

The in Silico Approach to Identify a Unique Plant-Derived Inhibitor Against E6 and E7 Oncogenic Proteins of High-Risk Human Papillomavirus 16 and 18

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Abstract

Background: Globally, the human papillomavirus (HPV) remains the foremost cause of cancer mortality among women. There is a need to identify natural anti-cancerous compounds that can fight against life-threatening infections by HPV. Various kinds of natural plant-originated compounds have been used in the traditional system of medicine for cancer therapy. Different studies have reported the effective inhibition of HPV infection enacted by certain natural compounds. Out of all the different HPV types, HPV-16 and 18 are the ones mainly associated with causing cervical cancer; furthermore, the E6 and E7 oncoproteins of these two high-risk HPV types typically interact with tumor protein 53 (p53) and retinoblastoma tumor suppressor proteins (pRb) of human host which consequent to cancer formation.

Objectives: The goal of this study is to identify unique plant-originated compounds to utilize in order to combat the high-risk human papillomavirus oncoproteins using docking measures.

Materials and Methods: Twelve natural compounds jaceosidin, withaferin A, curcumin, epigallocatechin-3-gallate (EGCG), artemisinin, gingerol, ursolic acid, ferulic acid, berberin, silymarin, resveratrol, and indol-3-carbinol were docked against E6 and E7 oncoproteins of high-risk HPV types 16 and 18 using a protein-ligand docking software called AutoDock4.2.

Results: Out of these 12 natural compounds, withaferin A was found to inhibit all four oncoproteins with minimum binding energy.

Conclusions: These in silico findings indicate that withaferin A may be used as a common drug for cervical cancer caused by high-risk HPV types, perhaps by restoring the normal functions of tumor suppressor proteins.

Keywords: Human Papillomavirus, Oncogene Proteins, Molecular Docking, Plant Components

1. Background

An estimated 15% - 20% of all human cancers worldwide are caused by viral infections (1); the human papillomavirus (HPV) accounts for 5.2% of all cancers (2, 3). Global cancer fatality in women is mostly due to cervical cancer, with an estimated 0.527 million new cases and a 0.265 million annual mortality rate (4). HPV-16 and 18 are responsible for 62.6% - 15.7% of cervical cancer cases (5); they are also associated with oropharyngeal cancers (89% - 95%), anal cancer (93%), vulva/vaginal cancers (80% - 86%), and penile cancer (63% - 80%), among others (6). Consequently, these two HPV types 16 and 18 are the most recent targets of anti-cancer drug designing efforts. Out of the eight types of proteins expressed in HPV, E6 and E7 proteins are reported as cooperative viral oncoproteins due to their expression in all HPV types (7). These two proteins have been well known to interact with tumor suppressor proteins p53 and pRb of

human host that leads abrogate to cervical cancer (8).

Although for over thirty years, HPV has been known to be a causative agent for cervical cancer, a successful method of treatment against HPV infection still has yet to be established (9). In recent years, however, many natural plant origin compounds have been identified as promising sources of drugs for therapeutic and prophylactic uses in cancer (10, 11).

In our previous study, we already described the positive results of using curcumin, epigallocatechin-3-gallate (EGCG), jaceosidin (12-14), resveratrol (13, 14), indole-3-carbinol, withaferin A (12, 14), artemisinin, ursolic acid, ferulic acid, berberin, resveratrol, gingerol, and silymarin (14) as indications that these compounds are possible effective sources of cancer treatment.

2. Objectives

The current study purposes to examine the binding interaction of each of the above-mentioned plant-originated ligands with the oncoproteins (E6 and E7) of high-risk HPV (type 16 and 18), comparing the effectiveness of each ligand with that of the others, in order to discover an appropriate natural compound that can be further explored as a common drug against high-risk types of HPV.

3. Materials and Methods

3.1. Hardware and Software

The protein-ligand docking software AutoDock 4.2 (15) installed in Dell Workstation with 6 GB RAM, 500 GB storage capacity, and 2.26 GHz processor was employed in this study.

3.2. Structure of HPV Oncoproteins

Predicted structures of the HPV oncoproteins E6 and E7 from the human papillomavirus types 16 and 18 retrieved from the in-house developed human papillomavirus proteome database (hpvPDB) (16) were selected as drug targets.

3.3. Ligand Preparation and Protein-Ligand Docking

The chemical structures of 12 natural compounds (artemisinin, WA, ursolic acid, ferulic acid, EGCG, berberin, resveratrol, jaceosidin, curcumin, gingerol, silymarin, and indol-3-carbino) were obtained from the PubChem compound database (17).

Receptor molecules (HPV oncoproteins) were prepared in the AutoDock 4.2 program (15), and protein-ligand docking was performed as per the standard methodology used by Kumar et al. (12). For preparing each receptor molecule, all hydrogen atoms were added to the carbon atoms of the receptor, and Kollman charges were also assigned using AutoDock Tools 1.5.4 (ADT). Non-polar hydrogens were also added for docked ligands. Gasteiger charges were assigned and torsions degrees of freedom were allocated by ADT. The Lamarckian genetic algorithm (LGA) was applied to model the interaction pattern between the receptor protein and selected inhibitors.

The grid maps representing the receptor proteins in the docking process were calculated using AutoGrid (part of the AutoDock package). A grid of 50, 50, and 50 points in the x, y, and z directions was centered on the p53 and pRb binding sites of E6 and E7 proteins. For all docking procedures, ten independent genetic algorithms running with a population size of 150 were considered for each molecule

under study. A maximum number of 25×10^5 energy evaluations, 27,000 maximum generations, a gene mutation rate of 0.02, and a crossover rate of 0.8 were used for the LGA. AutoDock was run in order to prepare corresponding Docking LoG (DLG) files for further analysis (12).

3.4. Visualization

For visualizing the structure files, AutoDock Tools was used.

4. Results

From our docking analysis, it was observed that all 12 natural ligands bind with HPV oncoproteins that might help the restoration of normal functioning of tumor suppressor proteins, and the lowest binding energy conformation was analyzed and tabulated (Table 1). The active site of the model was analyzed based on the docking interaction between the p53 and pRb binding site residues (12-14, 18-20) of HPV oncoproteins, and all natural ligands.

Out of the 12 natural ligands, withaferin A (WA) was the one found to effectively interact with all four oncoproteins of HPV using the lowest level of binding energy. WA was also observed to bind with the HPV-16 E6 protein using the lowest level of binding energy (-7.58 kcal/mol), and the inhibition constant was found to be $2.77 \mu\text{M}$. The three amino acid residues of HPV-16 E6, Ala53, Leu117, and Lys122 were observed to form hydrogen bonds with WA during protein-ligand interactions (Figure 1A). Furthermore, the binding energy of WA with HPV-16 E7 was observed to be a minimum of -7.56 kcal/mol with an inhibition constant of $2.88 \mu\text{M}$. WA formed three hydrogen bonds with three amino acid residues (i.e. Arg66, Asn53, and Glu80 from the HPV-16 E7 protein) (Figure 1B). In the case of the HPV-18 E6 protein, WA interacted with four amino acid residues from the receptor (Glu116, Asn113, Asn122, and Ser140) by forming hydrogen bonds (Figure 1C); the binding energy of the interaction was -5.85 kcal/mol, and the inhibition constant was $51.35 \mu\text{M}$.

Similarly, WA was observed to inhibit the HPV-18 E7 protein with a binding energy of -5.77 and an inhibition constant of $58.77 \mu\text{M}$ by forming only one hydrogen bond with Glu73 (Figure 1D).

5. Discussion

Few recent studies have observed the inhibitory effects of different natural compounds on HPV oncoproteins. Through their research, Kramer and Wesierska-Gadek (2009) revealed the antiproliferative action of resveratrol

Table 1. Docking Analysis Results of HPV Oncoproteins and Natural Ligands

Ligands	HPV-16 E6		HPV-16 E7		HPV-18 E6		HPV-18 E7	
	Binding Energy, kcal/mol	Inhibition Constant, μ M	Binding Energy, kcal/mol	Inhibition Constant, μ M	Binding Energy, kcal/mol	Inhibition Constant, μ M	Binding Energy, kcal/mol	Inhibition Constant, μ M
Withaferin A	-7.58	2.77	-7.56	2.88	-5.85	51.35	-5.77	58.77
Silymarin	-4.91	252.39	-4.65	393.2	-3.67	2040	-4.71	353.4
Ferulic acid	-4.57	445.71	-4.36	637.36	-5.18	158.26	-3.15	4950
EGCG	-4.13	935.14	-4.09	1010	-4.12	961.59	-2.76	9540
Indol-3-carbinol	-4.06	1060	-4.22	802.07	-4.98	223.5	-3.31	3750
Artemisinin	-4.04	1080	-6.47	18.09	-5.68	68.22	-4.67	376.18
Jaceosidin	-4.01	1150	-4.77	318.07	-4.27	745.68	-3.87	1460
Resveratrol	-1.85	44030	-9.26	0.1636	-4.31	693.75	-2.33	19570
Ursolic acid	-1.73	53630	-4.67	375.57	-5.31	127.95	-5.23	146.52
Berberine	-3.42	3120	-6.82	10.04	-4.12	958.17	-4.77	317.65
Gingerol	-3.25	4180	-3.41	3190	-2.86	8070	-2.31	20140
Curcumin	-3.09	5440	-6.32	23.25	-4.08	1020	-3.57	2410

by observing its long-term effects on the cell cycle progression of human HeLa cervical carcinoma cells (21). Lee et al. (2005) isolated jaceosidin from the methanol (MeOH) extract of *Artemisia argyi* and reported its inhibitory effects on the function of the E6 and E7 oncoproteins of HPV-16 (22). Mamgain et al. (2015) also observed the inhibitory effect of natural compounds such as curcumin, colchicine, ellipticine, daphnetin, and epigallocatechin-3-gallate, etc. on the HPV-16 E6 protein, using molecular docking (23). Our docking study also observed the interaction of all 12 natural ligands with HPV oncoproteins and amongst them, Withaferin A was found to effectively inhibit all four oncoproteins of HPV with minimum binding energy.

The active compound of WA (also known as *Withania somnifera* or "Ashwagandha") has been reported to engage in anti-cancer, radiosensitizing, and antiangiogenic activity (24, 25) against various cancer cells (26).

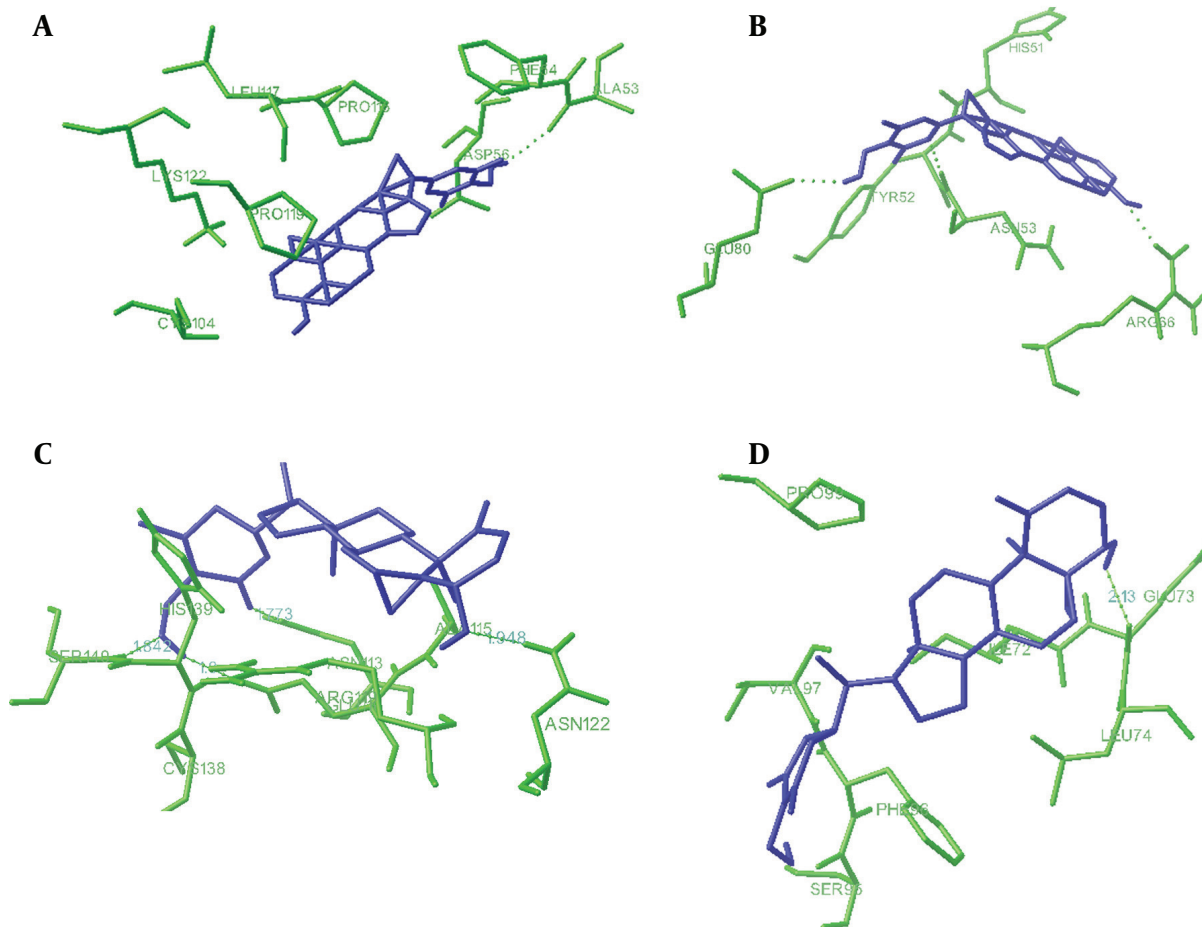
WA has also been reported to inhibit the nuclear factor- κ B-dependent, pro-inflammatory, and stress response pathways in the astrocytes and the activation of astrocytic TLR4 by bacterial lipopolysaccharide (LPS) challenge can promote nuclear factor κ B (NF- κ B)-dependent induction of tumor necrosis factor α (TNF α) besides cyclooxygenase 2 (COX-2), and inducible nitric oxide synthase (iNOS) (27). Lee et al. (2013) also reported WA as an effective approach for controlling metastasis and the invasiveness of tumors (28). Munagala et al. (2011) confirmed the successful inhibition of cervical cancer cells proliferation by WA through in vitro and in vivo study. In addition, they showed the

down-regulation of HPV E6 and the restoration of the p53 pathway using WA (29). This study also resulted in observations of WA as an effective inhibitor of HPV oncoproteins. This computational approach demonstrates the effectiveness of WA as an anticancer agent that needs to be explored further in order to learn more about how to use natural resources for designing novel drugs against cervical cancer.

Traditionally, different plant-originated compounds have been identified and tested as promising resources against cancer caused by HPV. Due to the recent advancement of bioinformatics and computational biology, it is now possible to validate those natural compounds as possible anticancer agents and identify additional common natural compounds that can fight against the proteins of different HPV types. For example, the high-risk HPV types 16 and 18 have HPV oncoproteins (E6 and E7) that need to be eliminated for various reasons, including the fact that they are capable of inactivating tumor suppressor proteins p53 and pRb by inducing their degradation. This in silico study revealed the effective inhibition of all four HPV oncoproteins by WA, which needs further in vitro and in vivo validation before it can be considered for becoming a common natural drug used for fighting cervical cancer.

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Figure 1. Interaction Profile of Withaferin A

A, HPV-16 E6; B, HPV-16 E7; C, HPV-18 E6; D, HPV-18 E7, showing the interaction of ligands with the active site residues of receptors by forming hydrogen bonds.

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Footnote

Authors' Contribution: Study concept and design: Satish Kumar; acquisition of data: Maheswata Sahoo and Tapaswini Nayak; analysis and interpretation of

data: Satish Kumar and Lingaraja Jena; drafting of the manuscript: Lingaraja Jena; critical revision of the manuscript for important intellectual content: Kanchan Mohod and Sangeeta Daf; study supervision: Ashok K. Varma.

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