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Imatinib interferes with survival of multi drug resistant Kaposi's sarcoma cells

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Abstract Multi drug resistance (MDR) is defined as the ability of tumor cells to become resistant to unrelated drugs. Tyrosine kinase inhibitor imatinib has been demonstrated to be effective in the treatment of certain tumors. In particular, imatinib inhibits Bcr-Abl kinase activity, c-kit and the phosphorylation of platelet-derived growth factor (PDGF) receptors. In this work, we show that imatinib inhibits PDGF phosphorylation not only in *wt* Kaposi sarcoma (KS) but also in multi drug resistant KS cells. This was associated with an increased apoptosis in *wt* cells and an increased autophagy in MDR-KS cells. These data add new insights to the possible use of imatinib in the overcoming of MDR in KS cells.

Keywords: Imatinib; Kaposi's sarcoma; Drug resistance; Apoptosis; Autophagy

1. Introduction

Receptor tyrosine kinases have been proposed as potential targets for antitumor therapy. Imatinib mesylate (also known as STI571 or Gleevec, and hereafter called imatinib) belongs to the group of new drugs classified as signal transduction inhibitors and has been approved as an effective treatment for Chronic Myeloid Leukaemia [1]. Imatinib inhibits Bcr-Abl kinase activity, causing apoptosis in Philadelphia$^+$ cells and inducing cytogenetic remissions in the majority of CML patients [1]. Additional tyrosine kinases are inhibited by imatinib: c-kit, the receptor for kit ligand (KL) and the two structurally similar platelet-derived growth factor receptors (PDGFRs), PDGFR-α and PDGFR-β [1,2]. Results of recent clinical studies have shown that imatinib therapy is well tolerated and leads to remission in patients with c-kit-positive gastrointestinal stromal tumors (GIST) that contain gain-of-function mutations in c-kit [3]. Imatinib has also been reported to inhibit the growth of several tumors, including Kaposi sarcoma (KS), all of which may express the PDGF/PDGFR or KL/c-kit autocrine growth loops [4–11]. Kaposi's sarcoma is a multi-focal angioproliferative disease that occurs in HIV-infected patients and is a leading cause of mortality and morbidity in the acquired immune deficiency syndrome (AIDS) [12]. A number of cytokines and growth factors have been implicated in KS progression [13,4]. In particular, activation of PDGF and c-kit receptors has been proposed to play a role in mediating the growth of AIDS-related KS [4]. Furthermore, although a reduced incidence and regression of KS have been reported in AIDS patients treated with antiretroviral therapies or with chemotherapy (e.g. by Doxorubicin), the drug toxicity and the appearance of multi drug resistance (MDR) represent the main cause of therapeutic failure. Here, we show that imatinib mainly induces apoptosis in KS cells and, more interestingly, autophagy in MDR-KS cells.

2. Materials and methods

2.1. Cells culture and treatments

The wild type Kaposi's sarcoma cell line (*wt*-SLK) and doxorubicin (DOX) resistant MDR$^+$ cells (SLK–DOX) were kindly provided by Dr. BM Lucia and Prof. R. Cauda (Catholic University, Rome, Italy). The cells were grown in RPMI-1640 supplemented with 10% FCS, 2 mM glutamine and antibiotics. SLK–DOX cells were selected from the original *wt*-SLK cell line by exposure to 50 ng/ml of the drug. Drug resistance was maintained by adding relevant concentrations of the drug every 4 weeks. Cells were cultured in drug-free medium for at least ten days before experimental procedures. Degrees of resistance were assessed in terms of MTT assay [14] and the P-gp and/or MRP function [15]. Cells were treated with imatinib (Novartis) at different concentrations (15, 25 and 35 μM) in the growth medium at 37 °C in a 5% CO_2 atmosphere for 48 h.

2.2. Cell growth

Cell proliferation was analyzed by performing growth curves both in *wt*-SLK and SLK–DOX cells. Cell number was determined by counting cells daily using the trypan blue (GIBCO, Loughbolough, UK) exclusion test.

2.3. Analytical cytology

For static and flow cytometry analyses, control and treated cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 30 min at room temperature. After washing in the same

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Abbreviations: KS, Kaposi sarcoma; MDR, multi drug resistance; DOX, Doxorubicin; PDGFRs platelet-derived growth factor receptors; KL, kit ligand

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buffer, cells were permeabilized with 0.5 Triton X-100 (Sigma Chemical Co., St. Louis, MO, USA) in PBS for 5 min. For PDGFR-β (phosphorylated and non-phosphorylated) monoclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) directed against these antigens were used. For Bcl-2 and Beclin-1 (Santa Cruz Biotechnology) polyclonal antibody directed against these antigens were used. After 30 min at 37 °C, cells were washed and then incubated with an anti-mouse fluorescein-linked or anti-rabbit fluorescein-linked whole antibodies (Sigma). For apoptosis detection the nuclei were stained with Hoechst 33258 (Sigma) at 37 °C for 15 min. For a qualitative analysis all samples were mounted on glass cover-slips with glycerol–PBS (2:1) and analyzed by intensified video microscopy (IVM) with a Nikon Microphot fluorescence microscope equipped with a Zeiss CCD camera. Regarding flow cytometry analyses, all the samples were recorded with a FACScan flow cytometer (Becton–Dickinson, Mountain View, CA, USA) equipped with a 488 nm argon laser. At least 20000 events have been acquired. The median values of fluorescence intensity histograms were used to provide a semi-quantitative analysis.

2.4. Acid compartment detection

Autophagic vacuoles were labelled with the autofluorescent drug, monodansylcadaverine (MDC, Sigma), by incubating cells with 0.05 mM MDC in phosphate-buffered saline (PBS) at 37 °C for 10 min. Acid compartments were also labelled by incubating the cells with 1 μM LysoTracker (LTR, Molecular Probes, Eugene, OR, USA) in the culture medium at 37 °C for 15 min. Cells were then fixed with 4% paraformaldehyde (w/v in PBS) for 1 h at room temperature (25 °C). Cells were analyzed by IVM.

Regarding flow cytometry analyses, all the samples were evaluated with a FACScan flow cytometer (Becton–Dickinson) equipped with a 488 nm argon laser. At least 20000 events have been acquired. The median values of fluorescence intensity histograms were used to provide a semi-quantitative analysis.

2.5. Morphometric analyses

Quantitative evaluation of apoptotic cells was performed counting at least 300 cells at high magnification (500×) at the fluorescence microscope after Hoechst labeling.

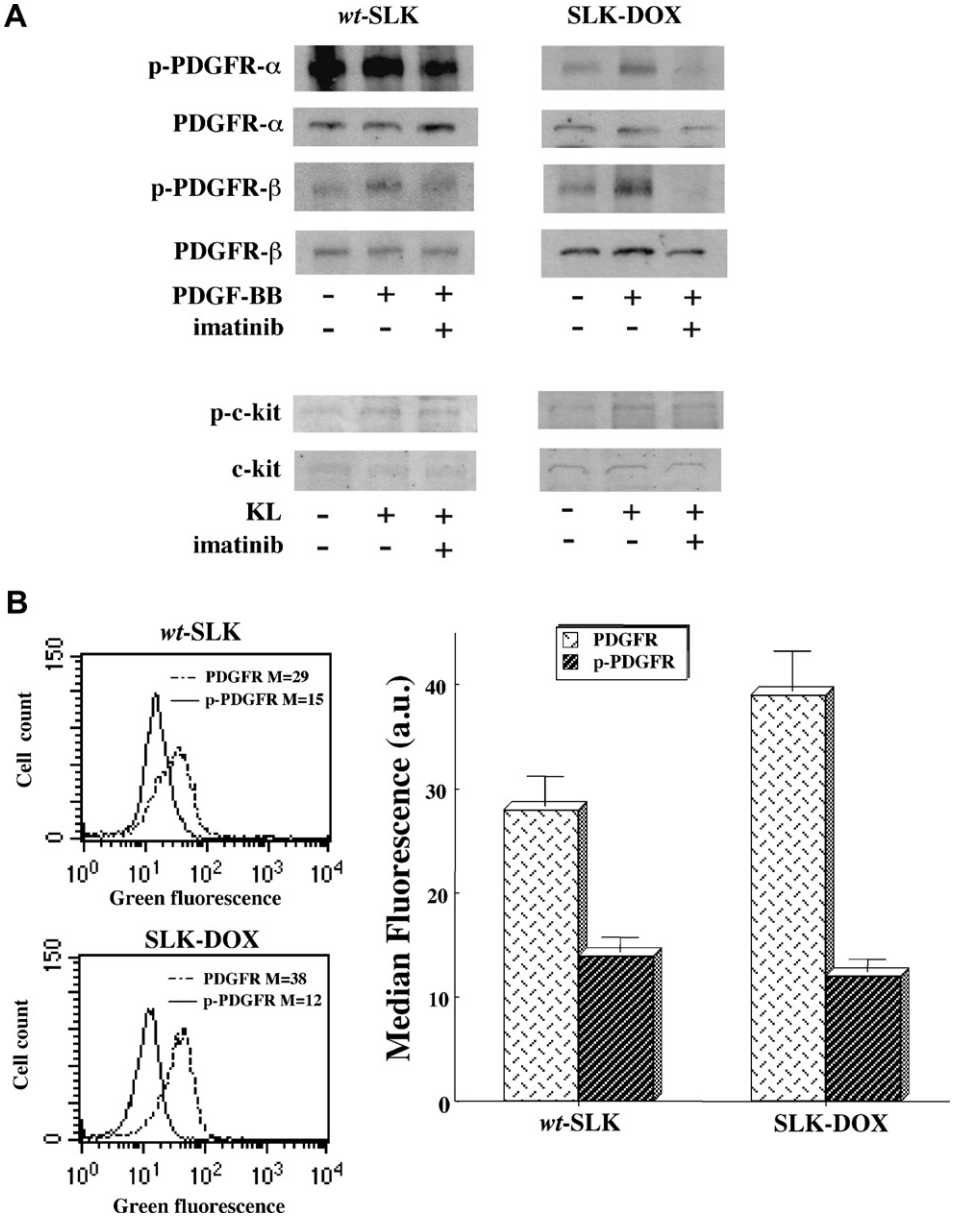

Fig. 1. Quantitative analysis of non-phosphorylated and phosphorylated PDGF receptors (α and β) and c-kit in both *wt* and resistant SLK cells by Western blotting (A). Flow cytometry evaluation of phosphorylated and non-phosphorylated PDGF receptors is shown in (B) as representative results (left panels) or as mean histograms (right panel, mean values ± S.D. from four independent experiments). Student's *t*-test to correlate samples was used.