Silencing of PKD1 Expression Increased Migration Ability of Human Osteosarcoma Cells

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INTRODUCTION

Osteosarcoma (OS) is one of high grade malignant bone tumors and is the most commonly encountered malignant bone tumor in children and adolescents. Significant numbers of patients in the disease eventually develop pulmonary metastases and succumb to the disease even after conventional multi-agent chemotherapy and surgical excision.

Protein kinase D1 (PKD1), founding member of a new family of serine/threonine protein kinases, plays a role in several cellular processes such as apoptosis, immune regulation, cell proliferation, oxidative stress signaling, adhesion and motility. Recent studies suggest that PKD1 expression is low in several cancers and that low expression of PKD1 may increase cell proliferation, migration and invasion of cancer cells. However, the role of PKD1 in musculoskeletal tumors has not been discussed.

Matrix metalloproteinases (MMPs) are considered to play important roles in the matrix degradation for tumor growth, invasion, and tumor-induced angiogenesis. Several solid tumors display enhanced expression of MMPs, and recently clinical trials have been initiated on MMP-inhibitors. Recent studies revealed that MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are present in large quantities in OS, and suggest that both MMP-2 and MMP-9 play a critical role on cell invasion and metastases of OS.

The purpose of this study was to evaluate the role of PKD1 on migration ability of human OS cells and to examine the relationship between PKD1 and MMPs in human OS cells.

MATERIALS and METHODS

Cells. We used three human OS cell lines in this study (KHOS, MG63 and KTHOS).

siRNA knockdown of PKD1. To examine the effect of PKD1 knockdown on cell migration ability, cells were transfected with either PKD1-targeting small interfering RNA (siRNA) or non specific control siRNA. After siRNA transfection, we evaluated the efficacy of siRNA transfection by quantitative real time PCR and western blotting analysis.

Cell migration assay. We evaluated the cell migration ability of PKD1-siRNA transfected cells using in vitro scratch wound healing assay.

Analysis of MMPs expression. To evaluate the effect of PKD1 knockdown on MMPs expression in OS cells, we performed quantitative real time PCR using both MMP-2 and MMP-9 specific primers.

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<0.01.

RESULTS

siRNA knockdown of PKD1 (Fig.1 and 2)

siRNA knockdown of PKD1 decreased PKD1 expression in both mRNA and protein level. Quantitative real time PCR showed that PKD1-siRNA decreased PKD1 mRNA expression to less than 50% of that of control in all three human OS cell lines (Fig.1).

Effect of PKD1 knockdown on cell migration ability (Fig.3)

siRNA knockdown of PKD1 increased cell migration ability of all three OS cell lines (Fig.3). After 16 hours in culture, healing of scratch wound of PKD1-siRNA transfected OS cells was nearly completed, and was significantly earlier than that of control cells (Fig.3b, d, f).

Expression of MMPs in PKD1 knockdown OS cells (Fig.4)

Both MMP-2 and MMP-9 production were significantly elevated in PKD1 knockdown OS cells compared with control.

DISCUSSION

In this study, we demonstrated that siRNA knockdown of PKD1 increased cell migration and elevated the production of both MMP-2 and MMP-9 in three human OS cell lines. These results suggest that PKD1 may negatively regulate cell migration ability of human OS cells, and low expression of PKD1 may contribute to malignant potential in human OS. Several studies have demonstrated that the negative regulatory effect of PKD1 on cell migration in various human malignancies (1, 2). Our results are consistent with previous reports.

Both MMP-2 and MMP-9 are considered to be particularly good targets for anticancer drugs because it degrades gelatins which are major components of basement membrane. Bjørnland K et al. revealed that the most invasive OS cell line secreted the highest amounts of MMP-2 and MMP-9 (3). However, the relationship between PKD1 and MMPs in OS has not been investigated. In this study, we demonstrated PKD1 as a novel regulator of secretion of MMP-2 and MMP-9 in human OS cells, which cause increasing of OS cell migration.

In summary, our results revealed that siRNA knockdown of PKD1, a protein of emerging translational interest in several human cancers, increased cell migration ability and production of MMP-2 and MMP-9 in human OS cells. These results suggest that PKD1 might be a potent therapeutic target for human musculoskeletal malignancies.

REFERENCE