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B-cell Epitope Mapping of Capsid L1 from Human Papillomavirus to Development Cervical Cancer Vaccine Through In Silico Study

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ABSTRACT

Human papillomavirus (HPV) is a virus that plays an important role in the occurrence of cervical cancer. The HPV gene is composed of two parts: early and late gene. The L1 protein has a conserved region composed of cysteine and lysine residues, both of which have involved in the binding process between virions and host receptors. Previous research has shown that vaccines can be developed based on epitopes that have conserved areas. This study is important to identify conserved protein

† Footnotes relating to the title and/or authors should appear here.
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sequences in L1 of HPV capsid, predict epitope mapping of B cells and antigenicity in the conserved region of L1 HPV capsid, as well as the similarity of amino acid residues of epitope composers with surface receptors of human body cells. The conserved areas were identified in L1 HPV as a potential epitope of B cells based on epitope mapping analysis of positions 23-46 and 97-119 with EGRGQPLGGSGHPNDDE DRDKQ and RHNGGPGPSGSSQFNKPYWAQGN peptides and each had a peptide length of 22-mer and 23-mer. The 97-119 epitope has a high antigenicity score and the similarity of the low amino acid residue sequence to the cell surface receptor of the human body, the 23-mer RHNGGPGPSGSSQFNKPYWAQGN peptide can be used as a reference for the development of cervical cancer prevention vaccine.

Keywords: Conserved Region, Epitope Mapping, HPV, Peptide, L1

INTRODUCTION

HPV infection has increased since 1960 due to increased cases of cervical cancer and the development of genital warts into carcinomas[1,2]. HPV types 6 and 11 were found to be 35% in genital warts whereas HPV types 16 and 18 found 63% in carcinoma[3,4]. Human papillomavirus (HPV) is a virus has played a key role in the occurrence of cervical cancer, HPV is a member of the family papovaviridae, genus papillomavirus[5,6]. HPV is 55 nm in diameter and that virus has a circular DNA type and icosahedral capsid (L1 and L2) composed of 72 capsomers HPV is composed of the double strand DNA (dsDNA) genome and two types of capsid proteins L1 and L2, HPV is a non-enveloped virus and replicates in the nucleus of an infected host cell[7]. The early proteins in HPV include E1, E2, E4, E5, E6, and E7[8]. Then, a late gene expressed by the late promoter acts as a protein-coding of L1 and L2 capsid[9].

Previous research was demonstrated that peptides designed under in silico analytical methods may be used as a candidate for nasopharyngeal carcinoma prevention vaccines[10]. In this study using L1 capsid protein, L2 has a low immunogenicity value that cannot trigger activation of Th1 and 2 cells[11]. L1 protein can be used as a vaccine against HPV infection as indicated in HPV vaccine that has been found previously, that is Cervarix and Gardasil containing capsid particles L1 of specific HPV types, the HPV vaccine is largely divided into 2 bivalent and quadrivalent vaccines, to date the method of making HPV vaccine is through recombinant DNA technology, one of which uses VLP, bivalent and quadrivalent HPV vaccine is a prophylactic vaccine[12,13].

B cell epitope mapping is a method to predict the region of proteins, that can be recognized and related to cell B response[14,15]. The epitope is a specific area of the part of the antigen binding to the paratope of the antibody and can also recognized by T and B cells, the epitope can be either self-epitope or outside[16,17]. This research uses linear B cell epitope prediction method, using web server tools on *iedb.org/bcell*[18]. A Bepipred method is a computational approach used to study and identify linear B cell epitope[19.20]. Based on this statement, the development of epitope conserved based vaccine for maximum cervical cancer prevention is necessary because to provide more protection against HPV infection by adding other HPV types that have been identified and not yet used in previous vaccine making, the importance of protein sequence identification conserved and predicted properties of antigenicity and epitope mapping on the L1 protein HPV underlying this study.

METHOD

Sample retrieval

Sequence data obtained from NCBI (*www.ncbi.nlm.nih.gov*). The search in a biological database is done by using keyword "L1 Human Papillomavirus", these sample collected from all high-risk strains of virus available in the database, then after the samples obtained successfully stored in FASTA format on a notepad[21,22].

Identification of Conserved Region on L1 HPV

Alignment is the process of aligning DNA and protein sequences, this process using software such as BioEdit and MEGA and alignment is done for sequence alignment of protein samples[23]. This study used MEGA 5.05 software to perform alignment of L1 HPV protein sequences and identification of conserved protein sequences as has been done in previous studies. After the alignment sequence process is complete then the next step is protein modeling, where this method aims to perform 3D construction of L1 capsid HPV[24]. Protein modeling in this study on methods that have been used by previous researchers. The conserved sequence modeled its 3D structure using the Swiss Model server (*www.swissmodel.expasy.org*), conserved protein modeling was done without the use of templates. After the modeling process is completed then the next 3D alignment between the conserve protein structure with a template in the form of L1 HPV protein is done in PyMol 1.1 software[25].

Prediction of B-cell Epitop Mapping and Antigenicity

This method has functions to determine or predict the epitope of B cells and their antigenicity. Epitope and antigenicity predictions are analyzed through facilities on the IEDB online webserver (*www.iedb.org*)[26,27].

Similarity Analysis

After the prediction of the B cell epitope, the peptide obtained was then further analyzed using basic local alignment tools protein (BLASTp) (*http://blast.ncbi.nlm.nih.gov/blast.cgi*)[28,29]. This analysis aims to compare the similarities with the protein sequences contained in the human body[30]. The results of the recommended analysis are the results of the analysis with a low similarity value to the human body cell protein, especially at the cell surface receptor itself[31]. This is done to avoid any autoimmune response from the patient's body that will receive the vaccine[32]. In the results of BLAST need to be considered for the score above 70%, because there must be sequences have similarities to human surfaces receptor, especially those on the cell surface[33].

RESULT AND DISCUSSION

The Position of Conserved Region on L1

The samples of L1 sequence from HPV HR type is obtained from the NCBI database were 13 types and 869 strains. The alignment process used the MEGA 5 program and produced a conserved protein sequence with a 173-mer sequence length, had residual C (cysteine) of 8 and 6 K (lysine) residues (Figure 1), then analyzed protein modeling on the web server Swiss Model for obtaining 3D protein structures. Homology modeling is a method of constructing 3D structures of proteins through protein template structures to produce target proteins using the web server *www.swissmodel.expasy.org*, which results in the structure of 3D protein[34,35]. Homology processes produce homologous protein structures that have conserved protein sequences, but sequences of similarity values below 20% are expressed to have different structures than templates[36,37]. The 3D model protein produced by the Swiss-Model server shows the identified query protein sequence value of 77.03% according to the HPV L1 capsid template consisting of 5 chains with 3ofl PDI ID (Figure 2).

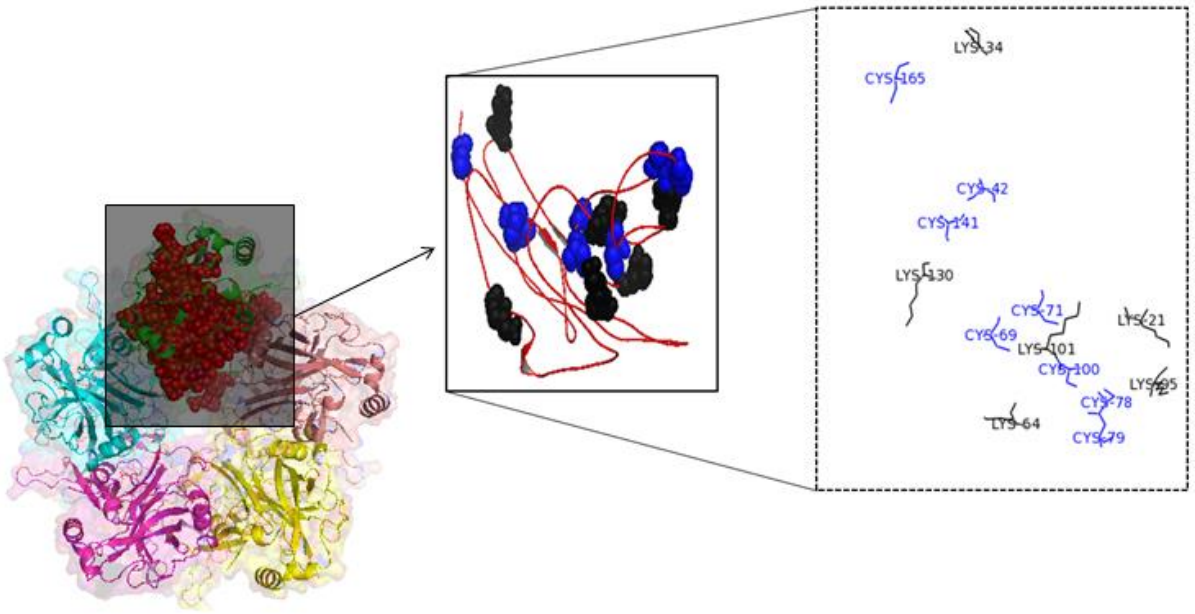

Figure 1. Visualization of the amino acid residue position of cysteine and lysine on the conserved region. The red color in the cartoons and spheres structure is the conserved region on the L1, the amino acid consist in blue and black spheres is cysteine and lysine.

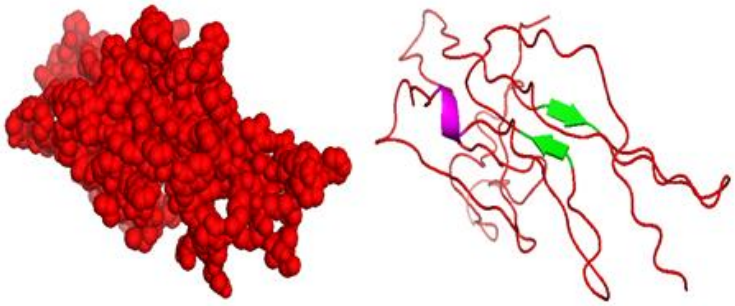

Figure 2. Visualization the model of peptides. Peptides are displayed in the sphere structure (left) and cartoon (right). Green, magenta, and red colors respectively show the structure of secondary proteins in the form of beta sheets, alpha helix, and coil.

B-cell Epitope Prediction

Epitope mapping is a method for predicting binding sites of an epitope, antibody, target antigen[38,39,40,41,42]. Identification by epitope mapping method was done for vaccine development and diagnosis[43,44,45,46]. Epitope prediction refers to the pathogenesis system of a particular disease to be designed for the vaccine, the system determines of epitope to be predicted based on the epitope of B cells or T cells. Epitope prediction can be analyzed by some IEDB software or web server[47,48]. This study used IEDB web server to analyze epitope mapping based on cell epitope B in conserved sequence in L1 HPV capsid protein using a BepiPred method[49,50]. Epitope or epitope mapping cell B prediction is a method for predicting the region of proteins that can be recognized as epitopes and related to cell B response. A BepiPred method is a computational approach used to study and identify linear cell epitopes B. The principle of linear epitope prediction uses the BepiPred method based on the parameters of the hydrophilicity scale and

secondary structure of the Levitt protein and using the statistical tools of the hidden Markov model. Scores of the results of linear epitope prediction analysis of BepiPred method show that there is a predicted protein sequence position of the B-cell epitope that is 25-46 sequence with 22-mer sequence length and 97-119 sequence with 23-mer length (Table 1).

Table 1. Peptide predicted by a BepiPred method. The 97-119 sequence position has a 23-mer length longer than of the 23-46.

No	Posisi Sekuens	Peptida	Panjang
1	23-46	EGRGQPLGGSGHPNDDEDRDKQ	22-mer
2	97-119	RHNGGPGPSGSSQFNKPYWAQGN	23-mer

In the epitope mapping graph, the BepiPred method of IEDB webserver output analysis shows that peptides with protein sequence positions 23-46 and 97-119 with the threshold of 0.510 are included in the yellow region (Figure 3). The residue with a score above the threshold (default 0.35) is predicted to be part of the yellow epitope and color in the graph (where the Y-axis shows the residual score and the X-axis shows the residual position in the sequence). 3D sequence structure obtained by homology modeling on web server *www.swissmodel.expasy.org*, then visualized protein structure and 3D alignment using PyMol software. Peptide position 23-46 with protein sequence EGRGQPLGGSGHPNDDEDRDKQ and position 97-119 with protein sequence RHNGGPGPSGSSQFNKPYWAQGN predicted as an epitope of B cells in chain A

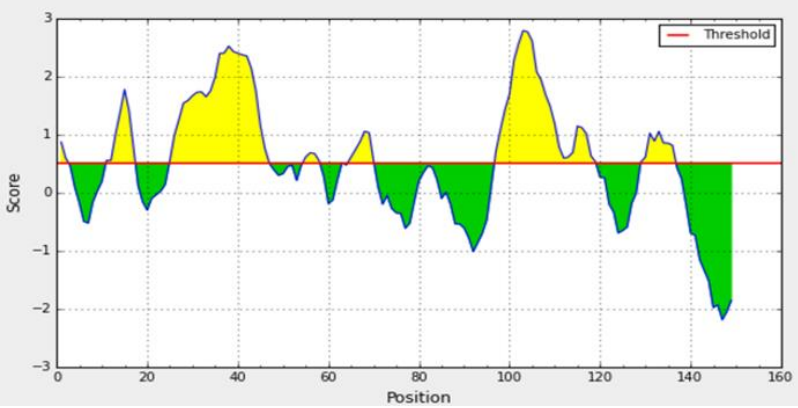

Figure 3. The result from the prediction of a BepiPred method. The yellow area shows a score above the positive threshold which is predicted as a B cell epitope.

Antigenicity is a property possessed by antigens that allow antigens to trigger B cell responses to produce specific antibodies, and the nature of this antigen also refers to immunogenicity. Peptides of 22-mer and 23-mer lengths are potential as epitopes, then compared their antigenicity values using the Kolaskar & Tongaonkar method on the IEDB web server and displayed in the PyMol software. The Kolaskar & Tongaonkar method is a computational protein antigenicity prediction method based on physicochemical properties and experimental data, with the accuracy of up to 75%[51]. The predicted antigenicity score using the Kolaskar & Tongaonkar method showed that the peptide of 23-mer sequence number 97-119 has

a high antigenicity score of 22-mer peptide position 25-46, the 23-mer peptide with protein sequence RHNGGPGPSGSSQFNKPYWAQGN predicted to trigger the formation of adaptive immune response by B cell.

Analysis of basic local alignment sequence tool (BLAST) in the vaccine design stage serves to perform data comparisons with proteins in the human body, it is done to avoid the autoimmune response from the body of patients who will receive the vaccine, on reading the results of BLAST maximum score of 70% query sequence similarities with proteins contained in the human body[52]. The results of the RHNGGPGPSGSRHNGGPGPSGS SQFNKPYWAQGN protein analysis with 23-mer length showed very low similarity score with the surface receptor of a human body cell that is >40. The epitope can be used as a reference for the development of HPV vaccine. This research shows that bioinformatics is a very useful tool in the vaccine design process and useful for analyzing the interactions of genes and analysis of herbal mediated cell apoptosis.

CONCLUSION

The positions of conserved protein sequence potentially B cell epitope 23-46 and 97-119 position with EGRGQPLGGSGHPNDDEDRDKQ and RHNGGPGPSGSSQFNKPYWAQGN peptide and each have a peptide length of 22-mer and 23-mer. The 97-119 epitope has a high antigenicity score and the similarity of the low amino acid residue sequence to the cell surface receptor of the human body. So the 23-mer RHNGGPGPSGSSQFNKPYWAQGN peptide can be used as a reference for the development of cervical cancer prevention vaccine.

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