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Chemoinformatics approach to Differential Drug Response of the Tyrosine Kinase Domain BCR-ABL to Imatinib in Chronic Myeloid Leukemia Patients

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Short Abstract: Chemoinformatics approach to differential drug response of the tyrosine kinase domain BCR-ABL to imatinib in chronic myeloid leukemia (CML) patients in order to investigate the effects of three substitutions (Tyr315Ile, Phe317Leu and Phe359Val) at direct drug contact sites in CML patients that develop imatinib resistance.

Long Abstract:

Chemoinformatics approach to Differential Drug Response of the Tyrosine Kinase Domain BCR-ABL to Imatinib in Chronic Myeloid Leukemia Patients

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The tyrosine kinase domain BCR-ABL is the fusion product of a reciprocal chromosome translocation between chromosomes 9 and 22, known as the Philadelphia (Ph) chromosome, and is present in the leukemic cells of more than 95% of patients with chronic myeloid leukemia (CML) (1). The tyrosine kinase inhibitor STI571 (imatinib/gleevec) is currently used in the treatment of CML patients. Differential drug response to this inhibitor in patients with CML has been associated with tyrosine kinase domain mutations (2) Fifteen different amino acid substitutions affecting 13 residues in the kinase domain have been identified in 29 out of 32 patients whose disease relapsed after an initial response to imatinib (3). Through computational analysis we have investigated the effects of three substitutions (Tyr315Ile, Phe317Leu and Phe359Val) at direct drug contact sites in CML patients that develop imatinib resistance.

The mouse (Mus musculus) tyrosine kinase domain structure in complex with imatinib (PDB ID: 1IEP) was obtained from the Protein Data Bank (PDB). The amino acid substitutions were introduced in the appropriate positions in the protein structure using the mutate tool of Deep View. The receptor geometry was optimized using the TINKER (4) package with the following parameters: AMBER99 force field, dielectric coefficient: 4.0, nobond cutoff: 14.0, and the steepest descent convergence method. Hence the most energetically favorable conformations for each structure were obtained.

The ligand (imatinib) was constructed using the program Ghemical (5), and its charges calculated with the AM1 (MOPAC 7.0) semi-empirical method through the ESP procedure.

Through the implementation of a genetic algorithm (GA) based on mechanisms of biological evolution, AutoDock 3.0 (6) allows efficient ligand positioning at the active site of the protein target. Rotation was considered between the bonds of carbon and the hydroxyl oxygen, and between carbons of the aromatic ring. In order to use the AutoDockTools program, hydrogens, Kollman atomic charges and solvent parameters were added. Hence AutoDock was used to predict how imatinib binds to the tyrosine kinase domain receptor, allowing comparisons between the mutants and the wild type receptor. AutoDock analysis was carried out with each mutant and imatinib, showing free energy of binding values greater than that obtained with the wild type (Table 1). Previous work on the biochemical characterization of imatinib resistance (2)has agreed with our results for the affinity between target and drug (Table 2).

Single nucleotide polymorphism (SNP) studies identify amino acid substitutions in protein-coding regions. Each substitution has the potential to affect protein function (7). Based on multiple alignment information, SIFT (Sorting Intolerant From Tolerant) predicts whether an amino acid substitution affects protein function, distinguishing between functionally neutral and deleterious amino acid changes on human polymorphisms. SIFT returns predictions on whether the substitutions are tolerant or intolerant based on the scores (8). SIFT predicted that two of the substitutions (Tyr315Ile and Phe359Val), which occur at highly conserved positions across species, are intolerant and therefore have a phenotypic effect (Table 1).

Table 1
TKD AutoDock GA (Kcal/mol) SIFT Score
Wild -7.71 -
Tyr315Il -2.86 intolerant (0.05)
Phe317Leu -7.16 tolerant (0.62)
Phe359Val -4.06 intolerant (0.00)

TKD: Tyrosine Kinase Domain

Table 2
TKD Biochemical IC50 (mM)
Wild 0.28
Tyr315Ile > 10
Phe317Leu 3.30
Phe359Val Not assessed

The results of our work indicate that the substitutions studied affect drug affinity at varying levels, suggesting the need for drug alternatives in the treatment of imatinib-resistant CML patients. Our results also confirm previous imatinib biochemical assays, showing varying degrees of resistance to imatinib caused by mutations in the receptor site.

Keywords: Chronic myeloid leukemia, Imatinib, SNP, automatic docking, Autodock, tyrosine

kinase, imatinib, Gleevec

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