INHIBITION OF PLATELET-DERIVED GROWTH FACTOR RECEPTORS AND C-KIT PHOSPHORYLATION OF MALIGNANT FIBROUS HISTIOCYTOMA CELLS BY IMATINIB MESYLAte DOES AFFECT TUMORIGENICITY IN VIVO

INTRODUCTION

Malignant fibrous histiocytoma (MFH) is the most common high grade soft tissue sarcoma with poor prognosis due to its chemoresistance and lack of understanding to distinct tumor entity. Some studies have shown that PDGFRs and c-Kit are expressed in MFH cell lines and this and lack of understanding to distinct tumor entity. Some studies have shown that PDGFRs and c-Kit are expressed in MFH cell lines and this receptor/ligand system may regulate the cell proliferation of MFH cell lines. Imatinib mesylate (STI571) was originally developed as a competitor for an ATP-binding site of the Abl protein kinase. In addition, imatinib mesylate was found to inhibit kinase activity of PDGFRs and c-Kit. Our previous studies have demonstrated an inhibitory effect of imatinib mesylate on the cell proliferation of MFH cell lines. Furthermore, although imatinib mesylate was associated with reduced PDGFRs and c-Kit phosphorylation, discrepancies remain between responses to imatinib mesylate in vitro and in vivo studies. The purposes of this study are to demonstrate the expression of PDGFRs and c-Kit in MFH cell lines and to test the inhibitory effect of imatinib mesylate on tumor growth of MFHs that show various patterns of PDGFRs and c-Kit expression using a xenograft model.

MATERIALS AND METHODS

Cell Culture. Four human MFH cell lines were used in this study (TNMY1, GBS-1, Nara-F and Nara-H). All cell lines were grown in culture medium consisting of minimum essential Eagle’s medium (Sigma-Aldrich Co., St Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich Co.), penicillin G (100 U/ml) and streptomycin (100 µg/ml). The cell lines were routinely maintained at 37°C in a humidified 5% CO2 atmosphere. For in vivo experiments, tumor cells were harvested by brief exposure to 0.25% trypsin.

Animal xenograft model. Male athymic nude mice (Charles River Laboratories, Inc., Tokyo, Japan) were used at 6 – 8 weeks of age. Animal maintenance was in accordance with institutional principles and procedures outlined in the Guide for the Care and Use of Laboratory Animals at our institution.

Implantation of tumors cells. To establish tumors, 12 million cells from Nara H, Nara F, TNMY1 and GBS-1 of MFH human cell line were injected s.c. into the back of the mice. Tumor growth was monitored, each day after implantation. Tumor dimensions were measured using a digital caliper and tumor volume was calculated according to the formula \( V = \frac{4}{3} \pi X^2 \times b \), where \( a \) represents the shorter diameter and \( b \) the longer dimension of the tumor. The mice were randomly assigned into two groups., Nara H group (n=12) and TNMY1 group (n=12) and from each of these group were randomly assigned into the imatinib mesylate group (treatment groups) and PBS group (control groups).

Treatment of tumor bearing mice. After tumors had reached ~100 mm³, the treatment was started. For each treatment a dose of imatinib mesylate (Novartis Pharma, Switzerland) at 100 mg/kg every 24 hours for each group compared to the control group.. Our previous studies showed an inhibitory effect of imatinib mesylate on tumor growth of the MFH cell line. In conclusion, our study demonstrated that imatinib mesylate has a similar effect as an inhibitor of receptor tyrosine kinase in vitro and in vivo.

Inhibition of PDGFRs and c-Kit Phosphorylation of human MFH cell lines in vivo. All PDGFRs and c-Kit were expressed at TNMY1 MFH xenograft with the same levels in the both the treatment and control groups.

RESULTS

mRNA expression of the receptor tyrosine kinase. TNMY1, Nara F and GBS-1 MFH cell line expressed mRNA of PDGFRA, PDGFRβ and c-Kit by RT-PCR. Neither PDGFRs nor c-Kit mRNAs were expressed in Nara H. Nara F cell line expressed low levels of PDGFRA .

Inhibition effect of imatinib mesylate for tumor growth. We used TNMY1 cell line (PDGFRs and c-Kit positive) and Nara H cell line (PDGF’s and c-Kit negative) to show the selectivity of imatinib mesylate inhibition effect for PDGFRs and c-Kit in MFH cell line. For TNMY1 xenograft, the tumor volume began to decrease on the seventh day after medication and the differences were significant between the treatment and the control group.

Inhibition of PDGFRs and c-Kit Phosphorylation of human MFH cell lines in vivo. All PDGFRs and c-Kit were expressed at TNMY1 MFH xenograft with the same levels in the both the treatment and control groups. Nevertheless, there was a significant reduction in expression of Phosphorylation of PDGFRs and c-Kit in the treatment group compared to the control group..

DISCUSSION

Our previous studies showed an inhibitory effect of imatinib mesylate on the cell proliferation of the MFH cell line which expresses PDGFR’s and c-Kit in a dose and time dependent manner. In this study, imatinib mesylate inhibited the growth of the MFH cell line, which expresses PDGFRs and c-Kit, but does not inhibit the growth of MFH cell line without PDGFRs and c-Kit in vivo. This inhibition is associated with the suppression of PDGFRs and c-Kit phosphorylation.

These results suggest that imatinib mesylate has a similar effect as an inhibitor of receptor tyrosine kinase in vitro and in vivo. As a potent tyrosine kinase inhibitor, imatinib mesylate may work at the kinase domain of the receptor to inhibit phosphorylation, but may not function at the receptor level expression in the MFH cell line. In conclusion, our data demonstrate that imatinib mesylate works to reduce tumor growth in the MFH cell line, which expresses PDGFRs and c-Kit associated with phosphorylation suppression.