In Silico Allosteric Network Predicts Variation in Response for Drugs Against Human Kinesin-5: Are All Anticancer Agents Created Equal?

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INTRODUCTION

Kinesin-5 is an essential motor protein for mitosis in eukaryotes.
Kinesin-5, also known as Eg5, is a plus-end directed motor protein. It contributes to the anti-parallel sliding of microtubules during mitosis [1]. Overexpression of Eg5 has been seen in pancreatic, lung, prostrate, breast, and bladder tumors [2]. Aberrant kinesin-5 activity results in apoptosis. It couples chemical work (ATP hydrolysis) with mechanical work to move along cytoskeletal tracks. As the active and microtubule (MT) binding sites are on opposite faces of the kinesin motor domain, mechanotransduction is allosteric.

Human kinesin-5 is a target for anticancer therapies; small-molecule screens uncovered Eg5 inhibitors, such as monastrol and STC, that targeted only kinesin-5 and none of the other members of the kinesin superfamily.
These anticancer therapies are allosteric inhibitors that bind within the same allosteric binding pocket within loop5. However, the inhibitors bind to different residues and therefore have a different statistical coupling analysis (SCA) network. SCA identifies co-evolution within a polypeptide chain by monitoring amino acid distributions across a sequence alignment via a series of perturbation experiments [3]. SCA has been experimentally validated in several protein families [for example, (4-9)].

The goal of this project is to determine which energetically-linked SCA residues are essential for inhibitor potency against this mitotic kinesin target.
The SCA algorithm will predict which residues that are distant from the inhibitor binding site can alter potency of an inhibitor. Such drug response prediction will be tested by variation in potency (half-maximum inhibitory concentration or IC_{50}) with two different inhibitors that bind to the same site of Eg5.

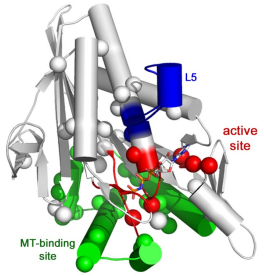


Fig. 1. The 56 coupled residues detected by SCA are represented as C_α spheres on a Kinesin-5 crystal structure (PDB 3HQD). Three functionally important sites within the motor domain are colored and labeled.

SIGNIFICANCE

Tailoring drugs to patients is one of the ultimate goals of precision medicine.
Patient-to-patient variability in drug response is a primary challenge facing development and use of medicines.
Use of molecular signatures for targeted genes has been useful in precision oncology [2].
Recent efforts to understanding such variability involves genotyping coupled with systematic measurement of dose-response across a large bank of cell lines [11].
Computational algorithms have advanced predicting drug response by integrating genetic features and chemical structure information.

Our hypothesis is that a kinesin residues identified by statistical coupling analysis (SCA) can predict inhibitor response to Eg5, without the need of empirical measurement.
Of the 56 SCA residues identified in kinesin mechanotransduction, 8 have known missense polymorphisms and 14 have synonymous polymorphisms in the human population (Table 1) [12]. In contrast, 6 residues in the L5 loop have missense mutations in the humans (data not shown).

Table 1. Missense and synonymous Eg5 polymorphisms found in human population. The Genome Aggregation Database (gnomAD) catalogue of human genetic variation was used to evaluate 15,708 whole genomes and 125,748 exomes; this totals 141,456 unrelated individuals who are part of various population genetic and complex disease-specific studies.

SCA residue	mutation type	2° structure	motor domain site
I319M	missense	α5	MT-binding
R327C	missense	L13	
R327H	missense	L13	
I332V	missense	β8	
S348N	missense	α6	MT-binding
E351Q	**missense**	**α6**	**MT-binding**
K357N	missense	α6	MT-binding
I359V	missense		necklinker

SCA residue	mutation type	2° structure	motor domain site
P27	synonymous	N-4	active site
E32	synonymous	α0	
Y82	synonymous	α1	
N98	synonymous	β3	
L160	synonymous	β4	central β- sheet
S237	synonymous	β6	central β- sheet
L261	synonymous	β7	central β- sheet
R281	synonymous	α4	MT-binding
A285	synonymous	α4	MT-binding
L293	synonymous	α4	MT-binding
H308	synonymous	L12	
T349	synonymous	α6	MT-binding
E351	synonymous	α6	MT-binding
A356	synonymous	α6	MT-binding

We selected a subset of SCA and non-SCA residues to measure the allosteric effects of STC and monastrol

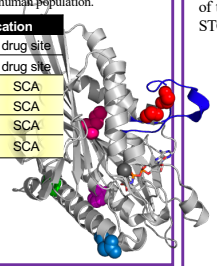
We selected a subset of SCA and non-SCA residues in various areas of the motor domain including the active and microtubule-binding sites to measure the effects of the inhibitors (Fig. 2).

We introduced five different mutations into wildtype Eg5 plasmid. We considered the mutations to be properly folded if expression and purification profiles were substantial and comparable to wildtype.

Using a bacterial expression system, the Eg5 mutations and wildtype were expressed, harvested, and then purified to >95% in a single chromatography step.

Table 2. Residues tested are shown in color in the Eg5 crystal structure (PDB 3HQD). Asterisk denotes mutation found in the human population.

Mutation	Location	
D130V	L5	drug site
A133D	L5	drug site
L263F	β7	SCA
D279G	α4	SCA
V298G	α4	SCA
E351Q*	α6	SCA



Mutations of L5 loop residues result in resistance to both inhibitors

Residues in the L5 loop upon mutation result in cancer cell resistance to targeted small molecule inhibition. Notably, these mutations do not prevent drug binding, just allosteric communication through the motor. We measures the ATP hydrolysis rate using a NADH-coupled assay (Fig. 3A)

Dose response curves of A133D and D130V confirmed that these L5 loop mutants have higher IC_{50} values for monastrol and STC (Fig. 3B).

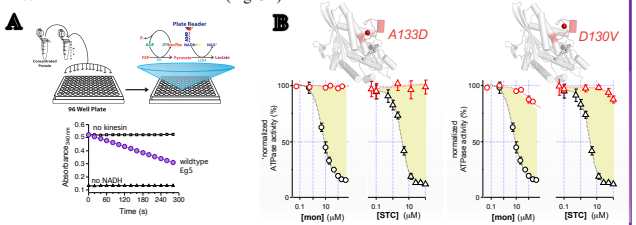


Fig. 2. (A) Scheme showing NADH-coupled reactions used to monitor ATP hydrolysis; pyruvate kinase and lactate dehydrogenase are used to regenerate ATP and monitor NAD+ production, respectively. Plot of NADH oxidation monitored at 340 nm in H2O at room temperature and pH 7.4. Reactions shown are wildtype Eg5 kinesin (gray with purple outline), with no NADH control, and with no motor control. **(B)** Dose response curves of WT (black), A133D (red, left), and D130V (red, right) in the presence of increasing concentrations of monastrol (circles) and STC (triangles).

Perturbation of the SCA network by mutation altered response to only one of the two inhibitors

We tested whether disrupting the SCA communication pathway would alter the concentration of the drug that decreases the maximal rates of kinesin catalysis by 50% (IC_{50}) would be altered. Mutation of these kinesin allostery network residues resulted in a ten-fold lower IC_{50} for STC, indicating that STC is a more potent inhibitor in three mutant proteins.

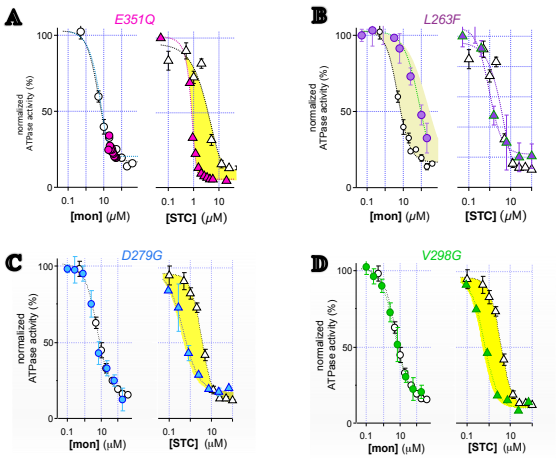


Fig. 3. Dose response curves of **(A)** E351Q, **(B)** L263F, **(C)** D279G, and **(D)** V298G in the presence of increasing concentrations of monastrol (mon) and STC. Dark yellow shading highlights that these beta-7 mutant that has a higher IC_{50} value for monastrol. Bright yellow shading highlights that these alpha-4 and alpha-6 helices mutants have lower IC_{50} values for STC.

Mutation of SCA residues at the microtubule-binding site gave rise to hypersensitivity to only one inhibitor, STC, whereas mutation at the motor core gives resistance to monastrol.

SCA residues in the microtubule-binding site mark an STC-exclusive allosteric pathway and may delineate the mechanism of STC-specific inhibition of Eg5 depolymerization activity [10].

CONCLUSIONS

Kinesin SCA network defines residue positions that give rise to resistance or hypersensitivity to allosteric inhibitors of the human Eg5 mitotic kinesin.

Although both monastrol and STC bind to the same L5 site, they do not have identical allosteric pathways of communication.

SCA network residues D279, V298 and E351 in the alpha-4 and alpha-6 helices are hypersensitive to only STC. These data demonstrate that an *in silico* map of mechanotransduction can be used to predict an individual's response to an anticancer therapeutic.

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