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Molecular mechanism of D816X mutation-induced c-Kit activation and -mediated inhibitor resistance in gastrointestinal stromal tumor

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ABSTRACT

The D816X (X = V, H, Y or F) missense mutation constitutively activates c-Kit kinase in gastrointestinal stromal tumor (GIST) and has been observed to cause acquired resistance against first-line and second-line kinase inhibitors. In the present study, the allosteric mechanism of D816X-induced c-Kit conformational change is investigated at molecular level. The Asp816 residue is located at the activation loop (A-loop) of c-Kit and the mutation can eliminate a negative formal charge from the loop region by substituting the acidic asparagic acid residue with neutral valine, histidine, tyrosine or phenylalanine. Here, we classify the c-Kit kinase into four states in terms of its mutation (wild type or mutant) and conformation (DFG-in or DFG-out). The wild-type kinase is electrostatically stabilized in inactive DFG-out conformation, whereas the D816X mutation can promote the conformational conversion to active DFG-in and then activate the kinase. Structural analysis reveals that the Asp816 residue in DFG-out is surrounded by a number of polar and positively charged residues within its first and second shells of protein context, and kinase conformational change to DFG-in brings this residue into a negative electrostatic potential environment. Dynamics simulation characterizes that the c-Kit conformational conversion from DFG-out to DFG-in can cause local unfavorable effect to type-II inhibitor, while the mutation-induced global structural rearrangement would participate in the favorable interaction of c-Kit with type-I inhibitor.

1. Introduction

Stem cell growth factor receptor (SCFR), also known as proto-oncogene c-Kit or CD117, is a type III receptor tyrosine kinase protein encoded by the human *KIT* gene [1,2]. A variety of activating mutations are frequently observed in c-Kit; they play a crucial role in cancer occurrence by conferring constitutive activation to the kinase [3]. These mutations can be classified into regulatory type, which affects regulation of the kinase molecule, and enzymatic pocket type, which alters the amino acid sequence directly forming the enzymatic site [4]. Leukemia was identified as the first cancer linked to c-Kit activating mutation [5], but later studies found that the kinase is mostly correlated with gastrointestinal stromal tumor (GIST) [6,7], with 95% of all GIST cases that are c-Kit-positive and 80% harboring c-Kit activating mutation [8].

Over the two past decades, c-Kit-targeted therapy using small-molecule kinase inhibitors have been established as a standard therapeutic strategy for c-Kit-positive cancers [9]; the FDA-approved inhibitors Imatinib and Sunitinib have been widely used as the first- and second-line treatments of GIST, respectively [10]. However, some activating mutations were clinically observed to cause acquired resistance to these kinase inhibitors, thus largely limiting the c-Kit-targeted therapy [11]. The *KIT* gene located on human chromosome 4q11 and contains 21 exons, and the most common resistance-related mutation developed during GIST treatment occurs at the nucleotide position 2447 in exon 17, which corresponds to amino acid residue Asp816 in the activation loop (A-loop) of c-Kit kinase domain, resulting in a missense mutation D816X (X = V in >95% cases; X = H, Y and F in <5% cases) [11]. The mutation can release the autoinhibitory state of wild-type kinase and can cause acquired resistance to many kinase inhibitors, including the Imatinib and Sunitinib [12] —— although the second-line Sunitinib has shown efficacy against certain first-line Imatinib-

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resistant c-Kit mutants, the D816X remains resistant to the Sunitinib. Gajiwala et al. showed that Sunitinib targets the autoinhibited conformation of wild-type c-Kit, while the kinase D816H mutant undergoes a shift in conformational equilibrium toward the active state [13]. Later, Laine et al. found that the D816V mutation can induce a long-range structural reorganization of the kinase juxtamembrane (JM) region by weakening its interaction with kinase domain [14].

Previous study found that A-loop phosphorylation plays a critical role in the activation of many protein kinases [15]. However, DiNitto et al. demonstrated that the phosphorylation at A-loop Tyr823 residue is not required for c-Kit activation [16], although this phosphorylatable residue is bound to the catalytic base Asp792 in inactivated kinase, blocking the access of substrates to the catalytic site [17]. The phosphorylation introduces a negative charge to A-loop, whereas the D816X mutation eliminates the negative charge of Asp816 residue in the loop. Considering that the removal-of-charge mutation can activate the kinase and the gain-of-charge phosphorylation is not required for the kinase activation, the negative charge in A-loop seems to play an important role in the regulation of c-Kit activity. Previously, computational modeling has successfully employed to characterize mutation-induced protein conformational change and drug response to the mutation [18–20]. Following these studies we herein attempted to elucidate the electrostatic effect of D816X mutation on c-Kit kinase protein, as well as to explore the binding energetics of type-I and type-II inhibitors to the modeled active and inactive conformations of both wild-type and mutant kinases.

2. Materials and methods

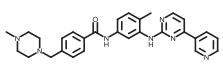
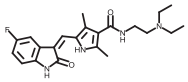
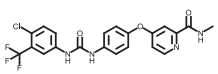
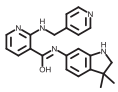
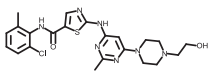
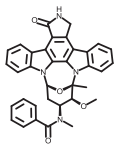
2.1. Collection of type-I and type-II c-Kit inhibitors

Two type-I and four type-II c-Kit kinase inhibitors are listed in Table 1. These inhibitors have reported to have moderate and high response (resistant or sensitized) to c-Kit D816V mutation at molecular or cellular level. The FDA-approved Imatinib and Sunitinib are cognate c-Kit inhibitors used for GIST and leukemia therapy, with sensitivity to wild-type c-Kit^{Asp816} but resistance to c-Kit^{Xxx816} mutant at molecular, cellular and clinical levels [21]. The Sorafenib and Motesanib have originally been developed to target VEGFR, PDGFR and Raf family kinases for treatment of diverse cancers, which were later found to inhibit c-Kit^{Asp816} but spare c-Kit^{Xxx816} [22,23]. The Dasatinib is an inhibitor of c-Src family kinases and also exhibits high inhibitory activity against c-Kit^{Xxx816} [24]. The PKC412 is an analog of the widely studied pan-kinase inhibitor Staurosporine, which has been found to selectively inhibit c-Kit^{Xxx816} over c-Kit^{Asp816} at cellular level [25]. These inhibitors can be classified into types I and II; the former (Dasatinib and PKC412) binds kinase in active DFG-in state and occupy the kinase active site, while the latter (Imatinib, Sunitinib, Sorafenib and Motesanib) induces kinase conformational conversion to inactive DFG-out conformation and extend to an additional hydrophobic region created by this conformation [26].

2.2. Structural retrieval and modeling

The crystal structures of wild-type c-Kit^{Asp816} kinase domain in active DFG-in and inactive DFG-out conformations were retrieved from the PDB database [27] with entry IDs 1PKG and 1T45, respectively (Fig. 1). We can directly modify the two wild-type crystal structures to c-Kit^{Xxx816} mutant in DFG-in and DFG-out states by virtual substitution of Asp816 residue with Val816, His816, Tyr816 or Phe816. The virtual substitution was carried out automatically by using the Voronoi-based BetaSCPWeb protein side-chain prediction server [28]. In this way, totally eight computationally modeled structures of four kinase mutants c-Kit^{Val816}, c-Kit^{His816}, c-Kit^{Tyr816} and c-Kit^{Phe816} in DFG-in and DFG-out conformations were obtained, which, plus the two crystal structures of c-Kit^{Asp816} DFG-in and c-Kit^{Asp816} DFG-out, define the

Table 1
Two type-I and four type-II c-Kit kinase inhibitors.

Inhibitor	Structure	Class	IC_{50} (nM)[a]		D816V response	PDB (kinase)[b]
			WT	D816V		
Imatinib		Type II	137	8100 [21]	resistant	1T46 (c-Kit)
Sunitinib		Type II	22	49 [21]	resistant	3G0E (c-Kit)
Sorafenib		Type II	2700	>5000 [22]	resistant	4ASD (VEGFR2)
Motesanib		Type II	36	>3000 [23]	resistant	3EFL (VEGFR2)
Dasatinib		Type I	79	37 [24]	sensitized	3G5D (c-Src)
PKC412		Type I	138	44 [25]	sensitized	4NCT (DYRK1)

[a] The reported inhibitory activity (IC_{50}) is measured at either molecular or cellular level.
[b] The co-crystallized kinase is given in parentheses.