Potential Natural Inhibitor Bioactivities against High-Risk HPV-16, HPV-18 and HPV-52 of E6 Oncoprotein through Molecular Docking

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ABSTRACT

Human papillomavirus (HPV) is known as the main cause of cervical cancer. Most sexually active women might be high risk infected by HPV. In Indonesia, HPV-16 and HPV-18 types are equally common in the general population associated with cervical cancer. HPV-52 is the most prevalent type in the general population worldwide. Some natural products have been identified as promising sources as inhibitor agents for treatment and prevention of cancers in recent years. Indonesia is abundant of original plants with bioactive compounds that play role as anticancer, such as red fruit, turmeric, god's crown, ground cherry and white turmeric. The aim of this research is to structure-based screen the interaction between protein E6 from HPV-16, HPV-18 and HPV-52 strains with natural compounds through molecular docking. Out of five natural compounds that we studied, we found the highest binding energy to interact with E6 HPV-16 is daphnoretin (-7.8 kcal/mol), HPV-18 and 52 is β-cryptoxanthin -7.8 kcal/mol and -8.4 kcal/mol. Molecular docking simulations provide a platform for capturing the structures, motions, and interactions of biological macromolecules in full atomic details. The accuracy of such simulations, however, is critically dependent on the force field—the mathematical model used to approximate the atomic-level forces acting on the simulated molecular system. The results show that the profile of E6 oncoprotein from HPV-18 and HPV-52 strains were stable, while HPV-16 strain was not stable due to the differences in the ligand interactions.

Keywords: High Risk HPV, E6 Oncoprotein, Natural Inhibitors, Molecular Docking

I. INTRODUCTION

Cervical cancer is known as the seventh global frequency, however it is ranked as the second highest cancer worldwide among women after breast cancer and it is the leading cause of high mortality in developing countries [1]. Widely spread of cervical cancer threat becomes major public health issue in women all over the world, especially in developing country including Indonesia [2]. The data from thirteen pathology centers in Indonesia shows that cervical cancer leads the first-ranked among all cancer (31% from 10 most common cancers among women) [3]. Data from several academic hospitals in 2007 showed that cervical cancer is the most common gynecologic malignancy followed by cancers of the

ovary, uterus, vulva, and vagina, respectively [4]. In a small group of women, the virus survives for years before it eventually converts some cells on the surface of the cervix into cancer cells. In the majority of cases, the infection does not cause any symptoms, but in some women, HPV infection shows progression in the development of cervical intraepithelial neoplasia, which can lead precancerous and cancerous lesions of the uterine cervix [5]. HPV has two types related to its risk, first type called low-risk HPV and the second called high-risk HPV. Low-risk HPVs only cause the genital warts, whereas high-risk HPVs cause lead to abnormal cell changes moreover cause genital cancers: cervical cancer as well as cancer of the vulva, and anus [6]. Based on mainly unscreened women population in Indonesia, it has been found that intermediate overall

prevalence of high-risk HPV, the top three highest HPV types are 52, 16, and 18, respectively with different age specific patterns in the three regions that cause cervical cancer. HPV 52 was the most prevalent type in Jakarta and Bali, while it is the second most prevalent type in Tasikmalaya. The high prevalence of HPV 52 is also reported worldwide including China, Taiwan, and Costa Rica [7]. In Indonesia, HPV 16 and HPV 18 are equally common in the general population, associated in cervical cancer. HPV 52 was the most prevalent type in the general population, suggesting that this type should be included when prophylactic HPV vaccination is introduced in Indonesia [8]. Around estimated 200,000 new cases cervical cancer was detected in Indonesia every year [10]. Many ways are used to treat cervical cancer, such as surgery, radiotherapy and chemotherapy. Surgery is generally only treated in early stage cervical cancer. Higher stage disease is usually treated with radiotherapy and chemotherapy [11]. Chemotherapy has many negative side-effects, due to it contains synthetic drug compounds that may induce high normal cells toxicity. It is very important to explore natural herbal with cytotoxic activity with low adverse effects. Indonesia has many original plants with anticancer activity, such as red fruit, [12], turmeric [13], god's crown, ground cherry and white turmeric [14-15], which empirically used for cancer treatment and can inhibit the progression of cancer. The aim of this research is to investigate the interaction mechanism between protein E6 from HPV-16, HPV-18 and HPV-52 with natural compounds which are potential as inhibitors through molecular docking approach.

II. METHODS AND MATERIAL

Protein preparation and homology modeling
The protein sequences of E6 HPV-16 (entry code: P03126) [16], E6 HPV-18 (entry code: P06463) [17] and 52 (entry code : P36814) [18] were selected from the universal protein source (UNIPROT). The homology model of protein E6 HPV-18 and 52 were built at SWISS-MODEL webserver program [19].

Validation of protein structure
The assesment of E6 HPV-16, 18 and 52 domain structure quality is done by a number of tools to check the internal consistency and reliability of the model structure. PROCHECK [20] analysis, which quantifies the residues in available zones of Ramachandran plot, is used to assess the stereochemical quality of the model.

Ligand preparation
The chemical structure of natural compounds are β-cryptoxanthin (red fruit) [12], curcumin (turmeric) [13], daphnoretin (god's crown) [21], luteolin (ground cherry) [22] and curcumenone (white turmeric) [23] were selected. These natural compounds are known as native Indonesian bioactive compounds.

Molecular docking of protein-ligand complex
Molecular docking experiment is performed using Autodock Vina program (Vina, The Scripps Research Institute) [24]. The AutodockTools is utilised to add partial charges using Gasteiger method and to arrange the polar hydrogen in the protein. The ligand is set to have flexible torsion angles at all rotatable bonds, while the protein is prepared as a rigid structure. Both protein and ligand are saved as output pdbqt files. Docking logs obtained from docking by Autodock Vina presented in ranked nine best docking modes based on the value of the lowest binding affinity (kcal/mol) according to thermodynamics law and delta Gibbs free energy (ΔG). Furthemore, ΔG value is used to calculate Ki (inhibition constant) value with equation:

$\Delta G = - R \times T (\ln KA)$; where $KA = e^{-(\Delta G / R \times T)}$ and Ki is expressed as $Ki = 1/KA$, which R is the ideal gas constant (1.98588×10^{-3} kcal mol^{-1} K^{-1}); and T is the absolute temperature in Kelvin (298.15K). calculated-Ki (cKi) value which is assumed to occur at room temperature.

III. RESULTS AND DISCUSSION

In preparing 3D protein structures of E6 from HPV-16, HPV-18 and HPV-52, we used SWISS-MODEL program webserver to build the structures which the sequences and templates acquired from UNIPROT. Approximately 75% identity of E6 HPV-16 and HPV-18, whereas E6 HPV-52 is 47% with the templates. Homology modeling was built by superimposing the desired protein with the template with the highest identity between the modeled protein and templates (Fig. 1).
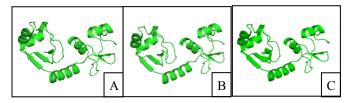
Figure 1. E6 protein structures of : A. HPV-16;
B. HPV-18; C. HPV-52

In assessing 3D protein structure, PROCHECK was used to check the reliability of the backbone torsion angles and the built model, which quantified the residue clusters in the available zones of Ramachandran plot, as shown in Fig. 2. Ramachandran plot analysis for the modeled E6 HPV-16, 18 and 52 were performed in Table 1. The quality structure of protein E6 HPV-16, 18 and 52 were further computed with calculation scores were 93%, 87% and 95%, respectively which indicated that the protein structures are acceptable as protein model for molecular docking process. Molecular docking between E6 HPV-16, HPV-18, HPV-52 and natural inhibitors had been performed with Autodock Vina. All complexes are depicted at Figure 3. From docking result, nine conformers had been selected to have high score and rank. From nine conformers, one conformer with the lowest affinity energy value from protein ligand complexes had been selected for further analysis (Table 2).
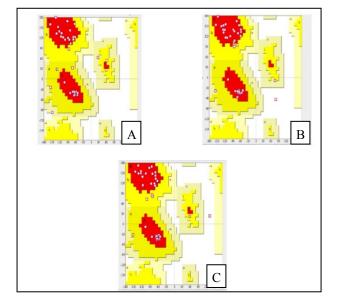

Figure 2. Ramachadran plot of : A. E6 HPV-16;
B. E6 HPV-18; C. E6 HPV-52

First approach mode only an amino acid sequence of a protein is submitted to build a 3D model. Template selection, alignment and model building are done completely automated by the server. In the 'alignment Out of five natural compounds that we studied, we found the highest binding energy to interact with HPV-16 E6 is daphnoretin (-6.8 kcal/mol). The chemical interaction between E6 HPV-16, 18 and 52 with ligands showed in Table 3. Swiss-model is a webserver program for automated comparative modeling of three-dimensional (3D) protein stuctures. It provides several levels of user interaction through its web-base interface, which the modeling process is based on a user-defined target-template alignment. Homology modeling of E6 HPV-16, HPV-18 and HPV-52 have extensively used to develop protein structure and function analysis. Based on the homology meodeling results of E6 HPV-16, HPV-18 and HPV-52, we can see the proteins have the similar models, but they have the difference in their coils. We assumed that the difference on their coil means refer to the ferocity of the virus when attach human servical cells. By modeled protein development, it is necessary to validate the E6 HPV-16, HPV-18 and HPV-52 model to ensure the good quality of its structure. The E6 HPV-18 showed the highest similarity with the template structure and high score in determining good quality of protein model after conducting energy minimization. Validation of protein structure using PROCHECK and the results are depicted in Ramachandran plot form. Ramachandran plot is a way to visualize backbone dihedral angles ψ against φ of amino acid residues in a protein structure. A Ramachandran plot can be used in two somewhat different ways. First way is to show theory which values or conformations of the ψ and φ angles are possible for an amino-acid residue in a protein. A second way is to show the empirical distribution of data points observed in a single structure. The colored plots represent the different regions described in Morris *et al*. (1992): the darkest areas correspond to the "core" regions representing the most favorable combinations of phi-psi values. Ideally, one would hope to have over 80% of the residues in these "favored" regions [25]. The percentage of residues in the "favored" regions is one of the better guides to stereochemical quality. The different regions were taken from the observed phi-psi

Tabel 1. Summary of Ramachandran plot analysis

Protein	Favored region (red)	Allowed region (yellow)	Gener region (cream)	Disallowed region (white)
E6 HPV-16	83%	13%	4%	0%
E6 HPV-18	88%	10%	0%	2%
E6 HPV-52	87%	11%	0%	2%

Table 2. Binding energy of molecular docking interaction of E6-inhibitor complexes

Inhibitors	Binding energy (kcal/mol)		
	E6 HPV-16	E6 HPV-18	E6 HPV-52
Daphnoretin	-7.8	-7.5	-7.3
β-cryptoxanthin	-6.4	-7.8	-8.4
luteolin	-6.6	-7.0	-7.0
Curcumenone	-5.7	-7.7	-5.9
Curcumin	-5.7	-7.0	-6.0

Table 3. Chemical interaction of E6 with potential inhibitor complexes

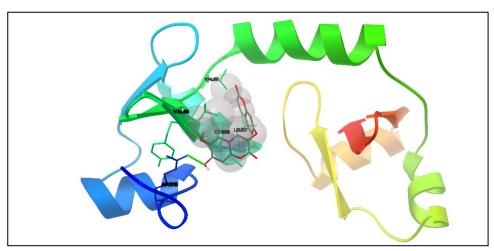| Protein	Potential Inhibitors	Hydrophobic interactions	cKi value (mM)
E6 HPV-16	Daphnoretin	Arg-15, Leu-57, Cys-58, Val-60, Val-69 (2 H-bonds)	1.8889×10^{-6}
E6 HPV-18	β-cryptoxanthin	Tyr-34, Phe-53, Leu-62, Ala-64, Ile-69, Thr-94, His-133, Tyr-134	1.8889×10^{-6}
E6 HPV-52	β-cryptoxanthin	Tyr-43, Lys-44, Arg-68, Ile-105, Cys-106, Cys-139, Arg-141	6.853×10^{-7}

Figure 3. Hydrophobic interactions between E6 HPV-16 with Daphnoretin
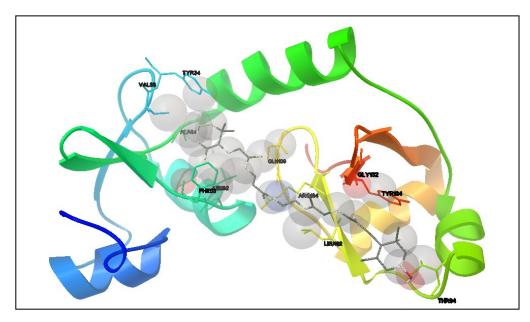
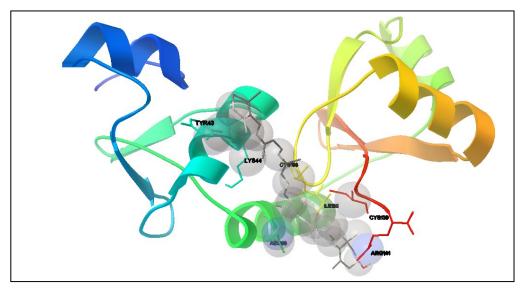**Figure 4.** Hydrophobic interactions between E6 HPV-18 with β-cryptoxanthin

Figure 5. Hydrophobic interactions between E6 HPV-52 with β-cryptoxanthin

distribution for 121,870 residues from 463 known X-ray protein structures. The two most favoured regions are the "core" and "allowed" regions which correspond to 10° x 10° pixels having more than 100 and 8 residues in them, respectively. The "generous" regions were defined by Morris *et al*. (1992) by extending out by 20° (two pixels) all round the "allowed" regions. In contrast, if we found very few residues in these "generous" regions, so they can probably be treated much like the "disallowed" region and any residues in them need to be investigated more detail to find what things might contribute the amino acid residues in disallowed position

[25]. The validation of modeled protein structure quality is important to verify protein structures determined by crystallography, NMR and homology modeling serve as supporting data for Ramachandran plot. Furthermore, molecular docking is conducted to simulate the interaction between protein targets with ligands as inhibitors. From docking study, as all natural inhibitors were found to be docked in various conformations and with varying binding energies, the lowest energy conformation is selected. The highest binding energy to interact with E6 protein HPV-16 is daphnoretin with binding energy is -7.8 kcal/mol, meanwhile HPV-18 and

HPV-52 is β-cryptoxanthin with binding energy -7.8 kcal/mol and -8.4 kcal/mol, respectively (Table 2). Thus, calculated Ki value of inhibitor capacities against E6 HPV-16, HPV-18, and HPV-52 are 1.8889×10^{-6} (daphnoretin), 1.8889×10^{-6} (β-cryptoxanthin), 6.8530×10^{-7} (β-cryptoxanthin), respectively (Table 3). These potential inhibitors are stabilised by hydrophobic interactions which present in binding site of the E6 protein of HPV (Fig. 3-5).

IV.   CONCLUSION

Homology modelling of HPV E6 were succesfully conducted. Validation of HPV E6 protein structures are acceptable as protein models for further structure and function analysis such as docking interaction. Molecular docking of HPV complexes E6 HPV-16, 18 and 52 have given the lowest afinity energy value which indicated the best interaction. As all natural inhibitors were found to be docked in various conformation and with varying binding energies, the lowest energy conformation was selected. Upon docking, the high-ranked binding energies of modeled structures of HPV-16, HPV-18, and HPV-52 E6 oncoprotein with natural inhibitors were obtained. Out of five natural compounds, we found the highest binding energy to interact with E6 protein HPV-16 is daphnoretin with binding energy -7.8 kcal/ mol, HPV-18 and HPV-52 is β-cryptoxanthin with binding energy -7.8 kcal/mol and -8.4 kcal/mol, respectively.

V. ACKNOWLEDGEMENT

VI.   REFERENCES

[1]. International Agency for Research on Cancer. Globocan 2008 fast stats [Internet] Lyon, France: International Agency for Research on Cancer; 2010.

[2]. World Health Organization. Cervical cancer screening in developing countries: report of a WHO consultation [Internet] Geneva: World Health Organization; 2002.

[3]. Ministry of Health of the Republic of Indonesia; Indonesian Pathology Association; The Indonesian Cancer Foundation. Histopathological data of cancer inIndonesia 2006. Jakarta: Ministry of Health of the Republic of Indonesia; 2006.

[4]. M. F. Aziz. 2009. Gynecological cancer in Indonesia. J. Gynecol Oncol. 20:8–10.

[5]. J. Doorbar, N. Egawa, H. Griffin, C. Kranjec, I. Murakami. 2015. Human papillomavirus molecular biology and disease association. Reviews in medical virology 25.S1 (2015): 2-23.

[6]. Center for Disease Control and Prevention. The link between HPV and cancer. 2015.

[7]. J. N. I. Vet, M. A. de Boer, B. E. W. M. van den Akker, B. Siregar, Lisnawati, S. Budiningsih, D. Tyasmorowati, Moestikaningsih, S. Cornain, A. A. W. Peters, G. J. Fleuren. 2008. Prevalence of human papillomavirus in Indonesia: a population-based study in three regions. British journal of cancer. 99.1: 214-218.

[8]. G. J. Fleuren, J. N. I. Vet, M.A. de Boer. 2008. Prevalence of human papillomavirus in Indonesia: a population-based study in three regions. British Journal Cancer . 99: 214-218.

[9]. F. Fehrmann, , D. J. Klumpp, L. A. Laimins. 2003. Human papillomavirus type 31 E5 protein supports cell cycle progression and activates late viral functions upon epithelial differentiation. J. Virol. 77:2819–2831.

[10]. Indraswati, Bestantia. 2005. Kanker Leher Rahim, Pembunuh No.1 Para Wanita di Indonesia dalam: *Sehat*. No.6 / Vol.3. Jakarta: PT Mitra Media Prima.

[11]. C. Dolinsky, C. Hill-Kayser. 2008. Cervical Cancer : The Basics. Trustees of the University of Pennsylvania.

[12]. Ratnawati, Hana, W. Wahyu, K. J. Diana, S. Sylvia. 2008. Cytotoxic activity of Buah Merah fractions (*Pandanus conoideus* Lam) towards cervical cancer cell in HeLa Cells Culture. Proceeding of The International Seminar on Chemistry 2008 (pp. 317-320).

[13]. Basnet, Natasa Skalko, Purusotam. 2011. Curcumin: An anti-inflammatory molecule from a curry spice on the path to cancer treatment.

[14]. Radji, Maksum, A. Hendri, H. Yahdiana, I. Cosphiadi. 2010. Uji sitotoksisitas buah merah, mahkota dewa dan temu putih terhadap sel kanker serviks.

[15]. C. C. Baker, W.C. Phelps, V. Lindgren, M. J. Braun, M. A. Gonda, P. M. Howley. 1987. Structural and translational analysis of human papillomavirus type 16 sequences in cervical carcinoma cell lines. J. Virol. 61:962–971.

[16]. K. Seedorf, G. Krammer, M. Durst, S. Suhai, W. G. Rowekamp. 1985. Human papillomavirus type 16 DNA sequence. Virology 145(1): 181-185.

[17]. S. T. Cole, O. Danos. 1987. Nucleotide sequence and comparative analysis of the human papillomavirus type 18 genome. Phylogeny of papillomaviruses and repeated structure of the E6 and E7 gene products. J. Mol. Biol. 193:599-608.

[18]. H. Delius, B. Hofmann. 1994. Primer-directed sequencing of human papillomavirus types. Curr. Top. Microbiol. Immunol. 186:13-31.

[19]. K. Arnold, L. Bordoli, J. Kopp, T. Schwede. 2006. The SWISS-MODEL workspace: A web-based environment for protein structure homology modelling. Bioinformatics 22: 195-201. DOI: 10.1093/bioinformatics/bti770.

[20]. R. A. Laskowski, M. W. MacArthur, D.S. Moss, Thornton JM. PROCHECK : a program to check the stereochemical quality of protein structures. *J Appl Crystallogr* 1993;26:283-291.

[21]. V. Lisdawati, S. Wiryowidagdo, L. B. S. Kardono. 2007. Lignan, isolasi dan elusidasi struktur senyawa, dan asam. "lemak dari ekstrak daging buah *Phaleria macrocarpa*. Bul. Penel. Kesehatan. 35(2): 115-124.

[22]. E. M. Sutrisna. 2007. Potensi Sitotoksik Tanaman Ceplukan (*Physalis Angulata* L) terhadap Sel Hela Cytotoxic Effects Of *Physallis Angulata* Plant On Hela Cell Line Cells. Pharmacon, 8(1): 1–6.

[23]. R. Lobo, K. S. Prabhu, A. Shirwaikar, A. Shirwaikar. 2009. Curcuma zedoaria Rosc. (white turmeric): a review of its chemical, pharmacological and ethnomedicinal properties. Journal of Pharmacy and Pharmacology. 61(1): Pages 13–21.

[24]. O. Trott, A. J. Olson. 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, Journal of Computational Chemistry 31: 455-461.

[25]. A. L. Morris, M. W. MacArthur, E. G. Hutchinson, J. M. Thornton. 1992. Chemical quality of protein structure coordinates. Proteins Struct Funct Genet 12:345-364.