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Designing HSP90 inhibitor drugs against mutations profiles of cancer patients

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Abstract

Background: Effective drug binding to its specific receptor determines the therapeutic effect. Tumor cells employ Heat Shock Protein 90 (Hsp90) to survive as Hsp90 prevents apoptosis. The protein is the top expressed protein among others. This expression rate further increases in tumor cells and provides a fine target for drug design. However, mutations and copy number variations, single nucleotide polymorphisms of the target gene as well as strong binding of therapeutic agents to the target provide adverse effects.

Aims: This work investigates HSP90 interaction with common inhibitor geldanamycin through key mutations to understand possible effects.

Methods: *In silico* analysis by YASARA software was employed to reveal interactions between HSP90 WT and its mutant along with geldanamycin.

Results: One key inhibitor-resistant critical residue interaction was monitored for each cytosolic HSP90 isoform. The residues are at critical positions during conformational changes and at the mutated regions of the protein. However, finely designed geldanamycin still tolerates its binding to HSP90.

Conclusion: HSP has no introns and may seem non-mutable, but designing inhibitors by considering structural alterations increases the effectiveness of the drug candidates and limits side effects through *in silico* experiments.

Keywords: HSP90; Geldanamycin; in silico; Cancer

1. Introduction

Cancer cells survive with distinct mechanisms. The cells not only induce mutations at checkpoints, tumor suppressor genes but also trigger the expression of Heat Shock Proteins (HSP). Hsp plays essential biochemical roles and in tumor biology as Hsps fold substrate proteins. The native state of a protein determines its biochemical activity, therefore, protein homeostasis is important to maintain their biologically active conformation [1]. Signaling molecules at tumor cells play key roles in proliferation, growth, and metastasis. Due to control mechanisms, cancer cells tend to force proteins to be mutated and the folding burden of these mutated substrate proteins forces more folder protein (Hsp) expression. The result of the process is two-fold: substrate protein folding to its native state and prevention of apoptosis through Hsp proteins [2]. Hsp family consists of different family members and is named after their molecular weight: Hsp27, Hsp40, Hsp60, Hsp70, Hsp90, Hsp100. Hsps coordinate and cooperate to form distinct functions. Hsp70 has 13 members and Hsp40 has 60 members known. Each forms different Hsp70-Hsp40 complexes for distinct functions [3].

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These universally conserved proteins among organisms ranging from Archaea to Homo sapiens are interesting to drug designers. Under physiological conditions and stress, Hsps or so-called chaperones not only fold proteins but also prevent protein aggregation and play essential roles in intracellular protein transport. Hsps are intronless and this may make them resistant to mutants. However, cancer cells are effective and may even mutate tumor suppressors. Therefore, mutational work on Hsps is essential [4].

Hsp structures are generally formed from two distinct domains: ATPase domain and substrate protein folding domain. Hydrolysis of ATP provides energy for conformational changes of substrate protein binding domain and opening-closing of the domain provides a closed space for proper folding. The closed environment provides a hydrophobic region and this is ideal for substrate protein folding. However, not all Hsp structures depend on ATP hydrolysis. However, coordination and cooperation of proteins are required [5]. Hsp70 process unfolded proteins by seven residues per action and submit that to Hsp90 where the 30 kDa substrate protein may be folded to the native structure. Hsp40 cochaperone along with Hip/Hop proteins play essential roles at Hsp70-Hsp90 coordination. Further, nucleotide exchange factors play a fine role in removing ADP from the pocket so that a new cycle may start. If the substrate may not be folded simultaneously then the substrate is forwarded to degradation by the proteasomal system. This process is decided through the Hsp system as well [6].

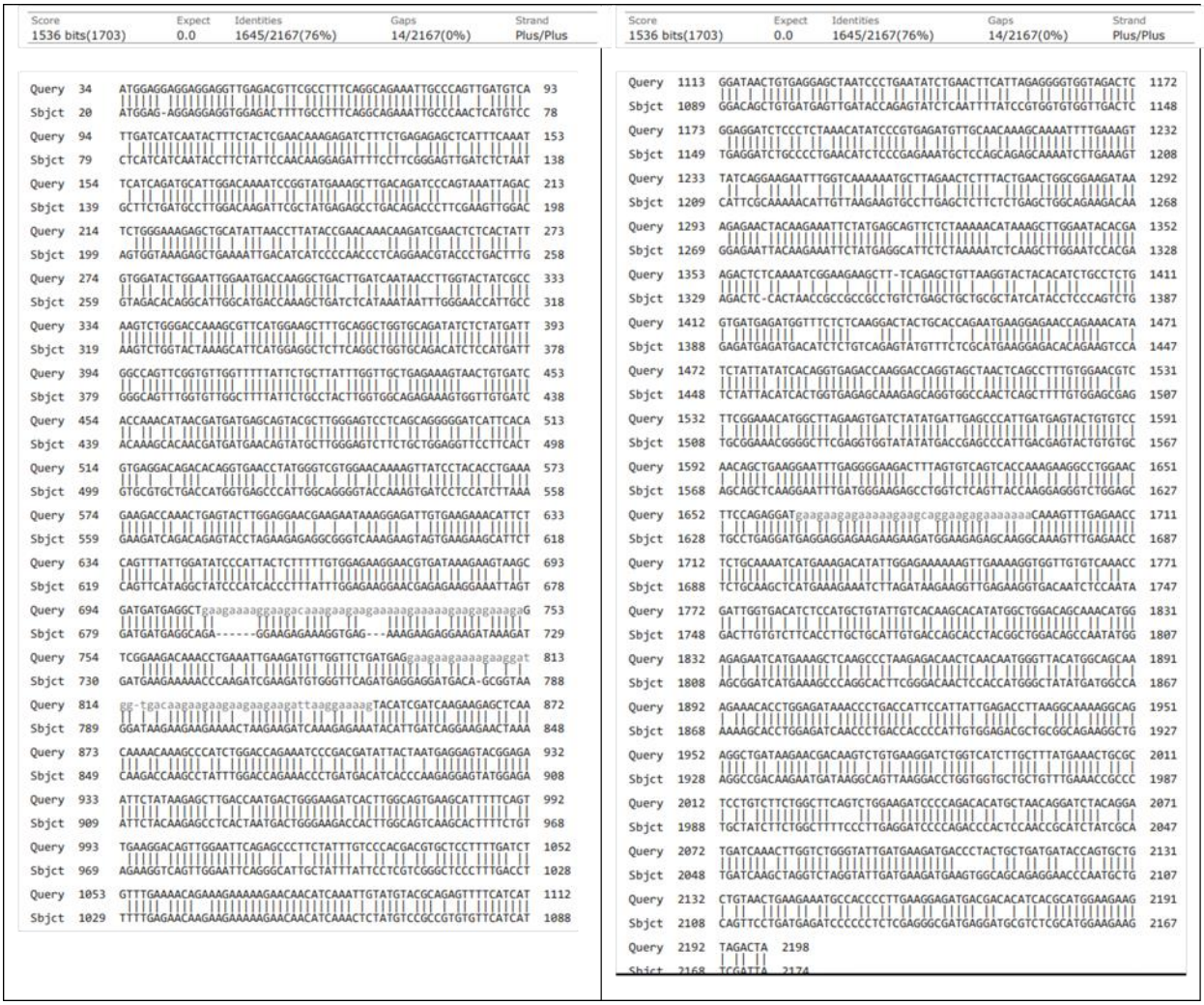


Figure 1 Hsp90AA1 and HSP90AB1 isoforms nucleotide sequence comparison

Hsp isoforms also exist at different parts of the cell-like cytosol, mitochondria, and ER. Further, distinct isoforms may exist even in the same cellular compartment [7]. Why there are redundant isoforms present in the cell is not elusive yet. However, inhibition of Hsp90 drives tumor cells to apoptosis and is a fine target for cancer treatment. Therefore, several drugs are tested and geldanamycin effectively inhibits the target [8].

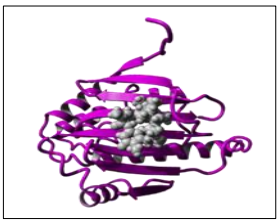
One of the biggest problems in drug design is the specificity of the target and several properties must be considered. The interaction between drug candidate and HSP90 must be strong enough but with a minimum number of hydrogen bonds [9]. The interactions must be at residues that are not mutated and conformationally unstable ones. Mutations or single nucleotide polymorphisms may affect corresponding proteins and the process may perturb drug binding [10]. Therefore, in silico experiments provide foresight in drug design. Another limitation is isoforms that are almost identical in sequence. HSP90AA1 (NM_001017963.3) and HSP90AB1 (NM_001271969.2) blast (Figure 1) shows that nucleotide sequences are pretty similar (%76) and at the amino acid level the similarity is higher. Therefore, designing specific drugs for HSP90 needs careful consideration of cavities that are similar in amino acid sequences.

2. Material and methods

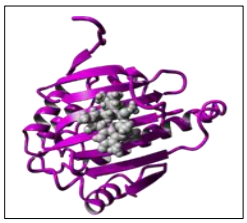
Yasara software was employed for docking geldanamycin aginst HSP90AA1 (PDB ID 3TOZ) and HSP90AB1 (PDB ID 6N8Y). The structures are compared to that of mutant structures (128 and 123) (Figure 2 and Figure 3). Comparison of binding energies and dissociation constants calculated with the commercial software.

3. Results

Previously reported inhibitor-resistant type HSP90 residue I123 from yeast model compared to the two isoforms and residues corresponding at HSP90AA1 and HSP90AB1 mutated. Binding energies and dissociation constant were calculated in the presence of geldanamycin (Table 1). The residues correspond to the ATPase domain and increase ATPase activity. This is an expected tumor behavior since accelerating ATPase function is beneficial to tumor cells. For this reason, the residues are essential to work on drug-mutated receptor interactions.

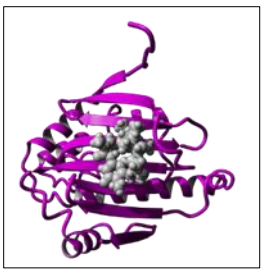


HSP90AA1-WT-Geldanamycine



HSP90AA1-I123T-Geldanamycin

Figure 2 HSP90AA1 WT and its mutant I123T interaction with geldanamycin



HSP90AB1-WT-Geldanamycine

HSP90AB1-I128T Geldanamycin

Figure 3 HSP90AB1 WT and its mutant I128T interaction with geldanamycin
Point mutations 128 and 123 were selected

Table 1 Binding energy and dissociation constant comparison of WT and mutant structures

Protein	Binding Energy [kcal/mol]	Dissociation constant [pM]
HSP90AA1-WT	6.9400	8183000.0000
HSP90AA1-I123T	6.9330	8280253.0000
HSP90AB1-WT	7.8130	1874973.2500
HSP90AB1-I128T	7.8180	1859216.7500

At the extreme end, mutated HSP90 isoforms may not perturb the effect of geldanamycin inhibition. ATPase activity is essential for substrate protein folding and since mutation in these residues is beneficial to tumor cells, designing a compound like geldanamycin is effective not only in unmutated both also at mutated cells.

These residues are reported to be inhibitor-resistant mutants and binding energies are similar to that of WT HSP90. This is important as HSP90 levels increases in stress conditions and tumorigenesis. The binding provides key insight into drug templates and innovative inhibitors such as less toxic and more soluble ones may be designed by considering the critical residues.

4. Discussion

Efforts in basic and clinical oncology in determining the molecular mechanism of this neoplastic disease require understanding finite details of the structure-function of inhibitor compounds. New therapeutic strategies have been developed and inhibition of cancer cells is one of the essential methods [11]. Novel drug treatment for the malignant disease must have a mechanism of action that works no matter what. Therefore, drug designing is difficult as several factors are involved. In silico studies provides a “pre-test” to understand the molecular mechanism. Several factors may be tested ahead of in vitro and in vivo experiments. The desirable attempt to relate the antitumor effect of the compound with molecular effect on relevant proteins is cumbersome due to distinct conformational changes. Further, mutations may bring further alterations. This burdens designing specific drug candidates. Geldanamycin has the potential to fulfill all these criteria through binding to HSP90 [12].

This work shows how HSP90 inhibitor geldanamycin is finely designed even in the presence of mutations the compound is prone to inhibit HSP90 ATPase action. HSP90 expression levels are difficult to control through two distinct cytosolic isoforms (HSP90AA1 and HSP90AB1). However, both isoforms and their corresponding mutants may be inhibited by geldanamycin.

Geldanamycin exerts its antitumoral effect by perturbing HSP90 ATPase action and driving tumor cells to apoptosis and disrupting the substrate protein folding-homeostasis.

5. Conclusion

Geldanamycin display superior activity even in the presence of mutants as evidenced by binding energies and dissociation constants. All drug candidates must be designed considering these properties to create an effective anticancer therapeutic.

Compliance with ethical standards

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

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