significantly lower than that in benign tumors (p=0.05, Fig 1), and PKCδ expression in high-grade malignant tumors such as osteosarcomas and MFHs was especially low. By immunoblot analysis, protein expression of PKC δ was detected in all human cell lines and the expression in osteosarcoma cell lines was weaker than that in MFH cell lines (Fig 2).

In MFH cells which were transfected with PKC δ siRNA, mRNA expression of PKC δ was decreased to 50 to 65% of that with control siRNA and the protein expression of PKC δ was also strongly reduced (Fig 3). Cell proliferation assay revealed that siRNA knockdown of PKC δ significantly activated cell proliferation after 72 hours of transfection (p<0.05, Fig 4).

DISCUSSION
Malignant musculoskeletal tumors are clinically aggressive and have a high metastatic potential, therefore the prognosis is still very poor, and current chemotherapy protocols are not very effective to improve the prognosis. PKC δ has recently attracted attention as cell proliferation regulator [2-3] and is known to play a role on tumor progression [1]. In this study, we demonstrated that mRNA expression of PKC δ in malignant musculoskeletal tumors was significantly lower than in benign tumors and that siRNA knockdown of PKC δ activated cell proliferation in all MFH cell lines. Taken together, these results suggest that PKC δ may play a role on cell proliferation in human musculoskeletal tumors and that PKC δ may be a useful therapeutic agent against malignant musculoskeletal tumors.

REFERENCES