Computational Investigations on p53-MDM2 Interaction and its Inhibition: a Significant Step in Cancer Therapy

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By

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CHAPTER 12

Summary and Future Prospects

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11.1. Overview of Results:

The main theme of this thesis involves studying the various sites of interaction between p53 and MDM2 molecules, as well as studying different small molecule inhibitors of p53-MDM2 interaction binding to either p53 or MDM2. Here we have investigated the molecular interaction in three out of six known sites of interaction between p53 and MDM2. We have also studied about how an inhibitor can be regulated at the molecular level, in order to stop tumor progression in cancer patients.

The thesis has been divided into two major parts. The first part deals with studying the conformational dynamics, stability, and the molecular interactions at one primary and two secondary sites of interaction between p53 and MDM2. The second part deals with regulation of the p53-MDM2 interaction inhibitor(s) as well as the conformational dynamics, stability, and the molecular interactions of the p53-Inhibitor and MDM2-Inhibitor complexes.

Within the first section, we first tried to study the binding and unbinding mechanism of the p53(TAD1)-MDM2(NTD) complex using PMF method. We found the PMF value to be minimum at a distance of separation of 12 Å with a dissociation energy of 30 kcal mol^{-1}.

Then we tried to study the structural characteristics and the dynamic properties of the p53(DBD)-MDM2(AD) complex as this site of interaction is significant for p53 ubiquitination. We found that there exists a strong binding affinity between p53(DBD) and MDM2(AD).

Then we tried to study the effect of the p53(CTD) on the dynamics of the MDM2(NTD), along with the N-Terminal Lid present in MDM2(NTD). We found that MDM2(NTD) inn its apo state predominantly exists in the closed conformation, which disfavors p53 TAD1 interaction. But the binding of the p53 CTD to this region was found to disrupt the intramolecular contacts made by the lid region and facilitate the conversion of MDM2(NTD) to an open conformation.

Within the second section, we began with studying the interaction profile between MDM2(NTD) and Idasanutlin in presence and absence of a photoremovable protecting

group (PPG). And we found that idasanutlin fits properly into the binding cavity present in MDM2(NTD). But when idasanutlin is bound to a PPG, it undergoes certain conformational changes for which idasanutlin can no longer fit properly into the binding cavity present in MDM2(NTD).

Then we tried to study the binding and unbinding mechanism of the MDM2(NTD)-Idasanutlin complex using PMF method. We found the PMF value to be minimum at a distance of separation of 11 Å with a dissociation energy of 17.5 kcal mol^{-1}. We also found that MET54 from MDM2 provide the highest energy contribution for the MDM2(NTD)-Idasanutlin interaction obtained from PRED analysis.

Then we tried to study the interaction profile, conformational dynamics, as well as stability of the MDM2(NTD)-XR-2 complex at the molecular level. We found that there persists a good binding affinity between MDM2(NTD) and XR-2, and XR-2 remains bound to the N-Terminal binding cavity of MDM2, where the TAD1 of MDM2 binds to.

Then we tried to study the interaction profile, conformational dynamics, as well as stability of the p53(NTD)-EGCG complex at the molecular level. We found that there persists a good binding affinity between p53(NTD) and EGCG, and EGCG remains bound to the TAD1 region present in the p53(NTD), which usually binds to the N-Terminal binding cavity of MDM2.

Then we tried to study the conformational dynamics, interaction profile, as well as stability of the p53(NTD)-RITA complex at the molecular level. We found that the RITA molecule which initially binds to 32-36 residues of p53(NTD), later during the MD simulation gets displaced and then gets bound to the TAD1 present in the p53(NTD), and then remains intact in the p53(TAD1) throughout the rest of the simulation time period.

11.2. Future Prospects:

This thesis gives a molecular level detail about the structural features of the p53-MDM2 complexes at different sites of interaction, as well as p53-Inhibitor/MDM2-Inhibitor complexes using *in silico* approaches such as molecular modelling, molecular docking, and molecular dynamics simulation. This work on p53-MDM2 interaction and its

inhibition has further possibilities to be worked upon, which comprise of:

a. Computational study on the other three sites of interaction between the p53 and the MDM2 molecules.

b. Design as well as regulation of p53-MDM2 interaction inhibitors, which bind to either p53 or to MDM2 using computational tools. Inhibitors can be either some small molecule ligands, or peptides, or circular RNAs (circRNAs), or microRNAs (miRNAs).