Nature Reviews Cancer | AOP, published online 10 November 2011; doi:10.1038/nrc3173

THERAPEUTICS

Another tool in the BCR–ABL kit?

Despite the success of treating chronic myelogenous leukaemia (CML) with the tyrosine kinase inhibitors (TKIs) imatinib, nilotinib and dasatinib, which inhibit the BCR–ABL fusion kinase, resistance and suboptimal responses in patients with advanced disease are still problematic. The ABL kinase contains an SH2 domain, which, in addition to mediating protein–protein interactions, facilitates the activation of the adjacent tyrosine kinase domain. However, the importance of activation by the SH2 domain in the already highly active BCR–ABL protein is unclear.

Giulio Superti-Furga and colleagues initially showed that an SH2 mutant (T231R) that had previously been identified in imatinib-resistant patients with CML increased BCR–ABL autophosphorylation, as well as *in vitro* and *in vivo* kinase activity, when expressed in HEK293 cells. They also investigated an I164E mutation that disrupts the SH2–kinase domain interaction; expression of this mutant reduced BCR–ABL autophosphorylation and kinase activity. Importantly, the I164E mutation did not affect the structural integrity of the SH2 domain or its canonical function (binding phosphotyrosine). In addition, induced dimerization of the ABL kinase domain with the SH2 domain strongly activated kinase activity, and this was blocked by the I164E mutation.

To determine whether this mechanism of activation is important for leukaemogenesis, the authors expressed BCR–ABL I164E in primary mouse bone marrow cells. Lethally irradiated mice that were injected with cells expressing BCR–ABL I164E did not develop leukaemia, and a defect in engraftment or haematopoietic differentiation was ruled out. The investigation of downstream signalling events showed that phosphorylation of signal transducer and activator of transcription 5 (STAT5) was reduced following expression of BCR–ABL I164E, but ERK and AKT phosphorylation remained intact, indicating that the disruption of the SH2–kinase interface downregulates specific signalling pathways.

Cells expressing BCR–ABL I164E were more sensitive than those expressing wild-type BCR–ABL to imatinib and nilotinib. Moreover, BCR–ABL proteins with TKI-resistance mutations (including T315I) showed restored sensitivity to nilotinib when the I164E mutation was introduced, indicating that the SH2–kinase interface could potentially be targeted for therapeutic benefit. To do this, the authors identified a monobody (a single domain-binding protein based on the fibronectin type III domain), 7c12, that specifically binds the ABL SH2 domain. Although 7c12 inhibits kinase activity of wild-type, but not I164E, BCR–ABL, the authors improved the potency of this monobody by linking it to a previously discovered SH2-binding monobody, HA4, which blocks phosphotyrosine binding. HA4–7c12 inhibited *in vitro* kinase activity of BCR–ABL at a level comparable to that of the I164E mutant, and blocked BCR–ABL activation and induced apoptosis in a CML cell line. Furthermore, HA4–7c12 inhibited transformation of primary mouse bone marrow cells by BCR–ABL and increased apoptosis of primary cells from patients with either chronic or accelerated phase CML.

Although the necessary intracellular delivery of monobodies will probably prevent their clinical development, these results establish a rationale for targeting the SH2–kinase interface in BCR–ABL, which may be feasible with small molecules.

Sarah Seton-Rogers

> “mice that were injected with cells expressing BCR–ABL I164E did not develop leukaemia”

ORIGINAL RESEARCH PAPER Grebien, F. *et al.* Targeting the SH2-kinase interface in Bcr-Abl inhibits leukemogenesis. *Cell* **147**, 306–319 (2011)