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Imatinib mesylate radiosensitizes human glioblastoma cells through inhibition of platelet-derived growth factor receptor

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Abstract

Imatinib mesylate is a small molecule inhibitor of the c-Abl, platelet-derived growth factor (PDGF) receptor and c-Kit tyrosine kinases that is approved for the treatment of Philadelphia chromosome-positive chronic myeloid leukemia (CML) and gastrointestinal stromal tumors. Glioblastoma multiforme is a highly malignant primary brain tumor that is usually treated with surgery and/or radiotherapy. Previous studies implicate an autocrine loop caused by high expression of PDGF and its receptor, PDGFR, in the proliferation of some glioblastomas. Here, we demonstrate that pretreatment of a human glioblastoma cell line, RuSi RS1, with imatinib significantly enhanced the cytotoxic effect of ionizing radiation. This effect was not seen in human breast cancer (BT20) and colon cancer (WiDr) cell lines. Whereas c-Abl and c-Kit were expressed about equally in the three cell lines, RuSi RS1 cells showed significantly higher expression of PDGFR- β protein in comparison to BT20 and WiDr. Imatinib treatment of RuSi RS1 cells decreased overall levels of cellular tyrosine phosphorylation and specifically inhibited phosphorylation of PDGFR- β , while c-Abl was not prominently activated in these cells. These results suggest that imatinib may have clinical utility as a radiosensitizer in the treatment of human glioblastoma, possibly through disruption of an autocrine PDGF/PDGFR loop.

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Keywords: Gleevec®; STI571; c-Abl; PDGF receptor; Radiation sensitizer

Introduction

Imatinib mesylate (Gleevec®, Glivec®, formerly STI571) is a 2-phenylaminopyrimidine compound that selectively inhibits the catalytic activity of the Abl, platelet-derived growth factor receptor (PDGFR) and c-Kit tyrosine kinases

[1,2], and is currently used for the treatment of several malignancies where there is constitutive activation of one of its kinase targets. These include Bcr-Abl in chronic myeloid leukemia [3], Tel-PDGFR- β in chronic myelomonocytic leukemia [4], FIP1L1-PDGFR- α in hypereosinophilic syndrome [5], and mutant c-Kit in gastrointestinal stromal tumors [6]. Glioblastoma multiforme is a highly malignant primary brain tumor with an extremely poor prognosis that is primarily treated by surgical resection or radiation therapy, with or without adjuvant chemotherapy [7]. The overexpression of PDGF receptor, predominantly the α -

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subtype, has been detected some in low- and high-grade astrocytomas as well as in virtually all cultured glioma cell lines [8]. The concomitant expression both of the receptor and its ligand forms an autocrine loop that may play a critical role in tumor progression [9]. Disruption of this loop by neutralizing anti-PDGF antibodies [10] or dominant-negative mutants of PDGF [11] or its receptor [12] leads to growth inhibition and revision of the transformed phenotype in cell lines, suggesting that PDGFR may be a target for therapy in glioblastoma. Consistent with this, imatinib inhibited the proliferation of BALB/c 3T3 fibroblasts engineered to express a PDGF-PDGFR autocrine loop and prevented intracranial tumor growth in nude mice inoculated with these cells [13]. In this study, we investigated a potential role of imatinib in radiotherapy of glioblastoma by determining the response of human glioblastoma (RuSi RS1), colon cancer (WiDr), and breast cancer (BT20) cell lines to combination treatment with imatinib and ionizing radiation. We observed that imatinib specifically increased the radiosensitivity of glioblastoma cells, and investigated the molecular mechanism of this effect.

Materials and methods

Cell lines

The human glioblastoma cell line RuSi RS1 was established in one of our laboratories (A.J.) from a 69-year-old glioblastoma patient, and cultured without antibiotics in DMEM medium with 10% fetal bovine serum, 1 mM sodium pyruvate, and 1% non-essential amino acids. The human mammary carcinoma-derived cell line BT20 (ATCC-CCL 218) and the human colorectal carcinoma-derived cell line WiDr (ATCC-HTB 19) were obtained from the ATCC and cultured in RPMI 1640 medium supplemented with 10% bovine serum and 1 mM sodium pyruvate. The HMC-1 cell line was the kind gift of Dr. J.H. Butterfield (Mayo Clinic, Rochester, MN).

Clonogenic assay

The effects of imatinib and radiation were tested in a clonogenic survival assay as described previously [14]. Briefly, cells were plated in triplicate at different densities (1×10^3 and 5×10^3) and precultured for 24 h, then treated with imatinib (Novartis Pharma AG, stored as a 10 mM stock solution in water at 4°C) for 30 min prior to X-irradiation at a dose rate of 2 Gy/min (100 kV, 10 mA; Müller MG 150, filter: 0.3 mm Ni). Irradiated cells were cultured in imatinib for 8–10 days, and the survival fraction determined after staining (2.5% crystal violet; 1% ammonium oxalate) and automated quantitation (Artek model 880, BioSys GmbH, Karben, Germany) of the surviving colonies. The cytotoxicity of imatinib alone was determined by treatment of 5×10^3 cells with imatinib for 30 min,

replating in imatinib-containing medium for 10 days, and quantitation of colony number.

Immunoprecipitation and immunoblotting

Immunoprecipitation was carried out as described [15] with polyclonal anti-Abl GEX4 and GEX5 [15], anti-PDGFR- β (15746E, Pharmingen, San Diego, CA), and anti-c-Kit (C-14, Santa Cruz Biotechnology, Santa Cruz, CA) antibodies. Immunoblotting was performed as described [16] with antibodies to phosphotyrosine (4G10) and Rad51 (3C10, both from Upstate Biotechnology, Lake Placid, NY), Abl (Ab-3, Calbiochem, San Diego, CA), PDGFR- α (C-20), and PDGFR- β (958, both from Santa Cruz), phospho-c-Abl Tyr245, and phospho-PDGFR- β Tyr751 (both from Cell Signaling Technology, Beverly, MA), and β -actin (AC-40, Sigma, St. Louis, MO).

Results and discussion

Synergistic effect of imatinib and radiation on RuSi RS1 but not BT20 or WiDr

The effect of irradiation with and without imatinib pretreatment on cell survival was tested in a clonogenic assay (Fig. 1) and quantitated by determination of the ID₅₀ and/or ID₉₀, defined as the dose of radiation required for a 50% or 90% decrease, respectively, in the survival fraction. In the absence of imatinib, RuSi RS1 cells were relatively radioresistant, demonstrating less than one logarithm decrease in survival fraction at the highest radiation dose (16 Gy), while BT20 and WiDr cells had ≥ 2 logarithm decrease in survival fraction at this dose. Treatment with imatinib alone at doses of 1.0 and 2.5 μ M decreased the clonogenic survival of RuSi RS1 cells by 26% and 78%, respectively (Fig. 1A) but had no effect on BT20 or WiDr cells (Figs. 1B and C). The cytotoxic effect of imatinib on RuSi RS1 cells was maximal at 2.5 μ M, and no further decrease in survival was observed at concentrations up to 7.5 μ M (data not shown). Pretreatment of RuSi RS1 cells with 1 μ M imatinib resulted in increased cytotoxicity following irradiation, with a decrease in the ID₅₀ from about 3.6 to 2 Gy, and in ID₉₀ from >16 Gy to 6.8 Gy, relative to unirradiated imatinib-treated cells (Fig. 1A). Escalating the dose of imatinib to 2.5 μ M resulted in no significant increase synergism with irradiation (Fig. 1A and data not shown). In contrast, there was no synergy observed between imatinib at any dose and irradiation with BT20 and WiDr cells (Figs. 1B and C). These results demonstrate that low-dose imatinib specifically enhances the radiosensitivity of glioblastoma cells but not breast or colorectal adenocarcinoma cells, and suggest that this effect is mediated by a tyrosine kinase that is highly sensitive to this inhibitor.

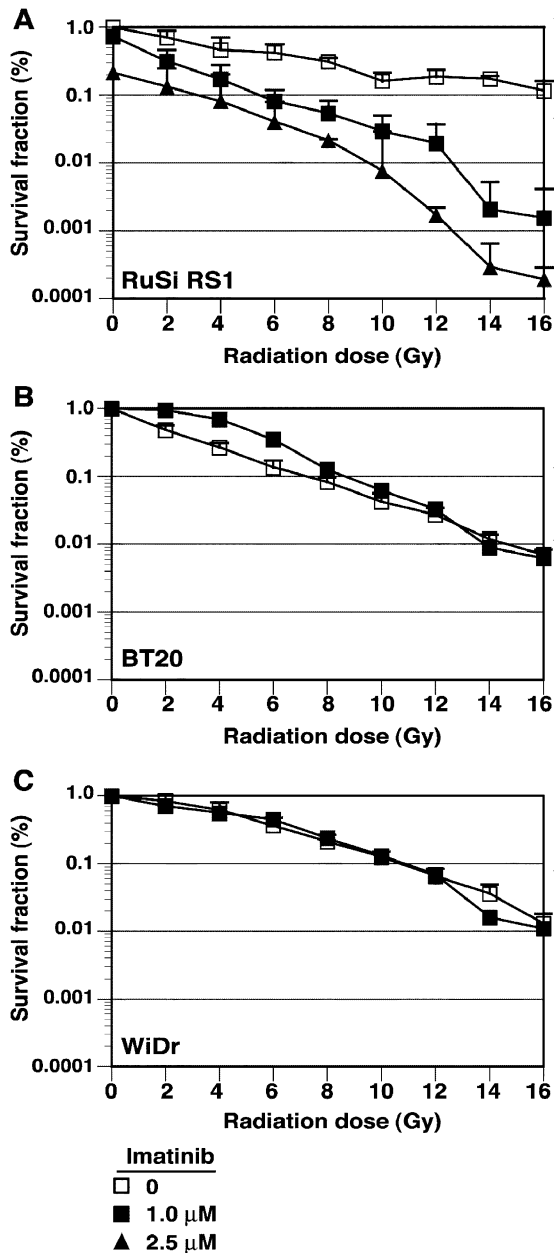


Fig. 1. Synergistic effect of imatinib and ionizing radiation on glioblastoma cells. (A) Clonogenic assay demonstrating the dose-dependent reduction of the survival fraction of RuSi RS1 cells when treated irradiation alone (□), irradiation and 1 μM imatinib (■), or irradiation and 2.5 μM imatinib (▲). (B, C) Clonogenic assay demonstrating no increased effect of irradiation and imatinib on the human mammary carcinoma cell line BT20 (B) and the human colorectal carcinoma cell line WiDr (C). Symbols as in panel (A); imatinib-treated samples in panels (B) and (C) were not done in triplicate. Error bars indicate SD.

Overexpression of PDGF receptor β in RuSi RS1 cells but not c-Abl or c-Kit

To assess the expression of the potential targets of imatinib in RuSi RS1, BT20, and WiDr cells, we performed Western blotting for PDGFR, c-Abl, and c-Kit. Whereas c-Abl and c-Kit were expressed at similar levels in the three

cell lines, RuSi RS1 showed significantly higher expression of PDGFR- β compared to BT20 and WiDr (Fig. 2). Although overexpression of the α form of PDGFR in glioblastoma has been most frequently observed by others [8,9], we did not detect expression of PDGFR- α in any of the cell lines (data not shown). The high expression of PDGF receptor in RuSi RS1 glioblastoma cells is consistent with the concept that the radiosensitization effect of imatinib may be mediated through the disruption of a PDGF autocrine loop [13], but effects on c-Abl and c-Kit are also possible.

The principal target of imatinib in RuSi RS1 cells is PDGFR- β , not c-Abl

To further identify the kinase responsible for radiosensitization, we assessed the effect of imatinib on tyrosine kinase signaling in the three cell lines. Imatinib treatment caused a dose-dependent inhibition of tyrosine phosphorylation of a variety of cellular proteins in irradiated RuSi RS1 cells, while radiation alone did not result in any change in phosphorylation (Fig. 3A). To determine the positions of PDGFR- β , c-Abl, and c-Kit, identical membranes were immunoblotted with antibodies to these kinases (Fig. 3B). Comparison to the anti-phosphotyrosine blot revealed that imatinib treatment moderately decreased the tyrosine phosphorylation of proteins co-migrating with c-Abl and PDGFR- β . To confirm whether imatinib was inhibiting the phosphorylation of these kinases, the membranes were stripped and re-probed with phospho-c-Abl- and phospho-PDGFR- β -specific antibodies that detect specifically the activated forms of these kinases (Fig. 3C). There was no detectable phosphorylation of Tyr245 of c-Abl in RuSi RS1 cells in the absence or presence of imatinib, whereas Bcr-Abl was highly phosphorylated in control K562 cells. In contrast, dose-dependent inhibition of tyrosine phosphorylation of PDGFR- β in RuSi RS1 cells was observed with imatinib treatment. To confirm these findings, we immuno-

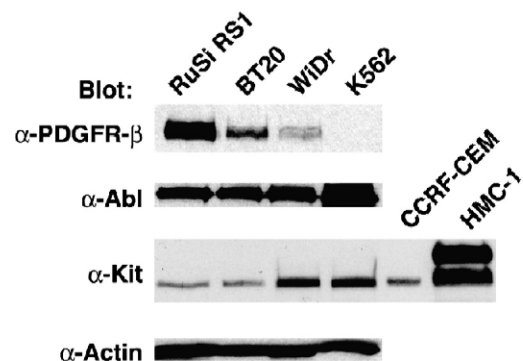


Fig. 2. Overexpression of PDGFR- β but not c-Abl or c-Kit in RuSi RS1 cells. Extracts from RuSi RS1, BT20, and WiDr cells were blotted with the indicated antibodies. Controls include the CML cell line K562 expressing Bcr-Abl, and human CCRF-CEM and HMC-1 cell lines expressing different levels of c-Kit.

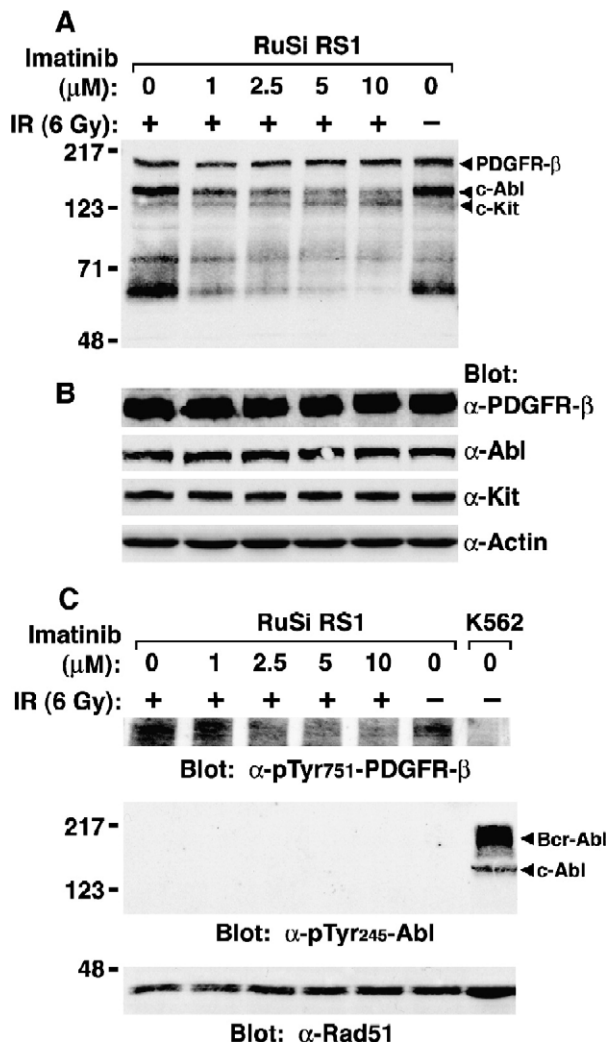


Fig. 3. Constitutive activation of PDGFR in RuSi RS-1 cells is inhibited by imatinib. (A) Anti-phosphotyrosine blot of extracts from RuSi RS-1 cells illustrating the dose-dependent inhibition of tyrosine phosphorylation by imatinib after irradiation with 6 Gy. Molecular mass markers are at the left. Arrowheads at right indicate positions of PDGFR- β , c-Kit, and c-Abl, as determined from direct blotting with antibodies to these proteins (B). (C) Immunoblotting with phospho-specific antibodies specific to activated PDGFR- β (top panel), activated c-Abl (middle panel), or antibodies to total Rad51 (bottom panel).

precipitated c-Abl and PDGFR- β directly from RuSi RS1 cells and blotted with anti-phosphotyrosine antibody. Significant inhibition of tyrosine phosphorylation of PDGFR- β was observed with 1 μ M imatinib that was nearly complete at 10 μ M (Fig. 4A), while c-Abl was tyrosine-phosphorylated at low levels that were only modestly decreased by imatinib (Fig. 4B). Together, these results suggest that the principal imatinib-sensitive kinase in RuSi RS1 cells that mediates the radiosensitivity response to imatinib is PDGFR- β , not c-Abl. While c-Abl can be secondarily activated downstream of PDGFR through phosphorylation of Tyr412 by c-Src [17], the lack of phosphorylation of Abl Tyr245 (Fig. 3C) and minimal change in overall Abl phosphorylation with imatinib (Fig. 4B) argue against a

significant role for c-Abl as a primary target of imatinib in glioblastoma.

Levels of Rad51 in glioblastoma cells are unaffected by irradiation or imatinib

Previous studies have suggested that the RecA homologue Rad51 may be induced by activated Abl in leukemic cells [18], while Rad51 expression may be increased by irradiation via an imatinib-sensitive mechanism in glioblastoma cells [19]. To assess the effect of irradiation and imatinib on Rad51 expression in RuSi RS1 cells, we performed immunoblotting with anti-Rad51 antibody. In comparison to K562 cells, RuSi RS1 cells showed only low-level expression of Rad51, and no significant change in Rad51 expression was apparent upon treatment with imatinib and/or irradiation (Fig. 3C, bottom). These results suggest that changes in Rad51 levels are not responsible for radiosensitization of glioblastoma by imatinib, at least in RuSi RS cells.

Conclusions

In summary, our findings demonstrate that imatinib mesylate can specifically increase the cytotoxicity of irradiation to glioblastoma cells, and argue that primary inhibition of PDGFR, rather than c-Abl or c-Kit, mediates this effect. Based on other studies, it is likely that an autocrine PDGF/PDGFR loop is largely responsible for the constitutive increased tyrosine phosphorylation in RuSi RS1 cells. Inhibition of this loop by imatinib only partially decreased the viability of glioblastoma cells, in contrast to the potent apoptotic effect of imatinib in Bcr-Abl-expressing cells [1]. However, low-dose imatinib treatment dramatically increased the sensitivity of glioblastoma cells to irradiation. The pathways downstream of PDGFR in this response are unknown, but candidates include Stat5, which is activated by PDGFR and implicated in radioresistance in Bcr-Abl-expressing cells [20]. Further studies, including in vivo models, are warranted to evaluate the effect of imatinib

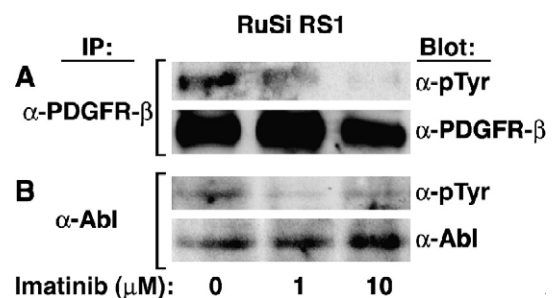


Fig. 4. Inhibition of activated PDGFR- β in RuSi RS-1 cells by imatinib. PDGFR- β (A) and c-Abl (B) were immunoprecipitated and blotted with anti-phosphotyrosine antibody (top panels) or the immunoprecipitating antibody (bottom panels).

on other glioblastoma cell lines and primary tumors and to promote its clinical development as a radiosensitizer in the treatment of patients with glioblastoma.

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