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Computational prediction of linear B-cell epitopes in the E5 oncoprotein of the human papillomavirus type 16 using several bioinformatics tools

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ABSTRACT

Human papillomavirus (HPV), one of the most common sexually transmitted infections worldwide, affects around 300 million new individuals each year. The HPV *E5* gen appear to have coevolved with the major HPV oncogenes *E6* and *E7*, and their presence in the viral genome correlates with risk of cancer. HPV-16 E5 is an oncoprotein with many functions during HPV-16 infection. The development of computational methods for epitope finding becomes an alternative to the wet experimental techniques, in order to save time and cost. In this work the bioinformatic tools: Predict Protein, BCPREDS (AAP, BCpreds and FBCPred), ABCpred, BepiPred, SVMTriP, LBtope, IEDB and LEPS were used in order to elucidate B-cell epitopes on HPV-16 E5. The HPV-16 E5 secondary structure was used to mapping the B-cell epitopes obtained in this study. A total of 202 B-cell epitopes and 44 B-cell epitopes with tumor activity were obtained with all together bioinformatics tools. Differences in ratio of these epitopes with the three α-helices of the secondary structure determined by Predict Protein were observed. The predicted epitopes in this study could facilitate the design of future therapeutic vaccines and the understanding of the immunological mechanisms of HPV-16.

Predicción computacional de epítopos de células B en la oncoproteína E5 del VPH-16 mediante el uso de diversas herramientas bioinformáticas

Palabras clave:
VPH-16
E5
Oncoproteína
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Células B
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RESUMEN

El virus del papiloma humano (VPH) es una de las infecciones de transmisión sexual más comunes en todo el mundo, que afecta a unos 300 millones de personas cada año. El gen *E5* del VPH parece haber coevolucionado con los principales oncogenes *E6* y *E7* y su presencia en el genoma viral se correlaciona con el riesgo de cáncer. VPH-16 E5 es una oncoproteína con muchas funciones durante la infección del VPH-16. El desarrollo de los métodos computacionales para el descubrimiento de epítopos se convierte en una alternativa a las técnicas experimentales húmedas, con el fin de ahorrar tiempo y costes. En este trabajo las

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herramientas bioinformáticas: Predict Protein, BCPREDS (AAP, BCpreds y FBCPred), ABCpred, BepiPred, SVMTriP, LBtope, IEDB y LEPS se utilizaron con el fin de elucidar epítopos de células B en la proteína E5 del VPH-16 (VPH-16 E5). La estructura secundaria de la VPH-16 E5 se utilizó para mapear los epítopos de células B predichos en este estudio. Un total de 202 epítopos de células B y 44 epítopos de células B con actividad tumoral se obtuvieron con todas las herramientas bioinformáticas en conjunto. Se observaron diferencias en la proporción de estos epítopos en las 3 α-hélices de la estructura secundaria determinada por Predict Protein. Los epítopos predichos en este estudio podrían facilitar el diseño de futuras vacunas terapéuticas y la comprensión de los mecanismos inmunológicos de VPH-16.

Introduction

Human papillomavirus (HPV), one of the most common sexually transmitted infections worldwide, affects around 300 million new individuals each year. About 14 types are classified as high-risk HPVs, among which HPV types 16 and 18 cause about 70% of all cervical cancers representing each year about half million new cases globally.[1,2]

Human papillomavirus type 16 E5 protein (HPV-16 E5) of 83-amino acid has been subjected to extensive analysis, but the molecular basis for its biological activities is still not well under- stood.[3,4] The HPV E5 genes appear to have coevolved with the major HPV oncogenes E6 and E7, and their presence in the viral genome correlates with risk of cancer, suggesting that the E5 proteins may contribute to human carcinogenesis.[5]

HPV-16 E5 acts synergistically with epidermal growth factor (EGF) in murine fibroblasts to induce anchorage independent growth and growth in low serum,[6,7] as well as to enhance DNA synthesis in primary human keratinocytes.[7] Furthermore, HPV16 E5 cooperates with HPV E7 to induce the proliferation of primary rodent epithelial cells.[8] HPV-16 E5 displays additional transforming activities, including the ability to render mouse cell lines tumorigenic,[9] enhance the ability of E6 and E7 to immortalize primary human keratinocytes,[10] and increase the motility and invasiveness of a human keratinocyte cell line.[11] The N-terminal hydrophobic domain of HPV-16 E5 is required for its ability to induce anchorage independent growth and invasiveness of human keratinocytes.[11,12] Despite its weak transforming activity in vitro, high-risk HPV E5 may play an important role in carcinogenesis. Analysis of natural variants of HPV-16 E5 revealed that those with the greatest mitogenic activity in vitro were derived from HPV-16 strains most commonly associated with cervical neoplasia,[13] and as noted above, the presence of an E5 gene in the viral genome is correlated with carcinogenic potential.[5,14] Importantly, expression of HPV-16 E5 in stratified squamous epithelia in transgenic mice causes epidermal hyperplasia and a higher frequency of spontaneous skin tumors.[15] Genetic studies demonstrated that EGF receptor (EGFR) signaling is required for E5-induced epidermal hyperplasia. Prolonged estrogen treatment of HPV-16 E5 transgenic mice caused cervical cancer.[16]

HPV-16 E5 activates many different cellular pathways involved mostly in the early stage of cervical carcinogenesis.[17] Different studies have pointed out the possibility of E5 targeting for cancer therapy of the uterine cervix. In animal studies, HPV-16 E5 delivered by an adenovirus vector reduces the growth of tumors and the E5 vaccine induces protection against tumors through $CD8^+$ cytotoxic T cells (CTLs).[18] The same research group has further demonstrated that the stimulating epitope was an E5 peptide (amino acids 25–33) and vaccination with this peptide carrying CpG oligonucleotides reduced tumor growth.[19] HPV-16 E5 modulates different cellular pathways and the targeting of these relevant pathways may lead in a near future to a possible therapeutic approach.[4,20–23]

Antigen-antibody interaction is a critical event in the immune process, and it can elucidate the underlying mechanism of immune recognition. The sites on antigens recognized and bound by B-cell produced antibodies are well known as B-cell epitopes.[24] The location of B-cell epitopes is useful for synthesizing peptides that can elicit the immune response with specific cross-reacting antibodies. For this reason, the identification of B-cell epitopes facilitates the design of the potentially safer peptide-based vaccines.[25] B-cell epitopes can be classified into two categories: linear (continuous) epitopes and conformational (discontinuous) epitopes.[26] Linear epitopes are formed by continuous amino acid sequences, while conformational epitopes consist of residues that are distantly separated in the sequences but spatially proximal. Recently, with the development of information science, computational methods for epitope recognition become an alternative to the wet experimental techniques, in order to save time and reduce cost.[25]

For all the above, this work aims to computational prediction of B-cell epitopes, in order to provide more information about role of HPV-16 E5 in the immune response and reducing the time and cost of future experimental trials.

Materials and methods

Database

To carry out this study, complete genome of HPV-16 was retrieved from National Center for Biotechnology Information (NCBI) database, whose accession number is NC_001526.2. Then with the use of the bioinformatics software Vector NTI 7.1 the gene encoding HPV-16 E5 was located and translated into its corresponding amino acid sequence for further analysis.

Computational prediction of the HPV-16 E5 secondary structure

Predict Protein (http://www.predictprotein.org) is an internet service for sequence analysis and the prediction of protein structure and function. Users submit protein sequences or alignments; Predict Protein returns multiple sequence alignments, PROSITE sequence motifs, low-complexity regions (SEG), nuclear localization signals, regions lacking regular structure (NORS) and predictions of secondary structure, solvent accessibility, globular regions, transmembrane α-helices, coiled-coil regions, structural switch regions, disulfide-bonds, sub-cellular localization and functional annotations. Upon request fold recognition by prediction-based threading, CHOP domain assignments, predictions of transmembrane strands and inter-residue contacts are also available. For all services, users can submit their query either by electronic mail or interactively via the World Wide Web.[27] This tool was used in order to locate the position of the B-cell epitopes on the secondary structure of HPV-16 E5 for further analysis

Tools used in the computational prediction of B-cell epitopes

BCPREDS server allows users to choose the method for predicting B-cell epitopes among several developed prediction methods. The current implementation of BCPREDS allows the user to select among three prediction methods: AAP, BCpreds and FBCPred. (http://ailab.cs.iastate.edu/bcpreds/). AAP is based on a novel amino acid pair (AAP) antigenicity scale using SVM (support vector machine) classifier,[28] BCpreds predict linear B-cell epitopes using SVM trained on the homology reduced data sets of B-cells epitopes.[29,30] and FBCpred predict flexible length linear B-cell epitopes using the subsequence kernel. The major difference between FBCPred and the other two methods is that FBCPred can predict linear B-cell epitopes of virtually any arbitrary length.[31]

ABCpred uses artificial neural networks for predicting linear B-cell epitopes. The predicted B-cell epitopes are ranked according to their score obtained by trained recurrent neural network (www.imtech.res.in/ragava/abcpred/).[32] BepiPred 1.0 server is an interesting tool that predicts the location of linear B-cell epitopes using a combination of a hidden Markov model and a propensity scale method (http://www.cbs.dtu.dk/services/BepiPred/).[33] SVMTriP predict linear B-cell epitope using the SVM method to integrate the Tri-peptide similarity and Propensity scores (SVMTriP) (http://sysbio.unl.edu/SVMTriP/).[34] LBtope is recent tool based on five datasets from Immune Epitope Database (IEDB) (Lbtope_Fixed, Lbtope_Fixed_non_redundant, Lbtope_Variable, Lbtope_Variable_non_redundant and Lbtope_Confirm dataset) and various models to discriminate B-cell epitopes from non-epitopes using SVM (http://www.imtech.res.in/raghava/lbtope/).[35]

Immune Epitope Database Analysis Resource (IEDB) is a repository of web-based tools for the prediction and analysis of immune epitopes. IEDB includes several tools for B-cells prediction as: Emini Surface Accessibility Prediction, Karplus & Schulz Flexibility Prediction, Parker Hydrophilicity Prediction, and Kolaskar & Tongaonkar Antigenicity,[36] which were selected in this work. (http://tools.immuneepitope.org/main/)

LEPS is a powerful tool that is the result of LEP (predict linear epitopes based on physico-chemical propensities combined with a mathematical morphology approach) and SVM combination. LEPS includes a number of methods for predicting linear epitopes on α-helix structures (α-helix/Deleage and Roux, α-helix/Chou and Fasman, α-helix/Levitt),[37] which were all selected in this research (http://leps.cs.ntou.edu.tw/).

VaxiJen is the first server for alignment-independent prediction of protective antigens. It was developed to allow antigen classification solely based on the physicochemical properties of proteins without recourse to sequence alignment. The server can be used on its own or in combination with alignment-based prediction methods. VaxiJen have several methods for antigen predictions, of which were selected, the model for prediction of tumor antigens, this model were used with a threshold of 0.5.[38] VaxiJen was used in order to check the oncogenic potential of all B-cell epitopes predicted (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html).

Results and discussion

In the secondary structure determined with Predict Protein three α-helix formed by amino acids 6–34, 36–53 and 56–81 are observed, respectively, occupying most of the protein, where their larger portions are buried solvent. The structure also shows that these α-helices have a mostly hydrophobic transmembrane helical portion (Fig. 1).

The prediction results of B-cell epitopes using various bioinformatics tools are shown in Tables 1–17. The peptides obtained with ABCpred, BCPREDS (BCpred, AAP, FBCPred), SVMTriP, BepiPred and IEDB analysis resource tools were ranked by their scores. A higher score is more likely that the predicted peptide corresponds to an epitope of B-cell. In the

Table 1 – Prediction results of B-cell epitopes with ABCpred.

Sequence	Start position	Score
LDTASTTLLACFLLCF	4	0.74
TLLACFLLCFCVLLCV	10	0.71 (**0.7042**)[a]
CLLIRPLLLSVSTYTS	26	0.70
LLWITAASAFRCFIVY	48	0.63
ASAFRCFIVYIIFVYI	54	0.60
CVLLCVCLLIRPLLLS	20	0.56
LLLSVSTYTSLIILVL	32	0.51

[a] Prediction score of tumor antigens.

Table 2 – Prediction results of B-cell epitopes with BCpred.

Start position	Epitope	Score
Fixed length epitope prediction:		
1	MTNLDTASTTLLACFL	0.933
1	MTNLDTASTTLL	0.948
60	FIVYIIFVYIPL	0.579

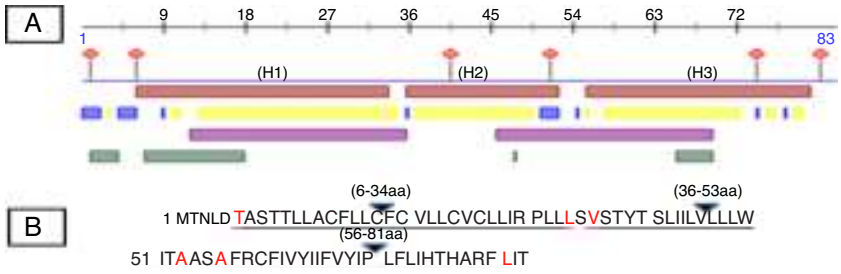

Fig. 1 – HPV-16 E5 secondary structure determined with predict protein bioinformatics tool. Red: protein binding region, brown: α-helix (H1-H2-H3), blue: solvent accessibility exposed, yellow: solvent accessibility buried, purple: helical transmembrane region, green: disordered region (A). HPV-16 e5 amino acid sequence and position of H1, H2 and H3 α-helices (B).

Table 3 – Prediction results of B-cell epitopes with AAP.

Start position	Epitope	Score
27	LLIRPLLLSVST	1
1	MTNLDTASTTLLAC	1
53	AASAFRCFIVYIIFVY	0.976

Table 4 – Prediction results of B-cell epitopes with FBCPRED.

Position	Epitope	Score
Flexible length epitope prediction:		
1	MTNLDTASTTLLAC	0.991

Table 5 – Prediction results of B-cell epitopes with SVMTriP.

Location	Epitope	Score
51–68	ITAASAFRCFIVYIIFVY	1.000
53–68	AASAFRCFIVYIIFVY	1.000
54–67	ASAFRCFIVYIIFV	1.000
9–20	TTLLACFLLCFC	1.000
72–83	AASAFRCFIVYI	0.807 (**0.8113**)[a]
74–83	IHTHARFLIT	1.000 (**0.7345**)[a]
55–64	SAFRCFIVYI	0.812

[a] Prediction score of tumor antigens.

Table 6 – Prediction results of B-cell epitopes with BepiPred.

Peptide position	Peptide
4–6	LDT

case of LBtope prediction, the probability is given in percentage, while with LEPS, the predicted peptides are returned by the program directly.

ABCpred
BCPREDS
SVMTriP
Bepipred
IEDB
LBtope
LEPS

With ABCpred, BCPREDS (BCpred, AAP, FBCPred), SVMTriP, BepiPred, LBtope, LEPS and IEDB analysis resource, a total of 202 epitopes were obtained; after an alignment performed with each of the epitopes individually (data not shown) in order to exclude any difference in ratio of these epitopes with the three α-helices determined in this study, a proportion given in percentage of 49.22%, 28.68% and 22.09% was observed for epitopes that were located within and at both

Table 7 – Prediction results of B-cell epitopes with Parker hydrophobicity prediction.

Peptide position	Peptide	Score
1–7	MTNLDTA	2.300
2–8	TNLDTAS	3.829 (**0.5489**)[a]
3–9	NLDTAST	3.829 (**0.5544**)[a]
4–10	LDTASTT	3.571 (**1.2055**)[a]
5–11	DTASTTL	3.571
6–12	TASTTLL	0.829
7–13	ASTTLLA	0.386
8–14	STTLLAC	0.286
9–15	TTLLACF	−1.957
30–36	RPLLLSV	−2.643 (**0.8296**)[a]
31–37	PLLLSVS	−2.314
32–38	LLLSVST	−1.871
33–39	LLSVSTY	−0.829
34–40	LSVSTYT	1.229
35–41	SVSTYTS	3.471 (**0.7835**)[a]
36–42	VSTYTSL	1.229 (**0.8502**)[a]
37–43	STYTSLI	0.614
38–44	TYTSLII	−1.457
49–55	LWITAAS	−1.614 (**0.8902**)[a]
50–56	WITAASA	−0.000
51–57	ITAASAF	0.114
52–58	TAASAFR	1.857 (**0.6164**)[a]
53–59	AASAFRC	1.314 (**1.0208**)[a]
54–60	ASAFRCF	−0.300 (**1.3342**)[a]
55–61	SAFRCFI	−1.743 (**0.6375**)[a]
72–78	FLIHTHA	−2.129
73–79	LIHTHAR	−0.214 (**1.0787**)[a]
74–80	IHTHARF	−0.214 (**1.1373**)[a]
75–81	HTHARFL	−0.386
76–82	THARFLI	−1.829 (**0.9468**)[a]
77–83	HARFLIT	−1.829

[a] Prediction score of tumor antigens.

Table 8 – Prediction results of B-cell epitopes with Karplus & Schulz flexibility prediction.

Peptide position	Peptide	Score
1–7	MTNLDTA	1.022
2–8	TNLDTAS	1.038 (**0.5489**)[a]
3–9	NLDTAST	1.044 (**0.5544**)[a]
4–10	LDTASTT	1.050 (**1.2055**)[a]
5–11	DTASTTL	1.049
6–12	TASTTLL	1.035
7–13	ASTTLLA	1.011
34–40	LSVSTYT	1.004
35–41	SVSTYTS	1.017 (**0.7835**)[a]
36–42	VSTYTSL	1.018 (**0.8502**)[a]
37–43	STYTSLI	1.011

[a] Prediction score of tumor antigens.

Table 9 – Prediction results of B-cell epitopes with Emini surface accessibility prediction.

Peptide position	Peptide	Score
1–6	MTNLDT	3.400
2–7	TNLDTA	3.471
3–8	NLDTAS	3.223
4–9	LDTAST	2.892
5–10	DTASTT	5.062
6–11	TASTTL	2.500
7–12	ASTTLL	1.428
8–13	STTLLA	1.428
30–35	RPLLLS	1.695
34–39	LSVSTY	1.851
35–40	SVSTYT	3.240
36–41	VSTYTS	3.240
37–42	STYTSL	3.600
38–43	TYTSLI	1.883
50–55	WITAAS	1.084
51–56	ITAASA	1.041
52–57	TAASAF	1.286
53–58	AASAFR	1.745
73–78	LIHTHA	1.162
74–79	IHTHAR	2.761
75–80	HTHARF	3.410
76–81	THARFL	2.067
77–82	HARFLI	1.004
78–83	ARFLIT	1.065

ends of the first, second and third secondary structure, respectively (Fig. 2).

In several cases it was observed that these epitopes were found between two α-helices, which were also considered to highlight the epitopes contribution by α-helices. On the other hand, it became remarkable the role of the first α-helix in the epitopes contribution. It is interesting that, when the antigenicity of the VPH-16 E5 was assessed by the method of Kolaskar and Tongaonkar (Fig. 3), a marked difference in antigenicity between the regions comprising the three α-helix was observed, being the first region corresponding to the first α-helix which shows the highest antigenicity.

When tumor activity was evaluated with the VaxiJen tool for each of the B-cell epitopes derived, it was observed that, of the 204 predicted B-cell epitopes only 44 of them showed tumor activity, also it was observed that most of these epitopes with tumor activity are more related to the first α-helix,

Table 10 – Prediction results of B-cell epitopes with lbtope fixed non redundant (nonredundant dataset).

Epitopes	SVM score	Percent probability of correct prediction
MTNLDTASTTL	0.78801963	76.27
MTNLDTASTTLL	0.84046082	78.02
MTNLDTASTTLLA	0.7496717	74.99
MTNLDTASTTLLAC	0.46571525	65.52
MTNLDTASTTLLACF	0.23677858	57.89
MTNLDTASTTLLACFLLC	0.12141822	54.05
MTNLDTASTTLLACFLLCF	0.17846699	55.95
MTNLDTASTTLLACFLLCFC	0.17056771	55.69
TNLDTASTTLLACFLLCFCV	0.061477745	52.05
DTASTTLLACFLLCFCVLLC	0.036981401	51.23
TASTTLLACFLLCFCVLLCV	0.01359391	50.45
STTLLACFLLCFCVLLCVCL	0.15035367	55.01
TTLLACFLLCFCVLLCVCLL	0.12954025	54.32
FLLCFCVLLCVCLLIRPLLL	0.022518435	50.75
LLCFCVLLCVCLLIRPLLLS	0.36590135	62.20
LCFCVLLCVCLLIRPLLLSV	0.1500738	55.00 (**0.5101**)[a]
CFCVLLCVCLLIRPLLLSVS	0.095927291	53.20
FCVLLCVCLLIRPLLLSVST	0.29869636	59.96
CVLLCVCLLIRPLLLSVSTY	0.39003	63.00
VLLCVCLLIRPLLLSVSTYT	0.40675774	63.56
LLCVCLLIRPLLLSVSTYTS	0.64595954	71.53
LCVCLLIRPLLLSVSTYTSL	0.42948099	64.32
CVCLLIRPLLLSVSTYTSLI	0.20060314	56.69
LLLSVSTYTSLIILVLLLWI	0.1107744	53.69
LLSVSTYTSLIILVLLLWIT	0.03027581	51.01
LFLIHTHARFLIT	0.012632301	50.42
FLIHTHARFLIT	0.29901812	59.97
LIHTHARFLIT	0.5239482	67.46

[a] Prediction score of tumor antigens.

because of the contribution of this structure with the formation of B-cell epitopes (Fig. 4).

The secondary structure determined in this work shows a high correspondence with those reported by Conrad and coworkers, which on analysis of the amino acid sequence of the HPV-16 E5 suggest it comprises 3 anchor-like α-helices (residues 8–30, 37–52 and 58–76), with only the first being long enough to span a lipid bilayer.[4,39] Moreover it has been shown that detection of HPV-16 E5 is very complex given its extreme hydrophobicity,[40] this physicochemical property is evident in the secondary structure determined in the present study where the buried regions of solvent accessibility constitute a considerable proportion of the HPV-16 E5.

Also in this structure it was observed that the region of solvent accessibility buried and the transmembrane helical region cover most of the secondary structure, suggesting that it could be the α-helix capable of crossing the lipid bilayer according to Conrad and coworkers.[39]

The fact that most of the epitopes predicted in this study were more concerned with the first α-helix, one could speculate that this structure is more involved in the interactions between HPV-16 E5 and B-cells with respect to the other two α-helices.

Epitopes predicted in this work with all tools, are promising candidates for B-cell epitopes, especially those with tumor activity, considering that all these tools are currently the most used worldwide for this purpose.

Table 11 – Prediction results of B-cell epitopes with lbtope_variable_nonredundant (nonredundant dataset).

Epitopes	SVM score	Percent probability of correct prediction
MTNLDTASTT	0.038882414 (**0.6473**)[a]	51.30
MTNLDTASTTL	0.16486973	55.50
MTNLDTASTTLL	0.45123994	65.04
MTNLDTASTTLLA	0.48761001	66.25
MTNLDTASTTLLAC	0.13535117	54.51
MTNLDTASTTLLACF	0.01321797	50.44
FLLCFCVLLCVCLLI	0.090004026	53.00
LLCFCVLLCVCLLIR	0.2574307 (**0.5830**)[a]	58.58
LCFCVLLCVCLLIRP	0.11659419 (**0.9881**)[a]	53.89
CFCVLLCVCLLIRPL	0.13064039 (**0.6522**)[a]	54.35
FCVLLCVCLLIRPLL	0.27147556	59.05
CVLLCVCLLIRPLLL	0.25446903	58.48
VLLCVCLLIRPLLLS	0.33288381	61.10
LCVCLLIRPLLLSVS	0.079813791	52.66

[a] Prediction score of tumor antigens.

Table 12 – Prediction results of B-cell epitopes with lbtop_confirm (confirm dataset).

Epitopes	SVM score	Percent probabilty of correct prediction
MTNLDTAST	0.36660214 (**0.5363**)[a]	62.22
MTNLDTASTT	0.74167997 (**0.6473**)[a]	74.72
MTNLDTASTTL	1.1148591	87.16
MTNLDTASTTLL	1.2623447	92.08
MTNLDTASTTLLA	1.0092323	83.64
MTNLDTASTTLLAC	0.57469904	69.16
MTNLDTASTTLLACF	0.53589833	67.86
TNLDTASTTLLACFL	0.29997316	60.00
NLDTASTTLLACFLL	0.22894045	57.63
VCLLIRPLLLSVSTY	0.13101464	54.37
CLLIRPLLLSVSTYT	0.27677213	59.23
LLIRPLLLSVSTYTS	0.2323884	57.75
LIRPLLLSVSTYTSL	0.398026 (**0.5204**)[a]	63.27
IRPLLLSVSTYTSLI	0.398026	63.27
RPLLLSVSTYTSLII	0.33018884	61.01
PLLLSVSTYTSLIIL	0.28889725	59.63
LLLSVSTYTSLIILV	0.2277135	57.59
LLSVSTYTSLIILVL	0.18839037	56.28
LSVSTYTSLIILVLL	0.18839037	56.28
SVSTYTSLIILVLLL	0.16713208	55.57
VSTYTSLIILVLLLW	0.069070288	52.30
STYTSLIILVLLLWI	0.3310608	61.04
TYTSLIILVLLLWIT	0.25891794	58.63
YTSLIILVLLLWITA	0.079420899	52.65
SLIILVLLLWITAAS	0.13729623	54.58
LIILVLLLWITAASA	0.040877495	51.36
ILVLLLWITAASAFR	0.0017060347	50.06
LVLLLWITAASAFRC	0.010196624	50.34
IFVYIPLFLIHTHAR	0.22777942	57.59
FVYIPLFLIHTHARF	0.18532451	56.18
VYIPLFLIHTHARFL	0.053064331	51.77
YIPLFLIHTHARFLI	0.17141819	55.71
IPLFLIHTHARFLIT	0.34987537	61.66
PLFLIHTHARFLIT	0.49772162	66.59
LFLIHTHARFLIT	0.44336093	64.78
FLIHTHARFLIT	0.5635044	68.78
LIHTHARFLIT	0.66572946	72.19
IHTHARFLIT	0.52119715	67.37
HTHARFLIT	0.22096653	57.37

[a] Prediction score of tumor antigens.

Table 13 – Prediction results of B-cell epitopes with lbtope_fixed (original dataset).

Epitopes	SVM score	Percent probability of correct prediction
MTNLDTASTTL	0.49606207	66.54
MTNLDTASTTLL	0.4815069	66.05
MTNLDTASTTLLA	0.38276016	62.76
MTNLDTASTTLLAC	0.25505304	58.50
MTNLDTASTTLLACF	0.10873719	53.62
MTNLDTASTTLLACFLLC	0.085390518	52.85
MTNLDTASTTLLACFLLCF	0.074603912	52.49
MTNLDTASTTLLACFLLCFC	0.033502249	51.12
LLCFCVLLCVCLLIRPLLLS	0.027928789	50.93
FCVLLCVCLLIRPLLLSVST	0.041765261	51.39
CVLLCVCLLIRPLLLSVSTY	0.045513519	51.52
VLLCVCLLIRPLLLSVSTYT	0.028816643	50.96
LLCVCLLIRPLLLSVSTYTS	0.24330717	58.11
LCVCLLIRPLLLSVSTYTSL	0.24095998	58.03
LIHTHARFLIT	0.2206929	57.36

Table 14 – Prediction results of B-cell epitopes with lbtope_variable (original dataset).

Epitopes	SVM score	Percent probability of correct prediction
MTNLDTAS	0.050255612 (**0.5877**)[a]	51.68
MTNLDTAST	0.17906443 (**0.5363**)[a]	55.97
MTNLDTASTT	0.35861795 (**0.6473**)[a]	61.95
MTNLDTASTTL	0.34206395	61.40
MTNLDTASTTLL	0.52040671	67.35
MTNLDTASTTLLA	0.44147341	64.72
MTNLDTASTTLLAC	0.17538365	55.85
MTNLDTASTTLLACF	0.028568973	50.95

[a] Prediction score of tumor antigens.

Table 15 – Prediction results of B-cell epitopes with alpha helix/Levitt.

Position peptide	Peptide
10–27	TLLACFLLCFCVLLCVCL (**0.5372**)[a]
74–80	ILVLLLWITAASAFRC
44–59	IHTHARF (**1.1373**)[a]

[a] Prediction score of tumor antigens.

Table 16 – Prediction results of B-cell epitopes with alpha helix/Chou and Fasman.

Position peptide	Peptide
12–16	LACFL
23–34	LCVCLLIRPLLL
43–59	IILVLLLWITAASAFRC
74–80	IHTHARF (**1.1373**)[a]

[a] Prediction score of tumor antigens.

Table 17 – Prediction results of B-cell epitopes with alpha helix/Deleage and Roux.

Position peptide	Peptide
12–27	LACFLLCFCVLLCVCL (**0.8676**)[a]
44–60	ILVLLLWITAASAFRCF
74–80	IHTHARF (**1.1373**)[a]

[a] Prediction score of tumor antigens.

However, according to the rule of thumb, epitopes that are present either in the coil or α-helix regions of the secondary structure of the protein are confirmed to be the epitopes,[30] which can support the results obtained here with all the tools used. Given that the HPV-16 E5 is largely constituted by α-helices and that all of the epitopes determined here were related in one way or another with that structure, it can be considered that the predicted epitopes here could contribute positively in future researches related to the design of future therapeutic vaccines and the understanding of the immunological mechanisms of HPV-16.

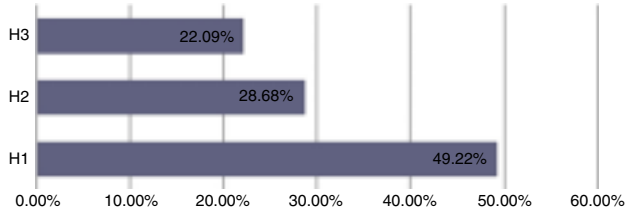

Fig. 2 – Relation given in percentage of the predicted B-cell epitopes in the three α-helices.

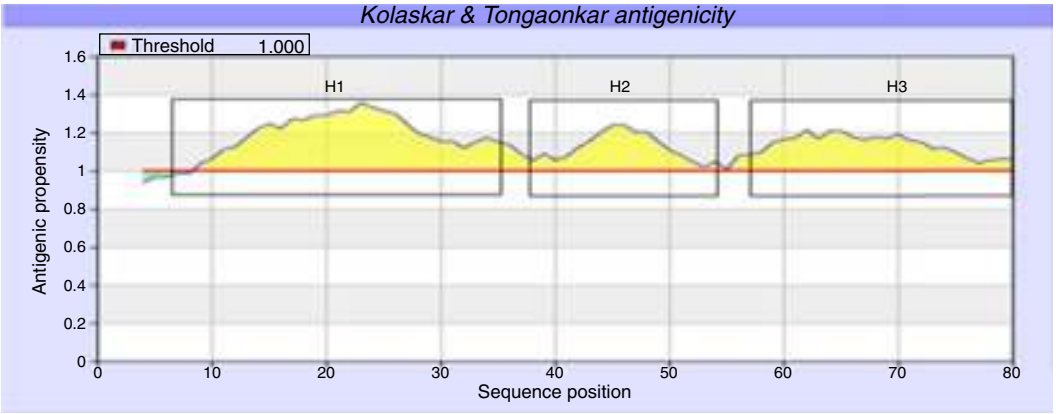

Fig. 3 – Antigenicity of the predicted epitopes with the method of Kolaskar & Tongaonkar. The yellow regions above red line are in correspondence with the number of B-cell epitopes (data not shown).

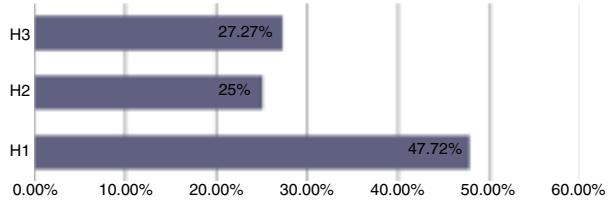

Fig. 4 – Relation given in percentage of the predicted B-cell epitopes with tumor activity in the three α-helices.

Conclusion

The differences in the ratio of these epitopes in the three α-helix structures of the HPV-16 E5, suggest that the first α-helix could be more related to the immune response of B-cell. The predicted B-cell epitopes in this study with all together bioinformatics tools could reduce the time and cost of the experimental trials aimed at finding these epitopes, as well as facilitates the design of future therapeutic vaccines and the understanding of the immunological mechanisms of HPV-16.

Conflict of interest

There is no conflict of interests regarding the publication of this article.

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