Molecular Dynamics Use in Personalized Cancer Medicine
Example of MET Y501C Mutation

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Abstract: We explored a possible new method for prediction of activating mutations in cancer-related proteins. This method is based on elucidation of flexibility of proteins associated in activating complexes. Based on the theory of intermediate binding complexes, the binding process is not only related to the three-dimensional structure of proteins, but also to the four-dimensional set of possible conformations allowed by the flexible regions of the involved members of the associated complex. Using molecular dynamics simulations, we found that an Y501C mutation in the MET gene might activate it. Using this information, a specific drug that functioned as a potent MET inhibitor was prescribed and had a salutary impact on the tumor.

1 INTRODUCTION

Elucidation of possible changes in protein activity based on an aberration in its sequence is one of the most important tasks of personalized medicine (Tsigelny et al., 2015). One of the most powerful databases in cancer medicine is Cosmic (Forbes et al., 2011). Even if one would extract only the 50 most frequent mutations in cancers in 600 proteins (which are used most frequently for cancer diagnostics), it would give 30000 aberrations. In some cases, such aberrations are described in the literature and related databases. Nevertheless, more than 90 percent of them are not covered. Here, we address the problem of how to elucidate possible activity changes that occur due to these mutations. Replacement of a residue in a pdb file may help if we have a simple case when the aberration effect is obvious: change of a charged residue to an oppositely charged residue in a salt bridge, or insertion of a hydrophobic residue instead of a hydrophilic residue that participates in hydrogen bonding etc. At the same time, the effect of a residue substitution might not be that obvious in other cases. Here molecular dynamics (MD) simulation might help elucidate the changes in the ensemble of conformers that could affect the activity of the protein or protein complex.

2 MET STRUCTURE AND FUNCTION

The tyrosine kinase MET is a receptor for the ligand–hepatocyte growth factor (HGF). It is known to be involved in cancerogenesis. When activated (in many cases because of a single amino acid replacement mutation), it affects cells in a number of organs creating invasive cancers (Stamos et al., 2004, Montesano et al, 1991). The MET receptor has significant structural similarity to Ron and Sea receptors (Ronsin et al., 1993; Huff et al., 1993, Stamos et al., 2004).

In order to be activated, MET requires binding of its ligand—hepatocyte growth factor (HGF) that is active only after proteolytic conversion to a two chain configuration (Stamos et al., 2004; Hartmann et al., 1992). Direct binding sites show that HGF-beta chains bind to the extracellular domain of MET with a K_d of about 90 nM. Accordingly, binding of HGF is crucial to activation of MET (Figure 1). Analysis of the MET–HGF interface shows a set of moderately complementary side chains on both sides.

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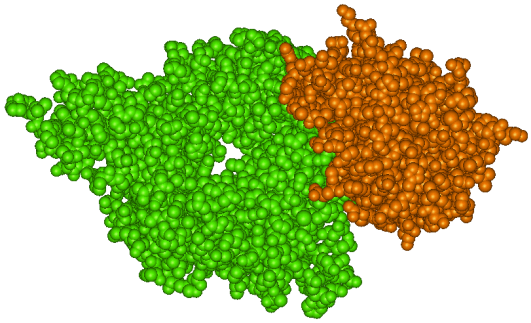

Figure 1: Interaction of the extracellular Sema domain of Met (green) with the HGF-beta chain (brown).

2.1 Sema Domain

The Sema domain of MET forms a conformation sometimes referred to as "seven-bladed beta-propeller" in the shape of a funnel with an inner diameter of 25 Å and a total diameter of around 50 Å. The blades of this propeller are formed by antiparallel beta-strands. The Sema domain is stabilized by the interactions between the C- and N-terminal residues, and the beta-propeller structure is stabilized by the seven disulphide bridges also found in a number of proteins with notable homology in the amino acids included in these domains. The HGF beta-chain associates with the Sema domain at the bottom face of the propeller with at least seven electrostatic pas interactions between the two proteins (Stamos et al., 2004).

In the studied case, there is a mutation Y501C in the Sema domain of MET. This residue is located at the interface between the C- and N-terminal of the Sema domain. From the general point of view used in elucidation of possible activity changes of the MET–HGF complex, a substitution of tyrosine to cysteine would not make any changes in activity unless cysteine is involved in a disulphide bond (not in this case). Another possibility is that this mutation happens in the N-C-terminal interface of the Sema domain and affects its flexibility.

3 FLEXIBILITY OF BINDING PROTEINS IMPROVES THEIR ASSOCIATION

As we pointed above, the activation of the MET–HGF complex significantly depends on interaction between these two proteins. As was shown by Levi and colleagues (2005), who studied more than 100 protein–protein complexes, the flexibility of the binding partners is one very important feature that often defines the process of protein–protein association. In other words, the binding process is not only related to the three-dimensional structure of proteins, but also to the four-dimensional set of possible conformations adopted by means of the flexible regions of the involved proteins. It is interesting to note that so-called transition-state conformational ensembles for general folding of proteins and their binding have similar characteristics (Levi et al., 2005). Taking into consideration the abovementioned concept, we hypothesized that increasing flexibility of the Sema domain of MET would increase its interactions with HGF and consequently improve binding between these proteins and thereby increase activation of the entire complex.

4 MUTATION Y501C AFFECTS THE FLEXIBILITY OF THE SEMA DOMAIN

In order to elucidate possible changes in flexibility of the Sema domain, we conducted 300 ns MD for the wild-type and mutated versions of the protein. Our results show significant increase in flexibility of several important parts of the Sema domain (Figure 2). The violet rectangles encompass the most flexible regions of the mutant structure. It is interesting to note that the maximum flexibility changes occur in the

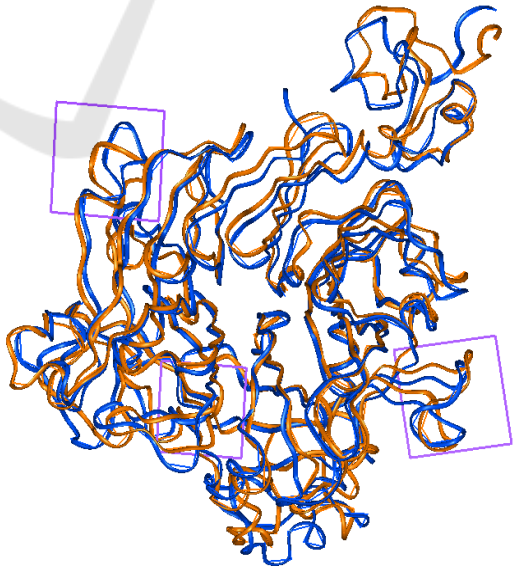

Figure 2: Superposition of the 300 ns conformers, with the wild-type represented by brown ribbons of the alpha-trace and the Y501C mutant of the Sema domain of MET shown as blue ribbons.

regions of the Sema domain that are in direct contact with the HGF protein when bound (Figure 3). Note that the regions around residues 150 and 210 of the Sema domain are in direct contact with HGF residues during its binding. These results suggest that the mutation Y501C of MET leads to a significant increase in the flexibility of the MET Sema domain in the regions contacting HFG. Such changes may improve the binding and consequently the activity of the MET–HGF complex.

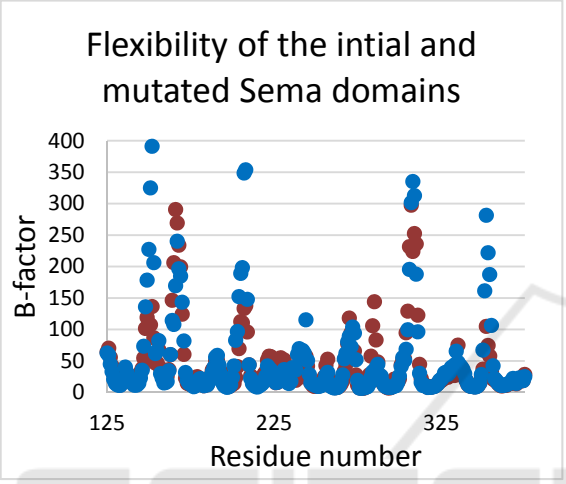

Figure 3: Flexibility (defined by B-factor values) of the Sema domain residues in the wild-type (brown) and Y501C mutant (blue) conformers as calculated from a 300 ns MD trajectory.

5 PATIENT TREATMENT BASED ON THE MD SIMULATIONS

A patient was diagnosed with hepatocellular carcinoma (HCC), with the MET Y501C (tyrosine to cysteine) missense mutation that was elucidated from circulating tumor DNA. Based on the results of MD simulation, we suggest that this mutation activates MET kinase and consequently has oncogenic effects. The patient received cabozantinib—a MET inhibitor. This drug administration caused significant (65%) reduction of alphapheto protein (AFP), a tumor marker for HCC.

6 CONCLUSIONS

Molecular dynamics simulations can be used to elucidate the four-dimensional ensembles of possible conformers and flexibility of the binding partners in protein–protein complexes and can help in decision making for physicians in cancer therapy.

7 METHODS

In order to generate the Y462C Sema domain mutant, Tyr462 in the wild-type protein was replaced by a cysteine while the rest of the protein structure remained unchanged. Disulfide bridges between the relevant cysteine pairs in each protein were generated. The SEMA domain of MET protein has been extracted from the complex with heparin (Stamos et al., 2004) pdb ID 1shy.

We conducted molecular dynamics (MD) simulations for both the wild-type and mutated versions of Sema domain of MET. The two proteins were each placed in an octahedral water box consisting of about 48,500 TIP3P water molecules and 9 neutralizing K^+ ions modelled by Joung/Cheatham ion parameters (Joung and Cheatham, 2008). The simulations were conducted using the GPU/CUDA-accelerated version of PMEMD implemented in the AMBER14 software suite (Case et al, 2014; Goetz et al., 2012; Salomon-Ferrer et al., 2013A, B), with the protein described by AMBER ff14 SB parameters. Each of the two protein systems were subjected to the following minimization/simulation steps: i) Unrestrained minimization for 10,000 steps; ii) Gradual constant volume heating from 0 to 100 K over 5 ps with restraints applied to the protein backbone; iii) Gradual constant pressure heating to 310 K over 100 ps with restraints applied to the protein backbone; iv) 300 ns unrestrained constant pressure simulation at 310 K.

The Langevin thermostat (Loncharich et al., 1992) was applied for regulation of temperature with a 1.0 ps^{-1} collision frequency, and the pressure was regulated isotropically during the second heating step and the production simulation by means of the Berendsen barostat (Berendsen et al., 1984) at a reference pressure of 1.0 bar. Bond lengths for bonds involving hydrogen were constrained using the SHAKE algorithm (Rycjaert et al., 1984), allowing for a time step of 2 fs. Periodic boundary conditions were applied, and the particle mesh Ewald (PME) method (Roe et al., 2013) was used for the evaluation of electrostatics.

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