STAT5 as a CML target: STATinib therapies?

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In this issue of Blood, Nelson and colleagues\(^1\) and Warsch and colleagues\(^2\) report that signal transducers and activators of transcription 5 (STAT5) is an attractive target to circumvent tyrosine kinase inhibitor (TKI) resistance in chronic myeloid leukemia (CML).

BCR-ABL activate many signaling pathways in leukemic cells, such as RAS, PI-3K and NF-κB. STAT5 was one of the first pathways to be described as being constitutively activated by p210 BCR-ABL and p190 BCR-ABL.\(^3,4\) STAT5 activation has been shown to correlate with functional effects such as antipapoptosis through activation of Bcl-X\(^\text{L}\)\(^6\) and drug resistance phenotype through activation of Rad51.\(^7\) BCR-ABL directly induces a tyrosine-phosphorylation and dimerization of STAT5 followed by nuclear translocation of the STAT5 dimers that then bind to consensus sequences through their DNA binding domain and promote activation of downstream target genes (see figure). In several experimental systems, STAT5 activation has been shown to be absolutely essential for leukemic cell survival,\(^8\) but there is no clear data with regard to its involvement in tyrosine kinase inhibitor-resistance of CML cells. The studies by Nelson et al.\(^1\) and Warsch et al.\(^2\) explore this important issue.

In the work by Nelson and colleagues, the authors used cell lines stably transfected with STAT5-responsive elements controlling a reporter (luciferase) gene and screened a chemical library. They identified the neuroleptic drug pimozide as a compound that appeared to specifically inhibit STAT5 phosphorylation (see figure) with no effect on STAT1 or NF-κB. This drug is a compound that has current Food and Drug Administration approval for the treatment of tics in Tourette syndrome.\(^9\) They showed that it acts in synergy with imatinib and nilotinib and surprisingly does not induce any dephosphorylation of BCR-ABL. On pimozide treatment there is also a down-regulation of the expression of several STAT5 target genes demonstrating a significant downstream effect. It appears therefore that pimozide is not a TK inhibitor but rather acts mainly by dephosphorylating STAT5. To determine the potential use of this compound to target specifically leukemic cells, experiments undertaken in cell lines have shown that pimozide has cytotoxic activity in leukemic cell lines but no cytotoxic effect in normal cells. Interestingly, pimozide also has growth inhibitory activity in CML cells with T315I mutation that confers resistance to all 3 TKIs currently in clinical use. Finally, pimozide also has activity against clonogenic CD34\(^+\) CML cells while sparing normal clonogenic CD34\(^+\) cells. The growth inhibitory effect of pimozide in CML cells seems to be the result of both cell-cycle arrest and increased apoptosis. The mechanism of action of the drug on STAT5 remains unclear; it remains to be determined if it involves the activation of a phosphatase that dephosphorylates STAT5, leading to its inactivation.

The role of STAT5 in TKI resistance in CML has also been studied by Warsch and colleagues, using a different approach. These authors used v-ABL transformed cell lines in which they overexpressed STAT5, STAT1, or STAT3. They show that STAT5 overexpression leads clearly to a TKI-resistant phenotype whereas STAT3 and STAT1 have no effect. High levels of STAT5 protected leukemic cells from TKI toxicity in the absence of JAK2 expression, suggesting that BCR-ABL (or v-ABL) induced JAK2 phosphorylation is not required for STAT5 activation in leukemic cells. The correlation with STAT5 expression and TKI sensitivity was further demonstrated using bone marrow cells from STAT5\(^{null/+}\) mice that express lower levels of STAT5 compared with wild-type STAT5\(^{+/+}\) mice. After retrovirus-mediated BCR-ABL gene transfer, STAT5\(^{null/+}\) cells exhibited increased sensitivity to TKI compared with their counterparts expressing higher levels of STAT5. Similarly, overexpression of STAT5A in v-ABL transformed B cells followed by imatinib treatment in vitro or in vivo led to imatinib-resistance only in cells with high STAT5A expression. Finally, in primary CML samples from patients in advanced CML, high levels of STAT5 mRNA levels have also been shown to correlate with TKI resistance and accordingly, STAT5A and STATB expression (2 highly homologous STAT5 gene products) were found to be increased in CML patients with advanced stage,
with or without ABL–kinase mutations. Overall, these results, in agreement with the report of Nelson et al, suggest that STAT5 phosphorylation is a marker of CML progression and it could be an attractive target to circumvent TKI resistance in CML.

The introduction of imatinib mesylate as a first line of therapy for CML has profoundly changed the prognosis of the disease but resistance occurs in 15%-20% of cases, and most importantly, the most primitive CML stem cells exhibit several mechanisms of resistance to TKI therapies. This explains the recent demonstration that human anti-2GPI antibodies from 3 patients with antiphospholipid syndrome (APS) into mice enhances the development of arterial thrombosis. In the Leiden thrombophilia study, anti-2GPI antibodies were associated with an odds ratio (OR) for thrombosis of 2.4, in comparison to increased viability of leukemic cells is an interesting concept especially in the context of bone marrow niche, which provides the leukemic cells with survival and/or quiescence signals. However, one of the important questions with STAT5 targeting will be the evaluation of a specific therapeutic window allowing targeting of leukemic cells without harming normal cells. In this context, the work by Nelson and colleagues clearly shows a selective effect with regard to leukemic progenitors compared with normal progenitors. From the clinical point of view, it will be important to determine the potential additive side effects of a given TKI and a drug such as pimozide, which might have cardiovascular and neurologic side effects. A clinical trial combining a TKI and a drug such as pimozide, which seems to induce the tyrosine phosphorylation and DNA binding activity of multiple specific STAT family members. Elevated anti-2GPI antibodies are thrombogenic.

REFERENCES

Comment on Arad et al, page 3453

Sutton’s law and anti-β2GPI antibodies

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In this issue of Blood, Arad and colleagues demonstrate that infusion of affinity-purified anti-β2-glycoprotein I (β2GPI) antibodies from 3 patients with antiphospholipid syndrome (APS) into mice enhances the development of arterial thrombosis after laser-induced vascular injury. This interesting observation directly demonstrates that human anti-β2GPI antibodies are thrombogenic.

Numerous targets of autoantibodies in patients with APS have been reported, although for most, their utility as predictors of thrombosis has not been rigorously validated. Antibodies against β2GPI were first identified more than 20 years ago, and have been the subject of several reports linking them to thrombosis. In the Leiden thrombophilia study, anti-β2GPI antibodies were associated with an odds ratio (OR) for thrombosis of 2.4, in comparison to an OR of 3.6 for the lupus anticoagulant and 1.4 for anti-prothrombin antibodies. A lupus anticoagulant in the absence of anti-β2GPI or anti-prothrombin antibodies was not associated with an increased risk of thrombosis, while in the presence of either of these antibodies the OR increased to 10.1. These findings are consistent with results of other studies demonstrating that prolongation of phospholipid-dependent coagulation assays in vitro by lupus anticoagulants is
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