

Targeting Genetically and Pharmacologically Rac GTPases in p190-BCR-ABL+ Acute Lymphoblastic Leukemia

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Significance and Background: The p190-BCR-ABL oncogene is associated with B progenitor (pro-B) acute lymphoblastic leukemia (ALL) of poor prognosis and poor response to tyrosine inhibitors (Imatinib). Rac GTPases (Rac1, Rac2 and Rac3) are molecular switches that integrate extra- and intracellular signals. Rac GTPases have been shown to be activated by BCR-ABL and a small molecule inhibitor (NSC23766), can specifically target Rac activation.

Methods: First, we analyzed the proliferation and survival of p190-BCR-ABL-expressing or control (empty vector)-transduced Ba/F3 cells in presence and absence of 100 μ g/mL NSC23766 and/or 1 μ g/mL Imatinib for 4-6 days. Second, we analyzed the effect of the same drugs on p190-BCR-ABL+ expressing B-ALL (bone marrow transduction/transplantation model) developed in a Rac-deficient murine model. Leukemic cells were cultured with NSC23766 and/or Imatinib and their content in B progenitors was analyzed by pre-B Colony Forming Unit (preB-CFU) assay and Witte-Whitlock long term cultures.

Results: NSC23766 distinctly inhibited cell expansion of p190-BCR-ABL expressing Ba/F3 cells (51% vs 28% in the control group and 38% in the Imatinib-treated group). The combination of NSC23766 and Imatinib decreased further the outgrowth of p190-BCR-ABL-transduced Ba/F3 cells (73%) due to both decreased proliferation (12.8-fold reduction in frequency of cells in S-phase) and decreased survival (50% apoptosis). Mice transplanted with p190-BCR-ABL WT cells developed leukemia in a period of 6-10 weeks (median survival \sim 50 days). Pre-B colony formation ability of primary murine p190-BCR-ABL+ ALL was completely abrogated by the combination NSC23766 and Imatinib (99.7% inhibition, Figure 1). While similar levels of inhibition were observed in delayed-onset Rac2-deficient ALL (median survival \sim 90 days), Rac1/Rac2-deficient p190-BCR-ABL ALL, as expected, showed an exquisite sensitivity to NSC23766 preB CFU formation inhibition (90.5%). Reversal of compactness of colonies from Rac1/Rac2-deficient, NSC23766-treated cells and only marginal inhibition of preB CFU expansion of Rac1/Rac2-deficient ALL in White-Whitlock assays, suggested that other stroma dependent and independent signaling pathways may be activated in the absence of Rac activation.

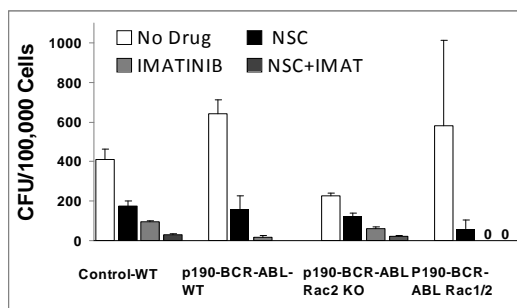


Figure 1. Genetic and pharmacological deficiency of Rac impairs p190-BCR-ABL+ leukemic progenitor outgrowth.

Conclusions: The results of this study indicate that Rac activation is necessary for the outgrowth of B-ALL induced by p190-BCR-ABL in vitro and in vivo and validate a new signaling pathway as a therapeutic target for BCR-ABL-induced B-ALL.