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Inhibitory Effect of STI571 on Cell Proliferation of Human Malignant Fibrous Histiocytoma Cell Lines

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Abstract. *Background: Malignant fibrous histiocytoma (MFH) is one of the most common high-grade sarcomas in bone and soft tissue and, due to its chemo-resistance, the prognosis of the disease is poor. STI571 is a tyrosine kinase inhibitor that was initially developed as a BCR/ABL inhibitor for chronic myeloid leukemia patients. STI571 also selectively inhibits platelet-derived growth factor receptors (PDGFRs) and c-kit. We examined the expression of PDGFRs and c-kit in human MFH cell lines, and the effect of STI571 on cell proliferation. Materials and Methods: Four human MFH cell lines (TNMY1, GBS-1, Nara-F and Nara-H) were used. mRNA expression of the receptor tyrosine kinases (PDGFRs and c-kit) was analyzed using reverse transcription-polymerase chain reaction, and the inhibitory effect of STI571 on cell proliferation was analyzed using the MTS assay technique. Results: PDGFRα mRNA was expressed in TNMY1 and GBS-1, and PDGFRβ and c-kit mRNAs were expressed in TNMY1, GBS-1 and Nara-F. All three of these mRNAs were absent in Nara-H. STI571 inhibited cell proliferation of TNMY1, GBS-1 and Nara-F in a dose- and time-dependent manner, but cell proliferation of Nara-H was not inhibited by STI571 at concentrations of 10 μM or less. Conclusion: STI571 significantly inhibited proliferation of the three human MFH cell lines that expressed mRNAs of target receptor tyrosine kinases. The inhibitory effect of STI571 on cell proliferation in these three cell lines might be due to decreased tyrosine kinase activity. STI571 might be a potent chemotherapeutic agent for human MFHs.*

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Key Words: Malignant fibrous histiocytoma, STI571, tyrosine kinase, c-kit, platelet-derived growth factor receptor.

Malignant fibrous histiocytoma (MFH) is one of the most common high-grade sarcomas that develop in bone and soft tissue, and the prognosis is poor due to its chemo-resistance. Although there are currently many chemotherapy protocols for MFH , there is little consensus concerning the doses and combinations of these chemotherapeutic agents (1-3).

Human malignant tumors overexpress a number of important growth factor receptors with intrinsic tyrosine kinase activities for cell proliferation, including platelet-derived growth factor receptors (PDGFRs) and c-kit. Autocrine and/or paracrine stimulation of the growth factor receptors by their corresponding ligands might have an important role in the cell growth of various human malignant tumors, including small cell lung cancer (SCLC) (4), gastrointestinal stromal tumor (GIST) (5-7), pancreatic cancer (8) and osteosarcoma (9). The receptor/ligand systems also regulate cell growth in MFH (10-13).

STI571 is a low-molecular-weight tyrosine kinase inhibitor developed as an ATP competitive inhibitor of BCR/ABL tyrosine kinase, which causes chronic myeloid leukemia (CML) (14-16). STI571 is also a selective inhibitor of PDGFRs and c-kit (17). STI571 is now clinically used for CML patients and KIT-positive GIST patients. *In vitro* and/or *in vivo* studies demonstrate that STI571 inhibits the cell proliferation of human malignant tumors that overexpress PDGFRs and/or c-kit, including SCLC (18), GIST (5-7), dermatofibrosarcoma protuberans (19-21), osteosarcoma (9), pancreatic cancer (8, 22, 23), Ewing's sarcoma (24) and neuroblastoma (25). We examined PDGFR and c-kit expression in human MFH cell lines, and the inhibitory effect of STI571 on MFH cells.

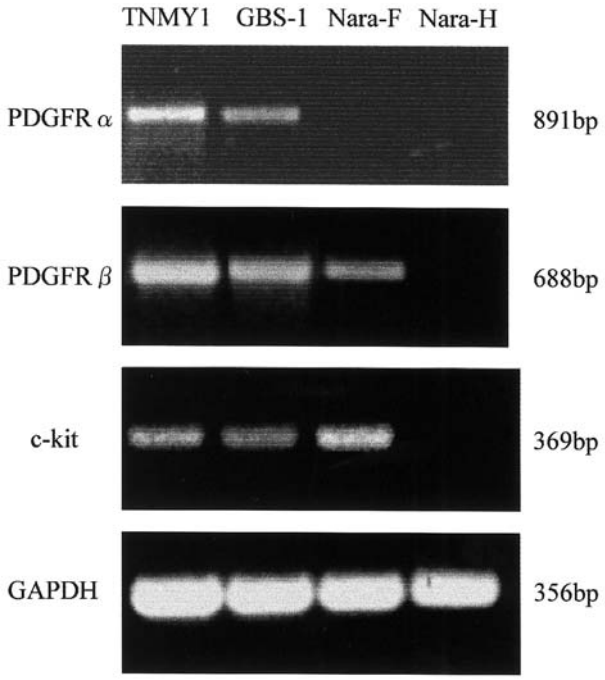

Figure 1. *PDGFR and c-kit mRNAs in MFH cell lines analyzed using RT-PCR. PDGFRα mRNA was expressed in TNMY1 and GBS-1, but not in Nara-F or Nara-H. PDGFRβ and c-kit mRNAs were expressed in TNMY1, GBS-1 and Nara-F.*

Materials and Methods

Cell culture. Four human MFH cell lines were used in this study (TNMY1, GBS-1, Nara-F and Nara-H). TNMY1 was previously established in our laboratory (26). GBS-1 was kindly 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 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), streptomycin (100 μg/ml) and L-glutamine (2 mM). The cell lines were routinely maintained at 37˚C in a humidified 5% CO_2 atmosphere.

mRNA expression of the 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, USA). Reverse transcription of RNA into cDNA was performed by incubating 1 μl of RNA in a reaction mixture containing 0.5 mg/ml of oligo (dT) primer, Reverse Transcription 10xBuffer, 10 mM dNTP Mix and AMV Reverse Transcriptase (Promega Corporation, Madison, WI, USA). Polymerase chain reaction (PCR) was performed using a Perkin-Elmer DNA thermal cycler (Perkin-Elmer, Norwalk, CT, USA). Receptor tyrosine kinases were examined by reverse transcription (RT-) PCR. The samples were preheated at 94˚C for 5 min, then cycled 40 times at 94˚C for 60 sec, 58˚C for 60 sec and 72˚C for 60 sec and finally 72˚C for 7 min. The sequences of the primers for human PDGFRα were: forward primer 5'- ATC AAT CAG CCC AGA TGG AC-3', reverse primer 5'- TTC ACG GGC AGA AAG GTA CT-3', expected size 891 bp (29); human PDGFRβ: forward primer 5'- AAT GTC TCC AGC ACC TTC GT-3', reverse primer 5'- AGC GGA TGT GGT AAG GCA TA-3', expected size 688 bp (29); human c-kit: forward primer 5'-CGT TGA CTA TCA GTT CAG CGA G-3', reverse primer 5'-CTA GGA ATG TGT AAG TGC CTC C-3', expected size 369 bp (30). The sequences of forward and reverse primers for GAPDH were as reported, expected size was 356 bp (31). After PCR amplification, 8-μl aliquots of the PCR products were electrophoresed in a 2.5% agarose gel, followed by ethidium bromide dye.

Inhibitory effect of STI571 on cell proliferation of MFH cell lines. 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 100μl culture medium with 10% FBS. After 48 h, the medium was refreshed with 1% FBS containing STI571 in the indicated concentrations (0, 0.1, 1, 5 and 10 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% CO_2. The percent viability of each well was calculated. At least three independent cultures were performed for each study.

Statistical analysis. The data obtained were statistically analyzed using analysis of variance. A value of $p<0.05$ was regarded as statistically significant.

Results

mRNA expression of the receptor tyrosine kinases. Figure 1 shows the mRNA expression of the receptor tyrosine kinases analyzed by RT-PCR. PDGFRα mRNA was expressed in TNMY1 and GBS-1, but not in Nara-F or Nara-H. The level of PDGFRα mRNA in GBS-1 was lower than that in TNMY1. PDGFRβ and c-kit mRNAs were expressed in TNMY1, GBS-1 and Nara-F. The PDGFRβ mRNA level was highest in TNMY1. The c-kit mRNA level was highest in Nara-F. Neither PDGFR nor c-kit mRNAs were expressed in Nara-H.

Inhibitory effect of STI571 on cell proliferation of MFH cell lines. We analyzed the inhibitory effect of STI571 on cell proliferation of the MFH cell lines using the MTS assay technique. STI571 significantly inhibited proliferation of TNMY1, GBS-1 and Nara-F in a dose- and time- dependent manner at concentrations of 5 and 10 μM (Figure 2A-C). IC_{50} values for these three cell lines ranged from 5 to 10 μM. ST157 (10 μM) inhibited cell proliferation of TNMY1 below 50% of control cell viability after 72 h of culture, whereas STI571(10 μM) inhibited cell proliferation of GBS-1 and Nara-F below 50% of control after 96 h of culture. STI571 at any concentration did not inhibit cell proliferation of Nara-H after 24 to 120 h of culture (Figure 2D).

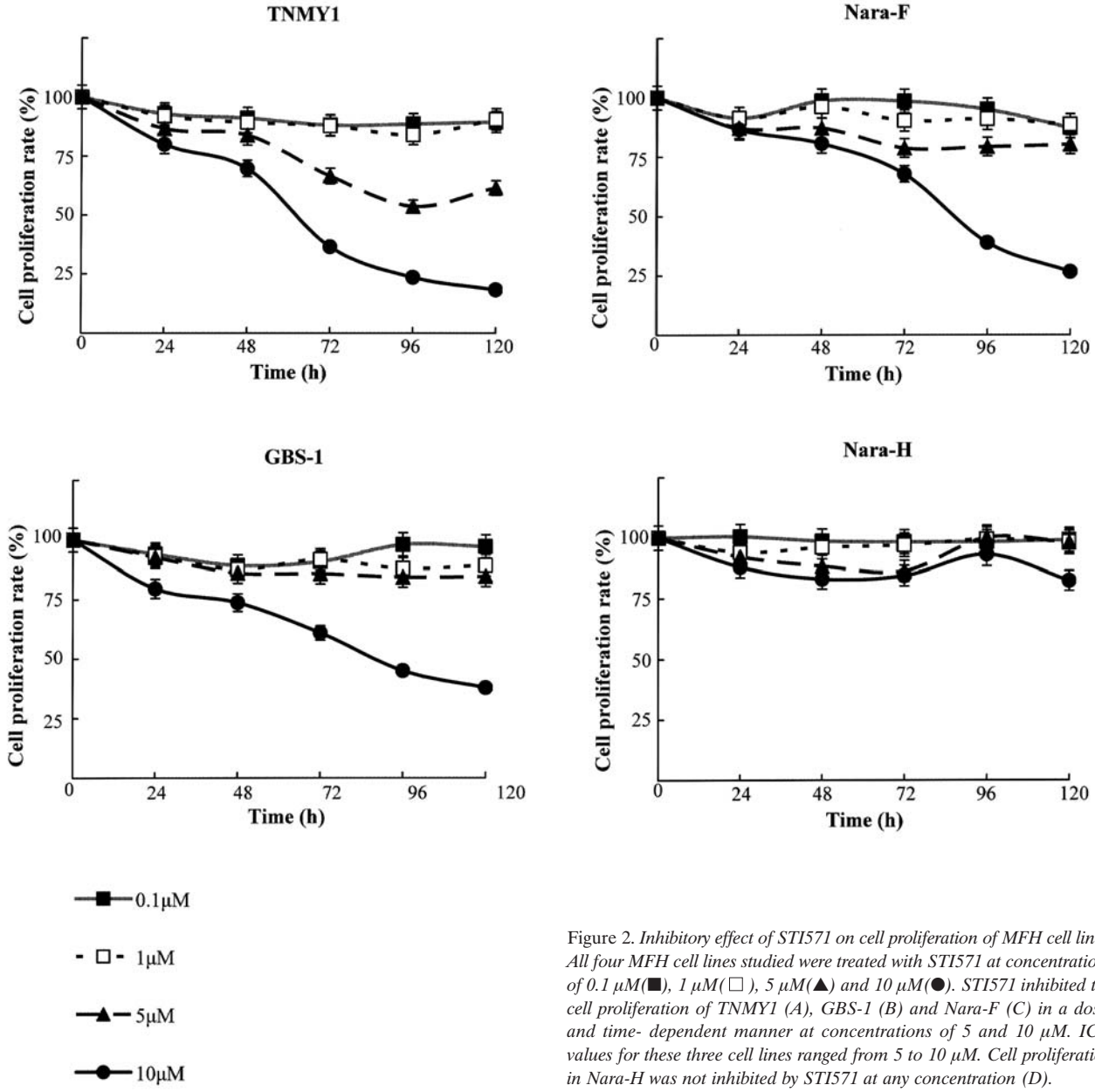

Figure 2. *Inhibitory effect of STI571 on cell proliferation of MFH cell lines. All four MFH cell lines studied were treated with STI571 at concentrations of 0.1 μM(■), 1 μM(□), 5 μM(▲) and 10 μM(●). STI571 inhibited the cell proliferation of TNMY1 (A), GBS-1 (B) and Nara-F (C) in a dose- and time- dependent manner at concentrations of 5 and 10 μM. IC_{50} values for these three cell lines ranged from 5 to 10 μM. Cell proliferation in Nara-H was not inhibited by STI571 at any concentration (D).*

Discussion

MFH is clinically aggressive and has a high metastatic potential to various organs. The prognosis of patients with MFH is poor due to its chemo-resistance. Although many chemotherapy protocols including doxorubicin, epirubicin, cisplatin and ifosfamide are currently used for MFH, these chemotherapy protocols do not sufficiently improve the prognosis of patients with MFH. The clinical response rate of these chemotherapy protocols for MFH is approximately 20% (1-3).

STI571 is a molecular targeting drug and an ATP competitive inhibitor of BCR/ABL and other tyrosine kinases. Clinical studies indicate that STI571 is effective for CML and KIT-positive GIST patients (14-16). In the present study, we investigated the effectiveness of STI571 on MFH cells, because recent studies reported that MFHs overexpress various growth factors and their receptors that have intrinsic tyrosine kinase activities (10,11).

Many studies demonstrated that growth factor receptor/ligand systems have an important role in cell

growth of human epithelial and mesenchymal malignancies, whereas only a few studies have examined the roles of PDGFRs and c-kit in cell proliferation of MFH in an autocrine or paracrine manner (10-13). Taniuchi *et al.* (11) immunohistochemically demonstrated that PDGFRβ expression might have an important role in the proliferation of fibrohistiocytic tumor cells. Abdiu *et al.* (13) also reported that two out of eight MFH cell lines fulfill the requirements of possible PDGF-related auto/paracrine regulation. We previously established the TNMY1 cell line in our laboratory and reported that the stem cell factor (SCF)/c-kit system had paracrine activity in TNMY1 cell growth (12). Several studies demonstrated that STI571 inhibits cell proliferation of human epithelial and mesenchymal malignant tumors *in vitro* and *in vivo* (5-9, 18-25). The effect of STI571 on human MFHs, however, has not been studied experimentally or clinically.

In clinical studies, the EORTC Soft Tissue and Bone Sarcoma Group demonstrated that STI571 is an active agent for KIT-positive GIST, but inactive for other soft-tissue sarcomas without a selected molecular target (32-34). Furthermore, activating mutations of PDGFRs or c-kit are associated with cell growth of human malignancies (35), and the presence of these mutations causes differences in sensitivity to STI571 (36). *In vitro*, STI571 inhibits cell proliferation of leukemic cells expressing TEL-PDGFR, which is the fusion protein tyrosine kinase that is constitutively activated in chronic myelomonocytic leukemia (14). STI571 also reduces *in vivo* and *in vitro* growth of dermatofibrosarcoma protuberans by inhibition of tyrosine kinase activity in PDGFRβ, and this tumor is characterized by COLIA1/PDGFB fusion protein (19-21). In GIST patients, activating mutations of c-kit correlated with a response to STI571. GISTs expressing wild-type c-kit have a significantly lower response rate to STI571 (7, 36), while GIST patients with gain-of-function mutations of PDGFRα (but without mutations of c-kit) are very sensitive to STI571 (37), suggesting that STI571 is less effective for human malignancies that do not express target tyrosine kinases activated by mutations.

Krystal *et al.* (18) reported that 70% of SCLCs express c-kit and SCF and establish an autocrine growth system. STI571 inhibits the growth of SCLC cell lines and the IC_{50} of STI571 for SCLC cells is 5 μM, which is significantly higher than the IC_{50} of 1μM for CML. In the present study, we demonstrated that STI571 inhibited the cell proliferation of three MFH cell lines at a concentration of 5 μM or higher. This is also a higher concentration than that administered to CML patients. Therefore, it is necessary to evaluate the presence of activating mutations of PDGFRs and/or c-kit in human MFH cell lines.

In conclusion, STI571 significantly inhibited the cell proliferation of human MFH cell lines *in vitro*. STI571 also had a dose- and time- dependent inhibitory effect on the cell proliferation of TNMY1, GBS-1 and Nara-F cells, which express PDGFRs and/or c-kit and target receptor tyrosine kinases. Cell proliferation of Nara-H cells without PDGFR or c-kit expression was not inhibited by STI571. These results suggest that STI571 is a selective inhibitor of receptor tyrosine kinases in human MFH cells and decreases tumor growth. Further studies are needed to explore the precise molecular mechanisms of the inhibitory effect of STI571 on cell proliferation in human MFH cells.

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