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Molecular Modeling and Docking Analysis of Jaceosidin Compound with HPV-16 E7 Protein

Sureshkumar BT[1,2], Gnanendra TS[3] and NatarajaseenivasanK[1]

[1] Medical Microbiology Laboratory, Department of Microbiology, Bharathidasan University, Tiruchirappali, Tamil Nadu,India.
[2]Department of Microbiology, Vivekanandha College of Arts and Sciences for Women,Tiruchengode,Namakkal,Tamil Nadu, India.
[3]Bioinformatics Division, OriGene Biosolutions, Salem, Tamil Nadu, India

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The expression of viral oncogenes E7 is thought to modify the cell cycle to retain the differentiating host keratinocyte in a state that favours the amplification of viral genome replication and late gene expression. HPV E7 acts as the primary transforming protein that competes for retinoblastoma protein (pRb) binding, freeing the transcription factor E2F to transactivate its targets, thus pushing the cell cycle forward.The new class of flavones Jaceosidin, pharmacologically active flavone derived from *Artemisia argyi* acts a putative oncogene inhibitor. Docking analyses suggest that these compounds bind in a hydrophobic pocket. Jaceosidin inhibits the binding between the E7 oncoprotein and the Rb tumor suppressor protein, and also the function of HPV-16 harboring cervical cancer cells, including SiHa and CaSki suggesting that this compound might be used as a potential drug for the treatment of cervical cancers associated with the human papillomavirus.

Keywords HPV-16, Early E 7 protein, Jaceosidin, Docking.

INTRODUCTION

Human Papillomavirus (HPV) is one of the most common virus groups in the world today affecting the skin and mucosal areas of the body. Over 140 different strains of HPV have been identified [1] which infect different parts of the body. The most visible forms of the virus produce warts (papillomas) on the hands, arms, legs and other areas of the skin.Human papillomavirus (HPV) is the most common viral infection of the reproductive tract. Most sexually active women and men will be infected at some point in their lives and some may be repeatedly infected.Cervical cancer is by far the most common HPV-related disease. Nearly all cases of cervical cancer can be attributable to HPV infection.

HPV belong to the Papovaviridae family. They consist of 72-capsomere capsid containing the viral genome. Capsomers are composed of two structural proteins: the 57 kDa late protein L1, which accounts for 80% of the viral particle, and the 43-53 kDa minor capsid protein L2. The HPV genome, a double-stranded DNA molecule consists of eight kilo base pairs (kbp) nucleotides. Arrangement of the 8-10 open reading frames (ORFs) within the genome is similar in all papillomavirus types and partly overlapping ORFs are arranged on a sole DNA strand. The genome can be divided into three regions: the long control region (LCR) without coding potential; the region of early proteins (E1-E8) & the region of late proteins (L1 & L2) [2]. Among all different viral proteins, it is found that E6 and E7 are necessary for HPV-induced malignancy [3]. One key activity of E7 is to overcome the pRB tumour suppressor block [4]. Binding of E7 to pRB and its related members result in the liberation of E2F transcription factors, which play a key role in promoting host cell and viral DNA synthesis. E7 also binds and activates cyclin complexes, such as p33-cyclin dependent kinase [5, 6] which control progression through the cell cycle. HPV E7 proteins of both low and high risk types have an ability to promote unscheduled DNA replication in spinous cells [7-11]. The viral function of E6 and E7 appears to be, at least in part, to control the cellular environment in a fashion favorable for replication of the viral genome, via transcriptional activation and induction of DNA synthesis as well as inhibition of cellular differentiation and promotion of cell growth [12]. *In vitro* studies have identified several biological properties of HPV-16 E7 which may be relevant to its function(s) *in vivo*, including: immortalization and transformation, alone or in cooperation with RAS or HPV-16 E6, of a variety of cultured cells and cell lines; binding to the under phosphorylated form of the retinoblastoma protein (pRb) as well as other "pocket proteins" including p107 and p103. Thus in this present study the homology modeling of HPV-16 E7 protein and docking studies of Jaceosidin was carried out as it might lead to the design of novel therapeutic agents against HPV.

Address Correspondence at Medical Microbiology Laboratory, Department of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappalli – 620 024, Tamil Nadu, India. Ph:+914312407082 E-mail: natarajaseenivasan@bdu.ac.in

METHODOLOGY

Target sequence and potential template search

The E7 protein sequence of HPV-16 was retrieved from the National Centre for Biotechnology Information (NCBI) [13] (NCBI protein code AF125673). NCBI-BlastP (basic local alignment search tool) [14] was used to search the homologous sequences against PDB and obtained homologous sequence was considered as the potential template structure for homology modeling. The atomic co-ordinate file of the template structure was obtained from the PDB [15]. The sequence alignment and alignment errors were refined by using ClustalW [16] program as homology modeling relies on the sequence alignment.

Homology modeling

The automated homology modeling software Modeler 9v9 [17] was used to built the model based on the final sequence alignment file of target and the template sequence.The atomic coordinate file of the template structure was used to

build the 3D model by generating the satisfaction of spatial restraints. A bundle of 20 models were calculated from the starting structure by random generation.The best model was selected by superimposing the model with its template using SUPERPOSE [18] and also based on the least RMSD value. This best model was energy minimized by applying 20 steps of each steepest descent and conjugates gradient using GROMOS [19] of Swiss PDB viewer and was used for further analysis.

Model assessment

The quality of the generated model was assessed by checking the stereo chemical parameters using PROCHECK [20], Verfiy3D [21] and ERRAT [22] at SAVES server [23].

Prediction of binding site

To determine the binding affinities between HPV-16 E7 and Jaceosidin, the amino acids in the binding site of the developed model of HPV-16 E7 protein was predicted through Q-site finder [24].

Ligand generation and flexible docking

The 3D structure of Jaceosidin molecule from *Artemisia argyi* (Chinese mugwort) was retrieved as SDF File from Pubchem Database. The obtained SDF structure was docked with the amino acids in the binding site of HPV-16 E7 protein using FlexX [25] with following parameters i) default general docking information's, ii) base placement using triangle matching, iii) scoring of full score contribution and threshold of 0,30 and no score contribution and threshold of 0,70. iv) Chemical parameters of clash handling values for protein ligand clashes with maximum allowed overlap volume of 2.9 A^{03} and intra-ligand clashes with clash factor of 0.6 and considering the hydrogen in internal clash tests. v) Default docking details values of 200 for both the maximum number of solutions per iteration and maximum number of solutions per fragmentation.

Prediction of ligand- receptor interactions

The interactions of Jaceosidin molecules with HPV-16 E7 protein in the docked complex were analyzed by the pose-view of LeadIT [26].

RESULTS AND DISCUSSION

Sequence analysis and potential template for comparative modeling

The BLASTP analysis of target sequence of the HPV-16 E7 protein, against PDB resulted that the structure of 2ewl-A chain structure of HPV-45 E7 protein as the homologous sequences with sequence similarity of 80% at an E-value of 1.10e-20**.**As both the sequences belong to HPV the resultant homologous sequence was selected as template structure for homology modeling.

Homology modeling

The sequence alignment file was used as input to build the initial model of HPV-16 E7 protein using Modeler 9v9 by applying spatial restraints from the initial structure, a bundle of 20 models were developed using random generation and the best model was selected for further analysis based on its structural compatibility (structure with lowest DOPE score). The modeled structure was shown in **Figure.1.**.

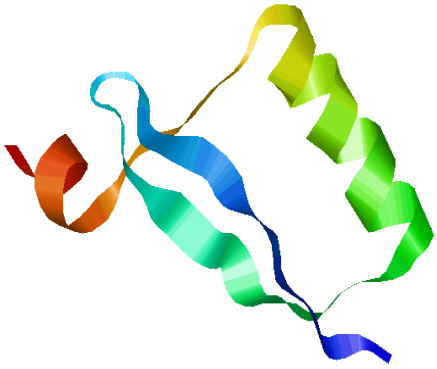

Figure.1: Modeled structure of HPV type- 16 E7 proteins

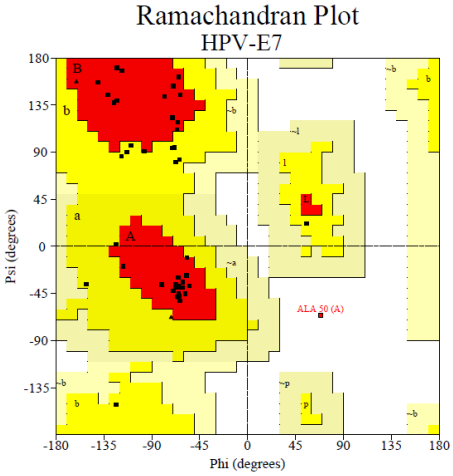

Figure.2: Ramchandran plot for stereo chemical parameters of modeled protein

Model Assessment

The overall stereo chemical quality of the model was assessed by PROCHECK, Verfiy3D, and ERRAT of SAVES server. The Ramachandran plot of the energy minimized model of HPV-16 E7 showed 90% of the residues in the most favorable region, 9.5% in the additionally allowed region, 0.0% in the generously allowed region and 0.5% in the disallowed region **Figure.2**.The Ramachandran plot of the all generated models of E7 Protein were analysed and considered the best model as it exhibited more number of residues in the most favorable regions and also the low number of residues in disallowed region. The total quality G-factor was -0.13. Further the overall quality factor and compatibility of an atomic model (3D) with amino acid sequence (1D) for the modeled protein HPV-16 E7 was observed as 83.328 and 97% from ERRAT and Verify3D respectively **Table.1**. The results of ERRAT and Verify-3D also confirm the model was reliable and of good quality.

Docking studies

The docking complex and the interactions of Jaceosidin docked with in the binding site of modeled E7 protein was shown in **Figure.3**. The docking interactions between the binding site residues of E7 protein (amino acids) and the Jaceosidin exhibited the binding energy of -21.1844 kJ/mol. The hydrophobic, h bond donor and acceptors of the binding sites were shown in **Figure.4.** The docking

Table 1: Validation of the model HPV-16 E7 by SAVES Server

Protein	PROCHECK					ERRAT	VERIFY-3D
	G-Factor	Ramachandran Plot					
		MFR	AAR	GAR	DAR		
HPV-16 E7	-0.13	90%	9.5%	0.0%	0.5%	83.328	97%

MFR-Most Favoured Region; **AAR**- Additionally allowed Regions; **GAR**-Generously allowed Regions; **DAR**-Disallowed Regions.

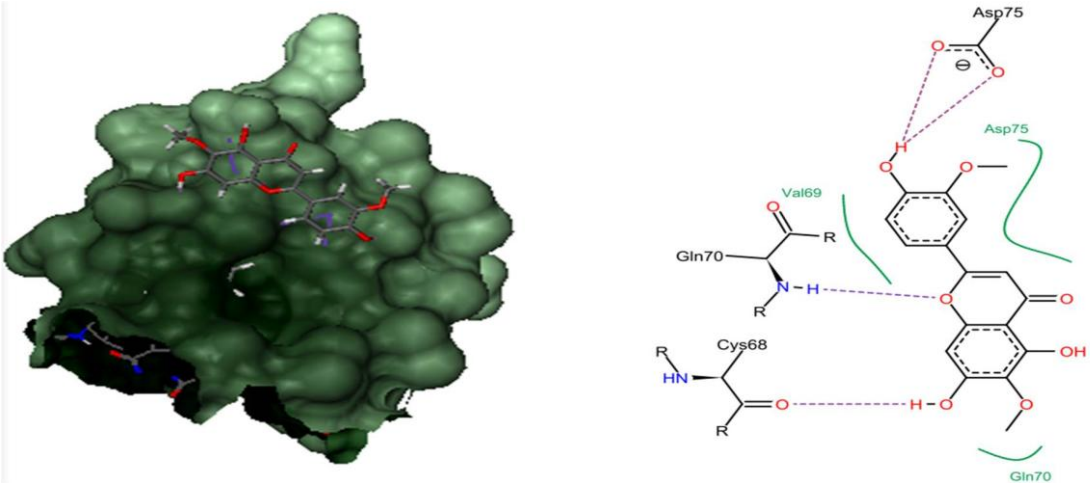

Figure.3 a. Docking complex of E7 protein and Jaceosidin with binding score of - 21.1844 kJ/mol **b.**The interaction of Jaceosidin by Hbonds and hydrophobic interactions.

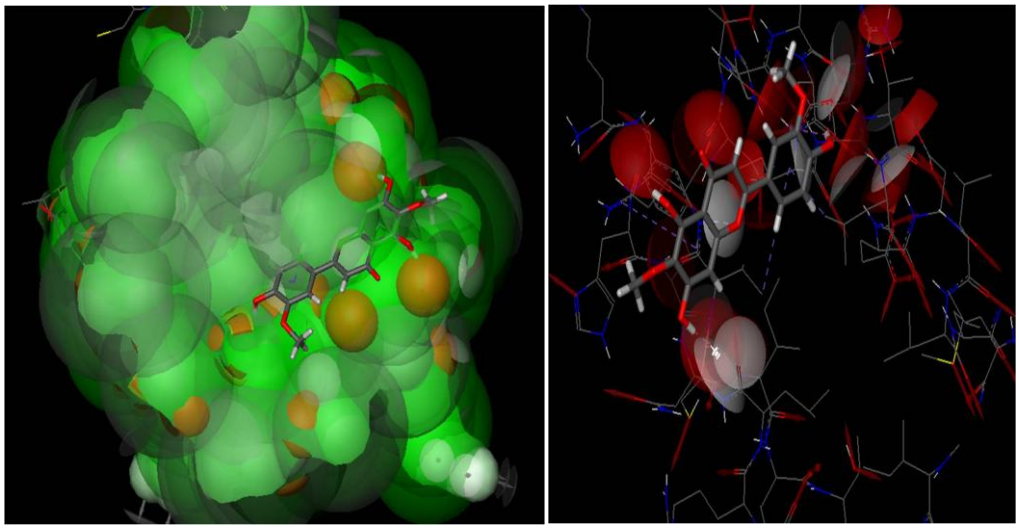

Figure.4 a. The Hydrophobic regions and aromatic centres in the active site of E7 Protein.**b.** The Hbond donor(red color) and acceptor(ash color) regions in the active site of E7 protein.

interactions revealed that Asp75, Gln70 and Cys68 are favouring the formation of Hbonds where as the residue, Val 69 along with Asp75 and Gln 70 it is favouring the hydrophobic interaction. The Hydrophobic regions and aromatic centers and Hbond donor (red color) and acceptor (ash color) in the active site of E7 Protein were predicted by using Hyde option from LeatIT. The result of this docking interactions envisaged that the amino acid Asp75 and Gln70 are essential in driving the both Hbond and hydrophobic interactions with Jaceosidin. Thus the understanding of 3D structure of HPV-16 E7 protein and its docking studies with Jaceosidin, which inhibits the binding E7 with pRb and also the suppression of cervical cancer cells might shed a light on the design of novel therapeutic agents that might be a significant for inhibition of HPV type 16.

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