GM-CSF protection of CML CD34$^+$ cells from the inhibitory effect of imatinib

Pongtep Viboonjuntra

Melissa White Leukaemia Research Laboratory
Division of Haematology
Institute of Medical &Veterinary Science
And
Hanson Institute

&

Department of Medicine
The University of Adelaide
South Australia

A thesis submitted to the University of Adelaide
in candidature of the degree of Doctor of Philosophy
February 2006

ABSTRACT

Imatinib mesylate has become the front line therapy for CML. It inhibits the ABL tyrosine kinase activity of the causative oncogenic fusion protein BCR/ABL. The efficacy of imatinib therapy has been impressive, with a majority of patients achieving complete cytogenetic responses. However, to date imatinib therapy has been unable to completely eradicate the leukaemic clone in the majority of cases. This suggests that leukaemic stem cells may not be totally dependent on the activity of BCR/ABL, or that there may be other factors supporting the survival of leukaemic cells. Cytokines of the IL-3 family are involved in the survival and growth of myeloid haemopoietic cells. A number of the signals generated are in common with the pathways used by BCR/ABL and previous published studies showed that the IL-3 family member GM-CSF was found in elevated levels in CML patient serum. Therefore, the central aim was to test whether GM-CSF could modulate the response of CML progenitors to imatinib. In addition, a further aim was to examine CML cell production of GM-CSF. When CML CD34+ cells were exposed to imatinib, spontaneous BCR/ABL driven cell division was strongly inhibited, and cells became apoptotic. However, the addition of GM-CSF reversed this effect, returning cell division and survival back to spontaneous levels. GM-CSF specificity was confirmed using the antagonist E21R. The cell division and survival of leukaemic CD34+ cells in cultures of total CML mononuclear cells were less sensitive to imatinib inhibition. However, inclusion of E21R in such cultures resulted in a similar pattern to CD34+ CML cells cultured alone. This suggested that non-CD34+ cells may be a source of GM-CSF. This was confirmed by sorting CD34+ and non-CD34+ CML cells and examining GM-CSF production, which was markedly more apparent in the non-CD34+ compartment. Finally, the effects of GM-CSF on the survival and proliferation pathways induced by BCR/ABL were examined, showing it may exert its effects through p-Erk and p-Akt mediated mechanisms. Our data suggest that GM-CSF can signal via pathways which are normally suppressed due to alternative activation of major substrates by BCR/ABL, as well as others which are not activated by BCR/ABL, leading to cell survival when kinase activity is blocked by imatinib.

TABLE OF CONTENTS