Research Article

EPITOPE PREDICTION FOR HUMAN PAPILLOMA VIRUS - TYPE 16 E7 PROTEIN EXPRESSED IN CERVICAL CANCER

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ABSTRACT

A cervical carcinoma that contained human Papillomavirus (HPV)-16 homologous DNA was analyzed. Each tumour cell genome contained a single, incomplete copy of HPV-16 DNA. The E6 and E7 open reading frames (ORFs) were completely conserved relative to other published HPV-16 sequences. Much of the non-coding region (NCR) was free of base changes, including complete conservation of several regulatory elements. Multiple mutations were identified in the remaining integrated HPV-16 DNA, which was composed of parts of The L1 and E1 ORFs. The extraordinary conservation of the E6/E7 DNA sequence, as compared with other regions of the integrated HPV-16 DNA, supports the role of E6/E7 in tumorigenesis. In the present work T-cell and B-cell epitopes were predicted for HPV Type-16 E7 protein expressed in cervical cancer of human. MHC-1 and MHC-2 epitopes were predicted by ANNPred and MHC2Pred servers respectively and B-cell epitopes were predicted by ABCPred server. In order to find most relevant epitopes among the MHC-1 and MHC-2 predicted epitopes; protein-protein docking studies were carried out. These predicted epitopes might be promising candidates for the vaccine-designing for human Papilloma virus-type 16 E7 protein expressed in cervical cancer.

Keywords: Human Papilloma virus (HPV), Epitopes, docking, modelling, vaccine designing.

INTRODUCTION

The immunology of human Papillomavirus (HPV) infections has peculiar characteristics. The long latency for cervical cancer development after primary viral infection suggests mechanisms that may aid the virus in avoiding the host Immunosurveillance and establishing persistent infections. In order to understand whether molecular mimicry phenomena might explain the ability of HPV to avoid a protective immune response by the host cell, sequence similarity between HPV16 E7 oncoprotein and human self-proteins was examined by computer-assisted analysis. Data were obtained showing that the HPV 16 E7 protein has high and widespread similarity to several human proteins involved in a number of critical regularity processes. In addition, multiple identical and different E7 peptide motifs are present in the same human protein. Thus, sharing of common motifs between viral oncoprotein and molecules of normal cells may be one cause underlying the scarce Immunogenicity of HPV infections.[1] E7 protein has both transforming and trans-activating activities. Disrupts the function of host retinoblastoma protein RB1/pRb, which is a key regulator of the cell cycle. Induces the disassembly of the E2F1 transcription factors from RB1, with subsequent transcriptional activation of E2F1-regulated S-phase genes. Inactivation of the ability of RB1 to arrest the cell cycle is critical for cellular transformation, uncontrolled cellular growth and proliferation induced by viral infection. Stimulation of progression from G1 to S phase allows the virus to efficiently use the cellular DNA replicating machinery to achieve viral genome replication. Interferes with histone deactivation edited by HDAC1 and HDAC2, leading to activation of transcription.[2] HPV16, in comparison to HPV types 6 and 11, is more often associated with malignant genital cancers in humans.[3] T-helper epitope of the E7 transforming protein of human papillomavirus 16 provides cognate help for several E7 B-cell epitopes from cervical cancer-associated human papillomavirus genotypes.[4] There is strong evidence implicating human papillomavirus type 16 (HPV16) in the genesis of human genital cancer.[5]

The aim of present study was to predict the T-cell and B-cell epitopes for human Papilloma virus type-16 E7 protein using the bioinformatics tools.[1]

MATERIALS AND METHODS

Tools used

ANNPred Server: ANNPred is an on-line web tool for the prediction of peptide binding to MHC class-1 alleles (http://www.imtech.res.in/raghava/annpred/). This prediction is based on Artificial Neural Networks (ANNs) for 30b MHC alleles. The method provides the option to specify the cut off score for prediction.[6] The predicted MHC binders are filtered to potential CTL epitopes by refining through Proteasomal matrices.[7]

MHC2Pred Server: The MHC2Pred is an SVM based method for prediction of promiscuous MHC Class 2 binding peptide (www.imtech.res.in/raghava/mhc2pred/).[8,9] The data for training has been extracted from MHCBN and JenPep database.[7] For the development of MHC binder prediction method, an elegant machine learning technique SVM has been used.[10]

ABCpred Prediction Server: ABCPred uses artificial neural networks for predicting linear B-cell epitopes. This is the

first server developed based on recurrent neural network using fixed length patterns. The aim of ABCpred server is to predict B cell epitopes in an antigen sequence, using artificial neural network (www.imtech.res.in/ragava/abcpred/). The target output consist of a single binary number as 1 or 0 (epitope or non-epitope).[11, 12]

Methodology

The FASTA and Amino acid sequence of HPV Type-16 E7 protein retrieved from the site of Swiss-Prot (Accession No. P03129) (http://expasy.org/sprot/). For the prediction of MHC Class-1 epitopes ANNPred online epitope prediction tool was used. The sequence was submitted to the ANNPred server in the FASTA format. The epitopes were predicted for different alleles (HLA-A2, HLA-A*0201, HLA-A*0202 and HLA-A*0203) of MHC Class-1. Epitope for different alleles (HLA-DR1, HLA-DR4, HLA-DR9 and HLA-DR11) of MHC Class-2 were predicted by using the MHC2Pred online tool. The B-Cell epitope were predicted by using the ABCPred online tool. The 3D-structure of small peptides was modelled by using various bioinformatics tools as ROSETTA Antibody modelling tool.

RESULTS

Result for MHC Class-1 binding epitope prediction

Table 1-4 shows the predicted T-cell MHC Class-1 epitopes for different alleles i.e. HLA-A2, HLA-A*0201, HLA-A*0202 and HLA-A*0203. These small sequences (epitopes) were obtained from the ANNPred Server.[6] This server allows the prediction of standard proteasomal and immunoproteasomal cleavage site in an antigenic sequence. It identifies the MHC binders who have cleavage site at the C-terminus.[7]

Table 1: MHC Class-1(HLA-A2) Binding Peptide Prediction

ALLELE: HLA-A2				
Threshold.5 as cut-off score				
Prediction method	**Rank**	**Sequence**	**Residue No.**	**Peptide Score**
ANNs	1	SPPVEHPVP	1	0.000
ANNs	2	PPVEHPVPR	2	0.000
ANNs	3	PVEHPVPRT	3	0.000

Table 2: MHC Class-1(HLA-A*0201) Binding Peptide Prediction

ALLELE: HLA-A*0201				
Threshold .5 as cut-off score				
Prediction method	**Rank**	**Sequence**	**Residue No.**	**Peptide Score**
ANNs	1	SPPVEHPVP	1	0.000
ANNs	2	PPVEHPVPR	2	0.000
ANNs	3	PVEHPVPRT	3	0.000

Table 3: MHC Class-1 (HLA-A*0202) Binding Peptide Prediction

ALLELE: HLA-A*0202				
Threshold .5 as cut-off score				
Prediction method	**Rank**	**Sequence**	**Residue No.**	**Peptide Score**
ANNs	1	SPPVEHPVP	1	0.000
ANNs	2	PPVEHPVPR	2	0.000
ANNs	3	PVEHPVPRT	3	0.000

Table 4: MHC Class-1(HLA-A*0203) Binding Peptide Prediction

ALLELE: HLA-A*0203				
Threshold .5 as cut-off score				
Prediction method	**Rank**	**Sequence**	**Residue No.**	**Peptide Score**
ANNs	1	SPPVEHPVP	1	0.000
ANNs	2	PPVEHPVPR	2	0.000
ANNs	3	PVEHPVPRT	3	0.000

Result for MHC Class-2 binding epitope peptide Prediction

Table 5-8 shows the small peptides (epitopes) for MHC Class-2 molecules predicted by SVM based MHC2Pred prediction server. This Table shows the prediction for different allele's i.e. HLA-DR1, HLA-DR4, HLA-DR9, and HLA-DR11.[8] All peptides having IC50 value less than 500nm has been considered as binders and peptides with IC50 value greater than 500nm has been considered as non-binders.[7]

Table 5: MHC Class-2(HLA-DR1) Binding Peptide Prediction

ALLELE: HLA-DR1				
Threshold 0.5 as cut-off score				
Prediction method	**Rank**	**Sequence**	**Residue No.**	**Peptide Score**
SVM	1	DLLMGTLGI	84	1.000
SVM	2	LYCYEQLND	12	0.594
SVM	3	RAHYNIVTF	39	0.367
SVM	4	PAGQAEPDR	31	0.160

Table 6: MHC Class-2(HLA-DR4) Binding Peptide Prediction

ALLELE: HLA-DR4				
Threshold 0.5 as cut-off score				
Prediction method	**Rank**	**Sequence**	**Residue No.**	**Peptide Score**
SVM	1	LDLQPETTD	3	0.702
SVM	2	STHVDIRTL	74	0.653
SVM	3	HVDIRTLED	76	0.543
SVM	4	VQASESDAC	59	0.526

Table 7: MHC Class-2(HLA-DR9) Binding Peptide Prediction

ALLELE: HLA-DR9				
Threshold 0.5 as cut-off score				
Prediction method	**Rank**	**Sequence**	**Residue No.**	**Peptide Score**
SVM	1	LMGTLGIVC	86	1.392
SVM	2	DIRTLEDLL	78	1.265
SVM	3	LLMGTLGIV	85	1.142
SVM	4	LQPETTDLY	5	1.141

Table 8: MHC Class-2(HLA-DR11) Binding Peptide Prediction

ALLELE: HLA-DR11				
Threshold 0.5 as cut-off score				
Prediction method	**Rank**	**Sequence**	**Residue No.**	**Peptide Score**
SVM	1	GQAEPDRAH	33	0.122
SVM	2	DEIDGPAGQ	26	0.092
SVM	3	YNIVTFCCK	42	0.087
SVM	4	DGPAGQAEP	29	0.084

Result for B-cell epitope binding prediction

Table 9 shows the prediction B-cell epitopes which are in the form of small peptide sequences. B-cell epitope were predicted using the ABCpred Server. All the predicted epitopes were ranked according to their respective scores which have been obtained by the trained recurrent neural network. Higher score of the peptide reveals the higher probability to be most reliable epitope.[11] In Table-9 highest score and 1st rank shown by sequence 'SPPVEHPVPRTE' and the lowest score shown by 'NESHMANPAPIL' sequence.

Table 9: Result of B-cell Epitope Prediction

Rank	Sequence	Start position	Score
1	**SPPVEHPVPRTE**	**1**	**0.81**
2	PILLMAVIRSTY	23	0.80
3	EPDRAHYNIVTF	89	0.78
4	VPRTEINESHMA	8	0.74
4	HEYMLDLQPETT	52	0.74
5	CYEQLNDSSEEE	67	0.71
6	TDLYCYEQLNDS	63	0.69
6	LDLQPETTDLYC	56	0.69
7	DTPTLHEYMLDL	47	0.67
7	MAVIRSTYPEGN	27	0.67
8	SVMHGDTPTLHE	42	0.65
9	NDSSEEEDEIDG	72	0.64
9	RSTYPEGNEPES	31	0.64
10	EEDEIDGPAGQA	77	0.62
11	NEPESVMHGDTP	38	0.59
12	**NESHMANPAPIL**	**14**	**0.53**

DISCUSSION

The present work was conducted to predict the epitopes for B-cell and T-cell of HPV Type 16 E7 protein expressed in human during cervical cancer. The epitopes were predicted in the form of small peptides using different bioinformatics software,[12] as shown in Table 1-4 for MHC class-1 epitope prediction, ANNPred Server was used. Four epitope were predicted for different alleles (HLA-A2, HLA-A*0201, HLA-A*0202, HLA-A*0203) of MHCclass-1 and rank has been given on the basis of log score.[6] As shown in Table 5-8 for MHC class-2 epitope prediction MHC2Pred server was used. Four epitopes were predicted by SVM method for different alleles (HLA-DR1, HLA-DR4, HLA-DR9 and HLA-DR11) of MHC class-2 and rank has been given on the basis of peptide score.[8] For B cell epitope prediction ABCpred server was used.[11] Top 16 ranked epitope has been shown in table 9.

CONCLUSION

T-cell and B-cell epitopes prediction gives peptide score and number of residues for different alleles or receptor sequences, which might be in future promising candidates for Antibody modelling and Vaccine design against human Papilloma virus(HPV) type-16 E7 protein expressed in cervical cancer.

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