Investigating Molecular Interactions of EBV Oncogene, EBNA1, by Heterologous Cloning and Peptide Array System

Mushtaq Hussain¹, Joanna B Wilson²

¹ Bioinformatics and Molecular Medicine Laboratory, Dow International Medical College Dow University of Health Sciences, Ojha Campus, Pakistan
² Institute of Cell, Molecular and Systems Biology, University of Glasgow

Abstract

Background: EBNA1 is an important latent genes that is involved in the oncogenesis associated with Epstein Barr Virus. The protein is highly promiscuous in terms of its interaction with the several proteins including EBP2 and USP7, a p53 stabilizer. Herein, we aim to investigate the interactions points of molecular complexes of EBNA1, an EBV oncogene, in order to design potential therapeutic peptides.

Methods: Full length EBNA1, EBP2 and USP7 models were constructed employing iterative threading. After proper refinements of models, EBNA1 dimer was constructed employing geometrical and structural constraints. Disruptor peptide was designed and its efficiency was assessed by molecular docking and confocal microscopy. In parallel, USP7 and EBP2 molecules were expressed in pET28c expression vector and after purification by affinity chromatography, the purified proteins were probed over EBNA1 peptide array (wild type and alanine substitution array) to resolve the fine interaction details. Finally, structural conformations of intermolecular complexes were examined by molecular docking.

Results: EBNA1 forms a dimer by engaging its C-terminal DNA binding domain where a proline loop of one molecule penetrated the C-terminal tail of another monomer. A disruptor peptide designed accordingly tend to bind in that region and found to inhibit EBNA1 dimerization resulting in the cellular apoptosis as observed in confocal microscopy and MTT assay. Peptide array systems showed the binding EBNA1 at the glycine and arginine rich regions especially ranging between 325 to 375 amino acids. Similarly, USP7 interactions were noticed between 441 to 450 amino acids where Ser447 holds a key position. Interestingly, phosphorylation of this serine completely inhibits the interaction. Molecular docking analysis further verify the binding sites and their draggability.

Conclusion: The present data shows molecular interactions of EBNA1 molecules at molecular resolution. The findings can be exploited to design inhibitors to curtail molecular interactions that leads to pathological consequences.

Keywords

EBV, EBNA1, USP7, EBP2, Cancer
Funding

Higher Education Commission, Govt of Pakistan

References