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Can structural features of kinase receptors provide clues on selectivity and inhibition? A molecular modeling study

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ABSTRACT

Cancer is a complex disease resulting from the uncontrolled proliferation of cell signaling events. Protein kinases have been identified as central molecules that participate overwhelmingly in oncogenic events, thus becoming key targets for anticancer drugs. A majority of studies converged on the idea that ligand-binding pockets of kinases retain clues to the inhibiting abilities and cross-reacting tendencies of inhibitor drugs. Even though these ideas are critical for drug discovery, validating them using experiments is not only difficult, but also in some cases infeasible. To overcome these limitations and to test these ideas at the molecular level, we present here the results of receptor-focused in-silico docking of nine marketed drugs to 19 different wild-type and mutated kinases chosen from a wide range of families. This investigation highlights the need for using relevant models to explain the correct inhibition trends and the results are used to make predictions that might be able to influence future experiments. Our simulation studies are able to correctly predict the primary targets for each drug studied in majority of cases and our results agree with the existing findings. Our study shows that the conformations a given receptor acquires during kinase activation, and their micro-environment, defines the ligand partners. Type II drugs display high compatibility and selectivity for DFG-out kinase conformations. On the other hand Type I drugs are less selective and show binding preferences for both the open and closed forms of selected kinases. Using this receptor-focused approach, it is possible to capture the observed fold change in binding affinities between the wild-type and disease-centric mutations in ABL kinase for Imatinib and the second-generation ABL drugs. The effects of mutation are also investigated for two other systems, EGFR and B-Raf. Finally, by including pathway information in the design it is possible to model kinase inhibitors with potentially fewer side-effects.

1. Introduction

Cancer continues to be a major cause of death among the human diseases. As per the latest statistics [1], new US cancer cases and deaths continues to be a major health concern, and for 2014 they are estimated to be around 1,665,540 and 585,720 respectively. Hence, there is an urgent need for novel cancer drugs with fewer side effects. Protein kinases are enzymes that help carry out the transfer of a phosphate group (phosphorylation) from ATP to substrate proteins [2–4] and play an important role in cancer and signal transduction pathways. So far, approximately 518 kinase genes [5,6] have been identified in the human genome, which amounts to about 1.7% of the total 20-25 K genes. Protein phosphorylation is, in most cases, initiated by extracellular signals and acts like a switch to control a substrate's binding affinity, receptor's enzymatic activity, and the cellular location of their products. These actions can, in turn, influence down-stream gene/protein functions. Kinases also act as effectors and participate in a wide-range of important cellular functions ranging from cell cycle modulation, DNA repair, immunity, growth, and apoptosis. Since kinase activity influences many cellular functions, its regulatory role is usually kept under tight control. Kinase phosphorylation is a reversible reaction, and enzymes called phosphatases catalyze the removal of phosphate groups from substrate proteins and make it ready for the next cycle [4].

Kinase dysregulation, in majority of cases, is the result of unchecked activity resulting in over-expression of proteins or downstream genes, and such a state of increased activity has often been positively correlated with many human diseases such as cancers, metabolic disorders, and infectious diseases. Several events that modify kinases and leave them in elevated active states have been identified [7]. A short list of these events include mutation [8], up-regulation (gene amplification) [9], simultaneous expression

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of multiple kinases [10], and increased expression of transporters [11].

Kinase dysregulation can be best understood by studying Chronic Myeloid Leukemia (CML). CML is a unique disease caused by unregulated kinase activity of a single malfunctioning gene, BCR-ABL [12], and is commonly invoked to understand the uncontrolled behavior of kinases. This disease is caused by the aberrant genetic material called the Philadelphia chromosome, formed when a section of DNA from chromosome-9 translocates to a region after the BCR gene in chromosome-22. This creates a fusion gene, BCR-ABL, which expresses a protein product also called BCR-ABL [6,12]. Unlike normal ABL, the fusion protein is constitutively active and does not require activation by external signals. Hence, BCR-ABL remains active and participates in cell-cycle pathways, and strongly influences cell-division and growth processes, leaving the affected cell in a highly unstable state.

Other than aberrant genetic material formation, mutations in and around the kinase active site can also influence kinase activity [13]. For example, it had been observed that a small fraction of Philadelphia-positive (ph+) patients who are undergoing Imatinib therapy can relapse and offer resistance to the therapy. This resistance has been attributed to the mutations in the active site that reduces the drug's ability to bind and inhibit activity. Other forms of resistance to treatment have also been documented. Notably, tumor cells tip the balance of regulating phosphorylation–dephosphorylation in their favor by specifically down-regulating the phosphatase to indirectly overcome receptor inhibition [7].

Malfunction arising from a single gene such as BCR-ABL, can in turn affect other down-stream pathway genes to create non-local effects. Since most cancers are associated with dysregulation of multiple genes involved in different pathways, the effect of malfunction can spread more rapidly than the single gene disease to even affect far-away expressed gene products. Constitutional rogue kinase activity or malfunction due to mutations can be controlled by using either small molecule inhibitor drugs or humanized antibodies. The later approach of using a humanized antibody, Trastuzumab, to target ERBB2 receptors for treating breast cancer has shown some potential, but the benefit is not universal [14].

In cancer treatment, the idea of targeting multiple receptors using one or more drugs is not new [3,7,11,15,16]. Imatinib, a drug known for inhibiting BCR-ABL, has also been identified to inhibit platelet-derived growth factor (PDGF), stem-cell factor (SCF) and c-KIT receptors. Imatinib (marketed as Gleevec [http://www.gleevec.com]) is currently being prescribed as a primary drug for treating both CML [12] and GIST [11]. The justification for inhibiting multiple, but related, genes involved in disease proliferation has been clearly illustrated [7] using the tyrosine kinase inhibitor PP121. PP121 was shown to inhibit PI3K and its downstream gene mTOR simultaneously, thus providing additional benefit of blocking both the negative feed loop initiator (mTOR) and the oncogene, PI3K.

Using experimental and modeling data, a class definition for the inhibitors (Type I/II) based on their chemistry and ability to trap the kinases in an active or inactive conformation [17–22] has been proposed. It is not clear whether the inhibitors force the kinases toward a particular conformation or the receptors influence the drug molecules to take a specific binding pose. Recent studies [17,19,21,23] have identified several key issues in kinase inhibition. Of significance is the work by Davis et al. [18,19]; the most elaborate study known to date. These authors used 72 inhibitors to create a selectivity profile for more than 80% of the known 518 kinases. The important points arising from this study [19] are that the inhibitor Type (I/II) does not guarantee selectivity and, most importantly [24], the structural features that are presented by the receptor during the binding often controls selectivity and therefore the choice of the best inhibitor. These findings will most likely have a profound impact on basic research and future kinase drug development.

Table 1
List of kinase target receptors used in this study.

PDB ID	Drug name	EC #	Gene	UniProt	Res. (Å)
1OPJ	Imatinib	2.7.10.2	ABL	P00520	1.75
3UE4	Bosutinib	2.7.10.2	ABL	P00519	2.42
3GVU	Imatinib	2.7.10.2	ABL	P42684	2.05
3CS9	Nilotinib	2.7.10.2	ABL	P00519	2.21
2GQG	Dasatinib	2.7.10.2	ABL	P00519	2.40
1T46	Imatinib	2.7.1.112	KIT	P10721	1.60
3MIY	Sunitinib	2.7.10.2	ITK	Q08881	1.67
3K54	Dasatinib	2.7.10.2	BTK	Q06187	1.94
1XBB	Imatinib	2.7.1.112	Syk	P43405	1.57
2PL0	Imatinib	2.7.10.2	LCK	P06239	2.80
2ZVA	Dasatinib	2.7.10.2	Lyn	P25911	2.60
2OIQ	Imatinib	2.7.10.2	cSRC	P00523	2.07
3G5D	Dasatinib	2.7.10.2	cSRC	P00523	2.20
3GP0	Nilotinib	2.7.11.24	MAPK	Q15759	1.90
3GCS	Sorafenib	2.7.11.24	MAPK	Q16539	2.10
1XKK	Lapatinib	2.7.10.1	EGFR	P00533	2.40
1M17	Erlotinib	2.7.10.1	EGFR	P00533	2.60
2ITZ	Gefitinib	2.7.10.1	EGFR	P00533	2.80
3BBT	Lapatinib	2.7.10.1	ERBB4	Q15303	2.80
1UWH	Sorafenib	2.7.11.1	B-RAF	P15056	2.95

To test these findings, receptor structure focused self- and cross-docking simulations were performed using the experimentally available small molecule kinase drug-bound PDB structures (19 in total) to analyze the respective binding modes and their corresponding estimated affinities. Our results qualitatively agree with the known kinase inhibition profiles and that of Davis et al. [18,19]. Based on our data set, we find that receptor features dictate ligand inhibition and Type II ligands in general are competitive for DFG-out conformation. On the other hand, Type I ligands show less selectivity. Lapatinib appears to behave like a Type II molecule based on its strong affinity for inactive receptor folds but based on its atypical receptor conformation (DFG-out and c-alpha helix conformation) requirements [19], we have decided to place Lapatinib along with Type-II ligands but without a Type classification. Additional simulations carried out on the wild-type and mutated protein system, ABL, confirm that the receptor structural features can qualitatively capture the change in binding affinities [13], and might be useful for identifying potent family-specific inhibitors. The influence of mutations on the binding affinity was further analyzed using EGFR and B-Raf kinase systems and the docking results show the change in the binding affinity and mean binding energy indicative of the negative effect of selected mutations on binding. Finally, using the common pathway information from our gene set, we explore the possibility of single drug or drug combinations that might be useful for advanced stage cancers.

2. Materials and methods

2.1. Kinase systems

Protein kinase structures co-crystallized with drug molecules were downloaded from RCSB-PDB (http://www.rcsb.org/pdb/) and used for docking simulations. Kinase families studied in this work include ABL, KIT, ITK, BTK, SYK, LCK, LYN, MAP, EGFR, erbB4, and BRAF kinases (see Table 1). The small molecules examined in this study are Dasatinib, Erlotinib, Sunitinib, Gefitinib, Bosutinib, Lapatinib, Sorafenib, Nilotinib, and Imatinib (see Fig. 1). Note that these are marketed small molecule drugs currently being used for the treatment of different types of cancers and tumors. Each kinase family was searched in RCSB-PDB database for the availability of 3D structures that have been co-crystallized with drug molecules