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Minireview

Exploiting the promiscuity of imatinib

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Abstract

The protein kinase inhibitor imatinib, also known as Gleevec, has been a notable success in treating chronic myelogenous leukemia. A recent paper in *BMC Structural Biology* reports a 1.75 Å crystal structure of imatinib bound to the oxidoreductase NQO2 and reveals insights into the binding specificity and the off-target effects of the inhibitor.

Imatinib (also known as STI571 or Gleevec) is one of the great success stories of cancer therapy and is a milestone in small-molecule drug discovery and molecular targeted therapies. Imatinib is the current first-line therapy for all stages of chronic myelogenous leukemia (CML), in which the chronic phase of the disease is characterized by the increased proliferation of the myeloid lineage and which is cytogenetically diagnosed by the presence of the Philadelphia chromosome. This contains a fusion gene encoding the oncoprotein BCR-ABL, in which a part of the BCR protein is fused to the non-receptor ABL tyrosine kinase, causing it to become constitutively active. The deregulated kinase activity of BCR-ABL accounts for the oncogenicity of the protein and is inhibited by imatinib. Since the approval of imatinib by the US Food and Drug Administration in May 2001, there has been a dramatic reduction in the number of bone marrow transplants for CML in the US; imatinib monotherapy has also been used successfully to induce a complete cytogenetic response in about 75-90% of newly diagnosed CML patients, although drug resistance is a problem with advanced stages of CML [1].

Protein targets of imatinib

Imatinib is not entirely specific and targets tyrosine kinases other than ABL, notably the receptor tyrosine kinases KIT and PDGFR (platelet-derived growth factor receptor). This lack of specificity has been exploited in the clinic, and imatinib has also been approved for the treatment of chronic eosinophilic leukemia (CEL), which is caused by a FIP1L1-PDGFRα fusion, and for gastrointestinal stromal tumors (GISTs), caused by mutations of KIT or PDGFRα (reviewed in [1]). There has been increasing interest in understanding other potential targets of imatinib to evaluate the specificity, safety and potential off-target effects of this first-in-class drug.

The traditional approach to identifying imatinib targets is through *in vitro* assays with a panel of recombinant kinases to measure inhibitor binding. This approach is limited by the pre-selection of test targets and tends to provide poor indicators for drug activity *in vivo*. Two recent studies circumvent this problem by using cell extracts from the CML cell line K562 to identify binding targets of imatinib [2,3]. In the first [2], imatinib was modified to allow attachment to solid support and incubated with cell lysates, and the bound proteins were identified by tandem mass spectrometry. The second approach [3] used seven broad-specificity kinase inhibitors attached to beads. These mixed kinase inhibitor beads (kinobeads) were incubated with cell lysates and bound proteins were again identified by tandem mass spectrometry. To identify

imatinib targets, cell lysates were pre-incubated with imatinib before binding to the kinobeads. Proteins that no longer bound to the kinobeads, because their binding sites were occupied by imatinib, were identified by comparative analysis [3].

Both approaches have confirmed the selective nature of imatinib: no more than five proteins from K562 cell lysates bound imatinib as compared with about 30 proteins for another BCR-ABL inhibitor, dasatinib. The explanation for this difference is that dasatinib targets the active kinase conformation, which is highly conserved and thus shared by other kinases, whereas imatinib targets the inactive conformation, which is unique to the ABL kinase [4]. Surprisingly, both approaches also identified the first non-tyrosine kinase target of imatinib, NQO2. Others may follow: indeed, a recent screen in yeast identified the vacuolar ATPase (V-ATPase), an evolutionarily conserved proton pump, as a target of imatinib [5], and imatinib may interact with other non-kinase targets that could have eluded detection by the K562 cell-based experiments. Structural studies, including one just published by Kuriyan and colleagues in *BMC Structural Biology* [6], show that this promiscuity reflects the flexibility of the inhibitor, a consideration that is likely to apply broadly to small-molecule inhibitors.

Flexibility of imatinib in binding to targets

The Kuriyan group has analyzed the crystal structure at 1.75 Å of a dimer of human NQO2 bound to imatinib [6]. The objective of the structural study was to cast light on possible side-effects attributable to imatinib binding to NQO2, and a spectrophotometric assay confirmed binding of imatinib to NQO2 with a concentration for 50% inhibition (IC50) of 82 nM, consistent with earlier reports [2,3] and well within the physiological range of the concentration of imatinib found in the serum of patients (about 1 μM; referenced in [6]). NQO2 is a cytoplasmic flavoprotein that is involved in the cellular response to oxidative stress, although its mechanism of action is not well understood. NQO2 is highly expressed in myeloid cells, and knockdown by RNA interference in K562 cells results in reduced proliferation (referenced in [6]). However, NQO2 knockout mice show myeloid hyperplasia and increased sensitivity to chemical carcinogenesis (referenced in [6]). The potential clinical side-effect(s) of inhibition of NQO2, despite the elucidation of the exact mechanism of imatinib inhibition, are thus not yet clear. The structure does show, however, that the structural flexibility that allows imatinib to bind to NQO2 is also the basis for its binding to other, more clinically relevant targets.

Imatinib interacts with NQO2 primarily through hydrophobic interactions, making no direct hydrogen bonds (Figure 1a). Steric constraints lead imatinib to adopt a compacted horseshoe shape that partially extends into the solvent, and the isoalloxazine ring of the NQO2 flavin cofactor in the active site stacks with the pyridine and pyrimidine rings of imatinib. The related kinase inhibitor dasatinib cannot make this stacking interaction, and this explains why it does not bind to NQO2 [6]. The structure also demonstrates why imatinib cannot bind the closely related NQO1, which has 49% identity and a similar structure to NQO2: steric hindrance from Phe232, Tyr128 and Pro68 are likely to occlude the imatinib binding site [6].

Comparison of the NQO2-imatinib structure with that of imatinib bound to ABL (Figure 1a,b) reveals the flexibility of imatinib binding and demonstrates the difficulty in designing a drug that has no off-targets. In the ABL-imatinib complexes (reviewed in [4]), the DFG motif (Asp381-Phe382-Gly383) that characterizes the activation loop of the kinase is rotated by 180° and adopts a flipped-out conformation (Figure 1b). This DFG-out conformation creates a binding pocket for imatinib, which on binding causes the activation loop to fold towards the active site and at the same time induces contraction of the phosphate-binding P-loop, which also binds to imatinib [4]. Overall, imatinib uses six hydrogen bonds and several van der Waals interactions to stabilize the complex. In the imatinib-ABL complex, imatinib adopts an extended conformation that is seen in several other kinase-imatinib structures [4] and that differs significantly from the compact, ring-stacking conformation seen in the NQO2 structure [6].

The structures of imatinib bound to the SYK kinase, or to the desmethyl imatinib analog bound to SRC kinase, however, are similar to the conformation seen in NQO2, which shows that this is a minor but not a unique conformation for imatinib [6]. Future drug designs will need to account for the distinct conformations that a small molecule inhibitor can adopt if they are to understand the full range of targets that a drug can bind.

Clinical effects of imatinib

The multi-target specificity of imatinib has had many clinical benefits. As mentioned earlier, imatinib has been approved for the treatment of CML, CEL and GIST because of its inhibition of the BCR-ABL, PDGFRα and KIT tyrosine kinases (Figure 2). Recent clinical and preclinical studies have expanded the use of imatinib for the treatment of other diseases, including systemic mastocytosis, which also involves the KIT and PDGFR tyrosine kinases, and (in

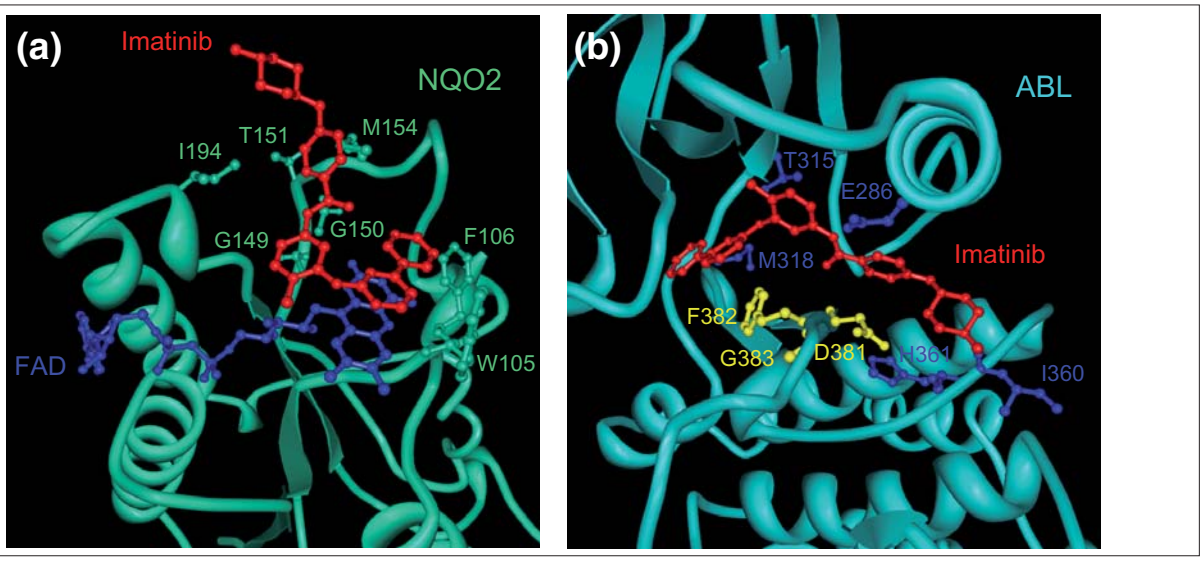

Figure 1
Atomic interactions of imatinib with **(a)** NQO2 (Protein Data Bank (PDB) code 1FW3) and **(b)** ABL kinase domain (PDB 1IEP). (a) A monomer of NQO2 (green) is shown bound to its cofactor FAD (blue) and to imatinib (red). Imatinib uses stacking interactions with FAD and makes hydrophobic contacts with both subunits in the NQO2 dimer. Only residues involved in hydrophobic interactions from a single monomer are depicted. (b) The ABL kinase domain (cyan) is depicted with the DFG motif (yellow) and residues involved in direct hydrogen binding (blue) either through side chains or the peptide backbone. D381 also makes a direct hydrogen-bonding contact with imatinib (red).

preliminary studies) fibrotic disorders (reviewed in [7]). It probably acts in fibrotic disorders through effects on the ABL tyrosine kinase, which has been implicated in TGFβ-induced fibrotic responses, and through the PDGFR: both of these are known to be involved in two major pro-fibrotic pathways activated in systemic sclerosis [7].

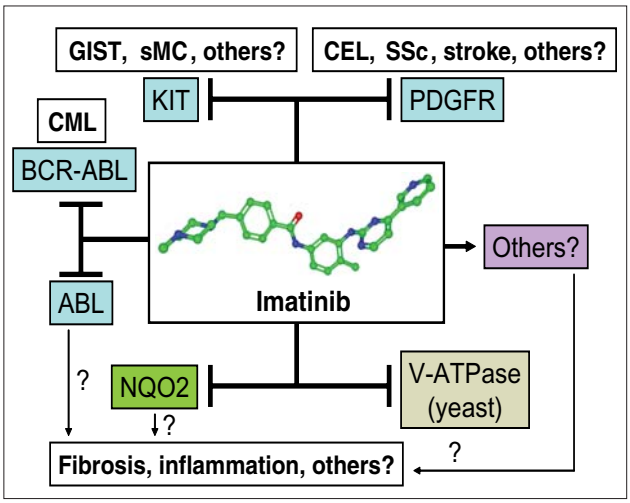

Figure 2
The promiscuity of imatinib allows its application in multiple diseases. The structure of imatinib (from PDB 1IEP) is shown in the middle, with carbon (green), nitrogen (blue) and oxygen (red) atoms displayed. Shaded boxes indicate imatinib targets; blue shading indicates targets that are tyrosine kinases. CEL, chronic eosinophilic leukemia; CML, chronic myelogenous leukemia; GIST, gastrointestinal stromal tumors; KIT, receptor for stem cell factor; PDGFR, platelet-derived growth factor receptor; sMC, systemic mastocytosis; SSc, systemic sclerosis; V-ATPase, vacuolar ATPase.

In mouse models, there is evidence that imatinib may be effective in the treatment of ischemic strokes [8] and in several inflammatory and autoimmune diseases [9]. The effects on inflammatory and autoimmune disease are consistent with phase I clinical studies and case reports detailing positive effects in rheumatoid arthritis, psoriasis, spondyloarthritis and Crohn's disease (referenced in [9]). A recent paper [9], again on a mouse model, reports that imatinib may be effective for the treatment of type 1 diabetes, largely through inhibition of PDGFR. Given the involvement of NQO2 in oxidative stress, it will be of interest to determine whether the inhibition of this oxidoreductase can contribute to the anti-inflammatory activity of imatinib.

Known mild adverse effects of imatinib include edema, muscle cramps, diarrhea and bone-marrow toxicity, and these do cause some patients to discontinue treatment [1]. Their cause is currently unknown. Cardiotoxicity has been reported as a potentially severe adverse effect of imatinib (reviewed in [10]): in this case the adverse effect seems to be due to inhibition of the primary target of imatinib, the ABL kinase, but the extent to which the cardiotoxicity results from imatinib treatment is controversial [10].

Imatinib has been shown to be a versatile drug with clinical benefit for treating CML, GIST and CEL and has potential for use in a variety of other diseases. Understanding how imatinib and other small-molecule drugs interact with their cellular targets is important for rational drug design and prediction of potential off-target effects. The limited promiscuity of imatinib may be optimal, allowing its use in a variety of diseases with mild adverse effects. Imatinib has revolutionized the treatment of CML and may be poised for more clinical successes.

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