Nutlin mediates MDM2-p53 binding interactions via novel additional site: a new window on drug discovery?

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Introduction
p53, a tumor suppressor gene, is highly regulated within the cell. One of its key inhibitors, MDM2, acts as an E3 ligase that primes p53 for degradation under normal cellular conditions. In certain tumor cells, MDM2 has been found to be overexpressed, leading to excessive p53 degradation. The lack of cellular damage response through apoptosis would promote further cancer proliferation. To negate MDM2’s over expression, scientists are looking for ways to stymie MDM2-p53 binding in cancer cells specifically.

One of the drugs that has been shown to reduce MDM2-p53 binding affinity in in-vitro studies is a nutlin inhibitor compound (nutlin). X-ray crystallography revealed that nutlin binds to MDM2 at the same site where p53’s transactivation domain binds to, suggesting that nutlin is a competitive inhibitor. However, X-ray crystallography and hydrogen-deuterium exchange studies have also revealed the presence of a secondary binding site!

In this study, we attempt to characterize the secondary site to elucidate its role in mediating MDM2-p53’s binding.

Methods

1. Prepare start files from various crystal structures (1RV1, 1YCR) and NMR structures (1Z1M).
2. Perform Molecular Dynamics Simulation using AMBER’s pmemd implementation.
3. Analyze data using various analysis programs including ptraj, mmgbsa (AMBER), Bio3D (R), Biopython (Python).
4. Visualize trajectory with Pymol and VMD.

Results

Binding propensity at secondary site
1. We simulated experimental and cellular condition by placing multiple nutlins randomly in the solution.
2. We noticed several nutlin associating with MDM2 at various sites, including the primary hydrophobic cleft and secondary site.
3. Nutlin aggregates in solution, and this effect was visible in the presence of MDM2 as well.
4. We simulated experimental and cellular condition by placing multiple nutlins randomly in the solution.
5. Nutlin mediates MDM2-p53 binding interactions via novel additional site!

Comparison between various binding ligand and binding sites, nutlin appeared to bind with the least affinity at the secondary site (Fig 5a).
1. Nonetheless, it had a negative binding enthalpy, which suggests that the interaction is favorable.
2. Using alanine scan analysis (Fig 5b), we identified Y100 and Y104 as major contributors to the binding at the secondary site. More specifically, they contribute via VDW interaction predominantly.

Allosteric Effect
1. We did not notice any significant changes in the dynamics and binding enthalpy of MDM2-p53 in the presence of nutlin.
   a. RMSF of sidechain and backbone had similar fluctuation.
   b. Binding enthalpy (BE) at the secondary site remained the same with and without nutlin.
   c. Alanine scan in the absence of nutlin showed that the mutation Y104A caused only a small change in binding enthalpy.

Transition
1. When we ran a simulation of nutlin bound to the secondary site of MDM2, we saw nutlin moving across MDM2’s surface (Fig 6).
   a. Off the 6 repeats, 3 showed the transition.
   b. The orientation of nutlin, as it moved along the surface, was not unique.
      a. The transition was likely mediated by non-specific forces, mainly hydrophobic interaction or solvent effects.
   c. Using alanine scan, we noticed that it is the hydrophobic residues that contribute most to the BE over the transition.
   d. By studying the surface of the MDM2, we noticed that the path was indeed populated by solvent exposed hydrophobic residues.
   e. Binding enthalpy decreased as nutlin moved towards the binding pocket.
      a. The solvent accessible surface area decreased accordingly, suggesting that this trend was indeed mediated by hydrophobic interaction (Fig 7).

Conclusion
1. Due to its hydrophobic nature, nutlin’s interaction with MDM2 was mediated by non-polar residues as a general consequence.
2. Hydrogen-Deuterium exchange with mass spectrometry revealed the presence of a secondary site.
3. Nutlin bound favorably to the secondary binding site. Residence time analysis may give further insight into the binding under non-equilibrium conditions.
4. Binding at the secondary site did not lead to any significant allosteric effect at the primary binding site.
5. The secondary site did however serve as an initial landing site, from which nutlin then slid into the primary binding site and acted as a competitive inhibitor.
6. FREY and NMR experiments may validate this occurrence.

References

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