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Vaccine construction for human papillomavirus (HPV) type 16 and 18 infection using *in silico* approach to combat cervical cancer

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Abstract

Human papillomavirus (HPV) is a virus that causes infection on the surface of the skin and has the potential to cause cervical cancer. This viral infection is characterized by the growth of warts on the skin in various areas of the body, such as the arms, legs, mouth, and genital area. Because the virus can endanger health, it is necessary to design an HPV vaccine to overcome this problem. In this study, we performed a study characterization of HPV types 16 and 18 sequences to obtain immunogenic epitopes retrieved from the National Center for Biotechnology Information (NCBI) web server. Then, epitope prediction was performed using the immune epitope database (IEDB) web server and selected to get the best vaccine candidate for HPV types 16 and 18. We recommend 16P1 as an epitope-based peptide vaccine candidate for HPV type 16 and 18P4 for type 18. Both vaccine candidates are antigenic, non-allergenic, and non-toxic. The 16P1 and 18P4 have the lowest global energy values among the other candidates. However, further research is needed to be able to develop the best vaccine (*in vitro* and *in vivo* experiments).

Keywords: Epitope-based peptide vaccine, human papillomavirus, Immunoinformatics

Introduction

Human Papillomavirus (HPV) is a virus that can cause infection by transmitting from skin to skin. HPV is the cause of nearly 500,000 cases of cancer including cervical cancer, anogenital cancer and oropharyngeal cancer[1]. HPV infection can be detected by DNA from infected skin samples, oral, and

anogenital from all people. Currently, there are about 200 types of HPV that have been fully characterized and have been classified into 3 genus namely *Alphapapillomavirus*, *Apapillomavirus* and *Gammapapillomavirus*[2].

Among several HPVs, the genus *Alphapapilomavirus* contains HPV types that are uniquely pathogenic[3]. Approximately 30 types of HPV can infect the genital area, 15 of which have a high risk, which is oncogenic or can cause cancer[4]. HPV type 16 is one of the most common and high-risk types, accounting for 56% of all cervical cancers[5]. HPV infection with a high level of risk is the most important aspect of cervical cancer[6].

About 15 mucosotropic HPV types associated with human malignancy are classified as high-risk HPV types[7]. There are HPV types 16 and type 18 which are the most common high-risk HPV types and cause about 80% of cervical cancers worldwide, of which 20% are caused by other types of viruses[8]. With these various explanations, the vaccine design process certainly needs to be done immediately to overcome these problems.

Vaccines are one of the largest contributors to global health[9]. With the vaccination process, of course, it can save the lives of many people from various diseases. For this reason, various studies are currently being carried out to get the best vaccine to later be able to treat and prevent various diseases caused by viruses and bacteria. one of the efforts that can be done is to design vaccines *in silico* or use an immunoinformatics approach which can later produce the best vaccine candidates before the in vitro process or clinical trials are carried out[10].

Development in the manufacture of the HPV vaccine is very necessary to do considering the nature of the virus which is very easy to mutate over time. mutations in viruses such as HPV can cause a virus to become resistant to drugs or vaccines given so that a vaccine design process is needed to get candidates that can overcome these problems[11]. Therefore, the epitope-based peptide vaccine is suggested as new potential candidates for effective vaccine of HPV.

Materials and methods

Sampling

The sequence of HPV Type 16 and 18 was obtained from National Center for Biotechnology Information (NCBI), USA.

Procedures

3D protein modeling

The 3D structure of protein sequence was modeled using the SWISSMODEL (https://swissmodel.expasy.org) with homology modeling approach[12,13].

Immunoinformatics prediction

This study predictions based on linear B-cell epitopes, including BepiPred, Emini Surface, and Kolaskar & Tongaonkar on the Immune Epitope Database (IEDB) web server (http://tools.iedb.org/main/bcell)[13]. Then we predicted the characteristics of candidate epitopes that can act as protective antigens using VaxiJen v2.0 web server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html)[14]. Prediction of similarity with proteins is performed on vaccine candidate peptide via the Basic Local Alignment Protein Search Tool (BLASTp) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Then, Allergenicity are predicted using AllerTOP v2.0 web server (https://www.ddg-pharmfac.net/AllerTOP/), and last step of immunoinformatics prediction is

determine the toxicity of vaccine candidate by using Toxinpred (https://webs.iiitd.edu.in/raghava/toxinpred/multi_submit.php)[15].

Protein docking

The molecular docking process was carried out to determine the interaction between the ligand and the receptor. In this study, candidate epitope from HPV which is a ligand and BCR is used. Peptides that have passed the previous selection need to be converted into pdb form so that the molecular docking process can be carried out using the Pepfold 3 web server (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3). BCR obtained from web server RCSB PDB (https://www.rcsb.org/). To carry out the molecular docking process, patchdock and firedock (https://bioinfo3d.cs.tau.ac.il/PatchDock/) web servers are used. The results of the molecular docking can be used to determine the best vaccine candidate[16].

Results

After carrying out the various stages of the required method, various data have been obtained which are then carried out the analysis process of the data.

Conserved Identification from HPV Type 16 and 18

A total of two HPV sequences were used in this study which had previously been obtained from the NCBI web server. The two sequences are protein sequences from HPV type 16 and HPV type 18. The sequences that have been obtained from NCBI can be seen, as follows:
**MEVTFIYILVITCYENDVNVYHIFFQMSLWLPSEATVYLPPVPVSKVVSTDEYVARTNIYYHAGTSR
LLAVGHPYFPIKKPNNNKILVPKVSGLQYRVFRIYLPDPNKFGFPDTSFYNPDTQRLVWACVGVEV
GRGQPLGVGISGHPLLNKLDDTENASAYAANAGVDNRECISMDYKQTQLCLIGCKPPIGEHWG
KGSPCNNVAVTPGDCPPLELINTVIQDGDMVDTGFGAMDFTTLQANKSEVPLDICTSICKYPDYI
KMVSEPYGDSLFFYLRREQMFVRHLFNRAGAVGENVPDDLYIKGSGSTANLASSNYFPTPSGSMV
TSDAQIFNKPYWLQRAQGHNNGICWGNQLFVTVVDTTRSTNMSLCAAISTSEPTYKNTNFKEYL
RHGEEYDLQFIFQLCKITLTADVMSYIHSMNSTILEDWNFGLQPPPGGTLEDTYRFVTSQAIACQK
HTPPAPKEDPLKKYTFWEVNLKEKFSADLDQFPLGRKFLLQAGFKAKPKFTLGKRKATPTTSSTST
TAKRKKRKL** which is a protein sequence of the HPV type 16 virus, and
**MCLYTRVLILHYHLLPLYGPLYHPQPLPLHSILVYMVHIIICGHYIILFLKSVNVFPIFLQMALWRPSD
NTVYLPPPSVARVVNTDDYVTRTSIFYHAGSSRLLTVGNPYFRVPAGGGNKQDIPKVSAYQYRVF
RVQLPDPNKFGLPDNSIYNPETQRLVWACAGVEIGRGQPLGVGLSGHPFYNKLDDTESSHAATS
NVSEDVRDNVSVDYKQTQLCILGCAPAIGEHWAKGTACKSRPLSQGDCPPLELKNTVLEDGDM
VDTGYGAMDFSTLQDTKCEVPLDICQSICKYPDYLQMSADPYGDSMFFCLRREQLFARHFWNRA
GTMGDTVPQSLYIKGTGMRASPGSCVYSPSPSGSIVTSDSQLFNKPYWLHKAQGHNNGICWHN
QLFVTVVDTTRSTNLTICASTQSPVPGQYDATKFKQYSRHVEEYDLQFIFQLCTITLTADVMSYIHS
MNSSILEDWNFGVPPPPTTSLVDTYRFVQSVAITCQKDAAPAENKDPYDKLKFWNVDLKEKFSLD
LDQYPLGRKFLVQAGLRRKPTIGPRKRSAPSATTSSKPAKRVRVRARK** is a protein sequence of the HPV type 18 virus.

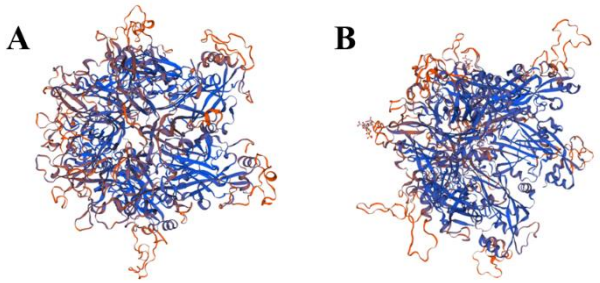

Figure 1. 3D structure from HPV (A) type 16; and (B) type 18 using SWISS-MODEL web server modeling

The B-cell Immunogenicity Predictions of Peptide Vaccine Candidate

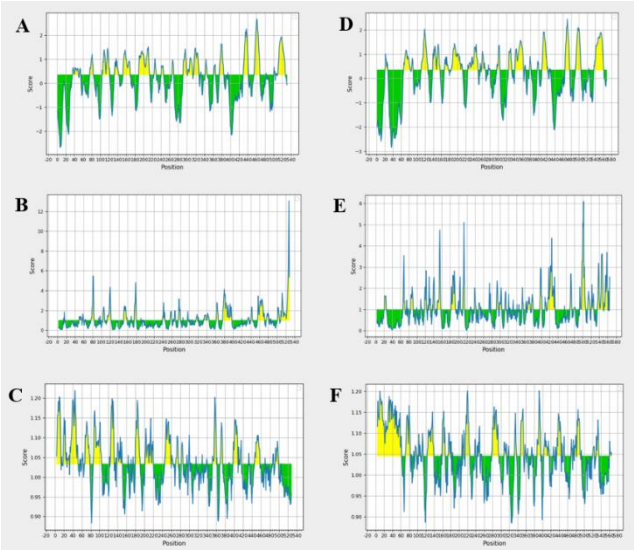

Figure 2. Results of B-cell epitope prediction. green areas showed negative prediction and yellow areas for positive prediction. (A) BepiPred for HPV type 16; (B) Emini Surfaces for HPV type 16; and (C) Kolaskar & Tongaonkar for HPV type 16, (D) BepiPred for HPV type 18; (E) Emini Surfaces for HPV type 18; and (F) Kolaskar & Tongaonkar for HPV type 18.

Table 1. HPV type 16 B-cell Immunogenicity predictions using BebiPred

Name	Peptide	Antigenicity	Similarity	Allergenicity	Toxicity
16P1	TVYLPPVPVSKVVS	ANTIGEN	Similar (40-50)	NON-ALLERGEN	Non-Toxin
16P2	PDPNKFGFPDTSFYNPDT	ANTIGEN	Similar (50-80)	ALLERGEN	Non-Toxin
16P3	VGRGQPLGVG	ANTIGEN	Similar (<40)	NON-ALLERGEN	Non-Toxin
16P4	DDTENASAYAANAGVD	ANTIGEN	Similar (50-80)	NON-ALLERGEN	Non-Toxin
16P5	PPIGEHWGKGSPCNNVAVTPG DCPP	ANTIGEN	Similar (80-200)	ALLERGEN	
16P6	YIKGSGSTANLA	NON-		NON-	

		ANTIGEN		ALLERGEN	
16P7	SNYFPTPSGSMVT	NON-ANTIGEN		NON-ALLERGEN	
16P8	STSEPTYKNTN	ANTIGEN	Similar (<40)	NON-ALLERGEN	Non-Toxin
16P9	FGLQPPPGGTLE	ANTIGEN	Similar (40-50)	ALLERGEN	
16P10	QKHTPPAPKEDPLK	NON-ANTIGEN		NON-ALLERGEN	
16P11	KRKATPTTSSTSTTAKRK	ANTIGEN	Similar (50-80)	ALLERGEN	

Table 2. HPV type 16 B-cell immunogenicity predictions using Emini Surfaces

Name	Peptide	Antigenicity	Similarity	Allergenicity	Toxicity
16P12	TSEPTYKNTNFKEYLRHGEEYD	ANTIGEN	Similar (50-80)	NON-ALLERGEN	Non-Toxin
16P13	KHTPPAPKEDPLKKYT	NON-ANTIGEN		NON-ALLERGEN	

Table 3. HPV type 16 B-cell immunogenicity predictions using Kolaskar & Tongaonkar

Name	Peptide	Antigenicity	Similarity	Allergenicity	Toxicity
16P14	TFIYILVITC	NON-ANTIGEN	-	NON-ALLERGEN	-
16P15	EATVYLPPVPVSKVVSTDE	ANTIGEN	Similar (50-80)	NON-ALLERGEN	Non-Toxin
16P16	LLAVGHPYFPI	ANTIGEN	Similar (<40)	ALLERGEN	-
16P17	ILVPKVSGLQYRVFRIYLP	NON-ANTIGEN	-	ALLERGEN	-
16P18	PLGVGISGHPLLN	ANTIGEN	Similar (40-50)	ALLERGEN	-
16P19	TQLCLIGCKPP	ANTIGEN	Similar (<40)	NON-ALLERGEN	Non-Toxin
16P20	CNNVAVTPGDCPPLELINTV	ANTIGEN	Similar (50-80)	ALLERGEN	-
16P21	EVPLDICTSICKYPD	NON-ANTIGEN	-	NON-ALLERGEN	-
16P22	LQFIFQLCKIT	NON-ANTIGEN	-	ALLERGEN	-
16P23	FVTSQAIACQKH	ANTIGEN	Similar (40-50)	ALLERGEN	-

Table 4. HPV type 18 B-cell immunogenicity predictions using BebiPred

Name	Peptide	Antigenicity	Similarity	Allergenicity	Toxicity
18P1	PSDNTVYLPPPSVARVV	NON-ANTIGEN	-	ALLERGEN	-
18P2	FRVPAGGGNKQDIPKV	NON-	-	NON-	-

		ANTIGEN		ALLERGEN	
18P3	LPDPNKFGLPDNSIYNPET	NON-ANTIGEN	-	NON-ALLERGEN	-
18P4	EIGRGQPLGVG	ANTIGEN	Similar (<40)	NON-ALLERGEN	Non-Toxin
18P5	YNKLDDTESSHAATSNVSEDVRDNVSVDY	ANTIGEN	Similar (80-200)	ALLERGEN	-
18P6	AIGEHWAKGTACKSRPLSQGDCPPLE	ANTIGEN	Similar (80-200)	ALLERGEN	-
18P7	GDMVDTGYGA	ANTIGEN	Similar (<40)	ALLERGEN	-
18P8	GMRASPGSCVYSPSPSGSIVT	NON-ANTIGEN	-	NON-ALLERGEN	-
18P9	STQSPVPGQYDATKF	NON-ANTIGEN	-	NON-ALLERGEN	-
18P10	NFGVPPPPTTSL	ANTIGEN	Similar (40-50)	NON-ALLERGEN	Non-Toxin
18P11	QKDAAPAENKDPYD	ANTIGEN	Similar (40-50)	ALLERGEN	
18P12	RRKPTIGPRKRSAPSATTSSKPAKR	ANTIGEN	Similar (50-80)	NON-ALLERGEN	Non-Toxin

Table 5. HPV type 18 B-cell immunogenicity predictions using Emini Surfaces

Name	Peptide	Antigenicity	Similarity	Allergenicity	Toxicity
18P13	PFYNKLDDTES	ANTIGEN	Similar (<40)	NON-ALLERGEN	Non-Toxin
18P14	PGQYDATKFKQYSRHVEEYD	NON-ANTIGEN	-	NON-ALLERGEN	Non-Toxin
18P15	DAAPAENKDPYDKLK	ANTIGEN	Similar (50-80)	ALLERGEN	Non-Toxin
18P16	GLRRKPTIGPRKRSA	ANTIGEN	Similar (40-50)	ALLERGEN	Non-Toxin

Table 6. HPV type 18 B-cell immunogenicity predictions using Kolaskar and Tongaonkar

Name	Peptide	Antigenicity	Similarity	Allergenicity	Toxicity
18P17	YTRVLILHYHLLPLYGPLYHPQPLPLHSILVYMVHIIICGHYIILFLKSVNVFPIFLQM	NON-ANTIGEN	-	NON-ALLERGEN	-
18P18	TVYLPPPSVARV	NON-ANTIGEN	-	ALLERGEN	-

18P19	IPKVSAYQYRVFRVQLP	NON-ANTIGEN	-	-	-
18P20	TQLCILGCAPA	NON-ANTIGEN	-	-	-
18P21	KCEVPLDICQSICKYPDY	NON-ANTIGEN	-	-	-
8P22	LTICASTQSPVPGQ	NON-ANTIGEN	-	-	-
8P23	LQFIFQLCTIT	NON-ANTIGEN	-	-	-
8P24	FVQSVAITCQ	ANTIGEN	Similar (<40)	ALLERGEN	-

Table 7. HPV type 16 and 18 peptides candidate.

Name	Peptide
16P1	TVYLPPVPVSKVVS
16P3	VGRGQPLGVG
16P4	DDTENASAYAANAGVD
16P8	STSEPTYKNTN
16P12	TSEPTYKNTNFKEYLRHGEEYD
16P15	EATVYLPPVPVSKVVSTDE
16P19	TQLCLIGCKPP
18P4	EIGRGQPLGVG
18P10	NFGVPPPPTTSL
18P12	RRKPTIGPRKRSAPSATTSSKPAKR
18P13	PFYNKLDDTES

Molecular Interaction between Peptide-BCR/Fab

Table 8. Global energy score of peptides vaccine candidate and BCR 5ifh.

Ligand	Receptor	Global Enery
16P1	5ifh	-66.04
16P3	5ifh	-51.28
16P4	5ifh	-48.54
16P8	5ifh	-48.22
16P12	5ifh	-21.04
16P15	5ifh	-58.50
16P19	5ifh	-48.95
18P4	5ifh	-47.04
18P10	5ifh	-43.19
18P12	5ifh	-4.65
18P13	5ifh	-17.69

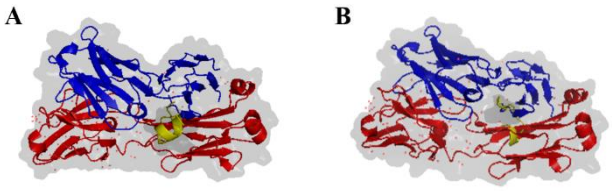

Figure 3. Visualization of the bond between the ligand and the receptor using Pymol software. (A) 16P1 and 5ifh bond visualization; and (B) 18P4 and 5ifh bond visualization.

Discussion

Conserved Identification from HPV Type 16 and 18

The initial identification process in this research is to do protein modeling that has been obtained previously using a swiss model web server. 3-dimensional modeling needs to be done to determine the structure of the protein to be used so that later it can facilitate the identification process further. 3-dimensional modeling on the swiss web server model is a homology modeling where the modeling works by matching the template of the protein sequence that we input with the 3D model of the protein that is available on the web server. From the process of modeling the protein sequence, the results of the 3-dimensional structure of the HPV type 16 and HPV type 18 virus protein sequences were obtained (Fig.1). With the virus protein model, a more in-depth analysis process can be carried out[14].

The B-cell Immunogenicity Predictions of Peptide Vaccine Candidate

The HPV type 16 and 18 protein sequences that have been obtained then need to be identified to determine the appropriate peptide so that later they can be used as vaccine candidates. By using the IEDB web server, various peptides were obtained with several tools provided on the web server such as BepiPred, Emini Surfaces, and Kolaskar & Tongaonkar parameters. By using these tools, a graph of the prediction results of b-cell epitope is obtained (Fig.2)[13].

The results of these readings are then processed to determine candidate peptides that meet the requirements. First is by selecting antigenicity with the VaxiGen web server and taking peptide candidates that are antigenic. The next step is to look for similarity with NCBI's BLASTp web server. Then allergenicity prediction was performed to determine whether the peptide could be allergenic using the AllerTop web server. The last step is to determine the toxicity using the Toxinpred web server to obtain the toxicity properties of the candidate peptides.

By selecting through several web servers that have been mentioned previously, various peptides from HPV types 16 and 18 have been named and the properties of these peptides have been known (Table 1-6). When the candidate peptide meets the required criteria, further processing can be carried out.

From the selection b-cell epitope of HPV type 16 and 18, 11 peptides were found that met the criteria as vaccine candidates which could then be carried out by a molecular docking process for further selection (Table 7)[17].

Molecular Interaction between Peptide-BCR/Fab

The molecular docking process is carried out using the Patchdock and Firedock web server. global energy was obtained from each candidate peptide docked with 5ifh BCR (Table 8). From the molecular docking results, 16P1 with 14-mer length has the lowest global energy among other candidate peptides, which means that the peptide can bind to the receptor well. 16P1 can be used as a candidate for

HPV type 16 vaccine because it meets the required criteria and has the lowest global energy, which is -66.04. Like 16P1, 18P4 peptide with 11-mer length is suitable for use as a candidate for HPV type 18 vaccine because the global energy is the lowest among other candidates, namely -47.04 and has met all the criteria needed to be used as a vaccine candidate[18].

From the results of vaccine candidates for HPV types 16 and 18 that have been obtained, then modeling using Pymol software is carried out to obtain visualization of the bond between the ligand and the receptor. From the results of the visualization, analysis can be done regarding the structure and form of the bond between the ligand and the receptor (Fig. 3).

Conclusions

In conclusion, we recommend 16P1 for HPV Type 16 and 18P4 for HPV Type 18 as epitope-based peptide vaccine candidate to deal with the HPV infection. 16P1 and 18P4 has a high level of immunogenicity and does not trigger autoimmune mechanisms. 16P1 and 18P4 also capable of forming BCR/Fab molecular complexes with the lowest binding energy for activation of transduction signal the direct B-cell immune response.

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Conflicts of Interest

There are not potential conflicts of interest.

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