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A Comprehensive in Silico Analysis for Identification of Immunotherapeutic Epitopes of HPV-18

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Abstract
Human papillomavirus (HPV) remains the major cause of cervical cancer, globally. High risk HPV (Hr-HPV) 16 and 18 together account for more than 70% of cervical cancer cases, whereas the hr-HPV-18 is the second most prevalent hr-HPV type, causing about 5.2% of all cancers worldwide. Considering the high prevalence and mortality rate, cervical cancer remains a noteworthy health problem among women. As of now, no registered immunotherapies are available after the HPV infection. Thus, developing an immunotherapeutic candidate against hr-HPV would be of major clinical benefit. Nowadays, the T-and B-cell peptide based targeted vaccines have been considered as the best candidate for vaccine development against viral infections. In this study, both prophylactic and therapeutic vaccine candidates against hr-HPV-18 were predicted. To achieve this, the prediction of T-and B-cell epitopes of major histocompatibility complex (MHC) were accomplished, that can be used for HPV immunotherapy. For MHC-I, a maximum number (20) of potent peptides were found, against HLA-B*51:01 (L1 = 9, L2 = 6, E2 = 4, and E4 = 1) having percentile value < 1 and, immunogenicity scores higher than 0.5, followed by HLA-A*11:01 (L1 = 8, E2 = 7 L2 = 2, and E6 = 1, E7 = 1); 19 epitopes. For MHC-II, the highest number of peptides found, against the HLA-DRB1*04:01 (L2 = 10, E5 = 7, and E4 = 4), HLA-DRB1*04:05 (E5 = 7, E2 = 5, E4 = 5, and L1 = 4) HLA-DPA1*01:03/DPB1*04:01 (E7 = 7, E6 = 5, L2 = 5, and E2 = 2), HLA-DRB5*01:01(E6 = 6, L1 = 6, and L2 = 6); peptides 21, 21, 19 and 18 respectively. For B-cell, total 94, 16 amino acid long B-cell epitopes were predicted. In conclusion, these predicted epitopes can be valuable candidates for in vitro or in vivo therapeutic vaccine studies against hr-HPV-18 associated cancer.

Keywords Hr-HPV-18 · MHC-I · MHC-II · B-cell · Epitope prediction · Immunotherapy

Introduction

Cervical cancer ranks as the 2nd leading cause of female cancer in India. 96,922 new cervical cancer cases with a crude incidence rate 14.9% are being diagnosed annually (Bruni et al. 2019). High Risk HPVs (Hr-HPVs) primarily 16 and 18 are the oncogenic HPV types are responsible for about 70% of all cervical cancer cases worldwide (Bruni 2019). According to Catalan Institute of Oncology and Information Centre on HPV and Cancer (ICO/IARC) statistics, 469.1 million women aged ≥ 15 years are at risk of development of cervical cancer in India (Bruni 2019).

HPVs are circular, double strand, non-enveloped DNA viruses of about ~ 8 kb (Doorbar et al. 2012) that can be divided into early, late, and long control regions (LCR) (Zheng and Baker 2006). Early region includes E1, E2, E4, E5, E6, and E7 protein coding part which are regularly in function and plays important role in HPV oncogenicity. E1 participates in HPV DNA replication process (Castro-Muñoz et al. 2019), along with E2; E2 also works as transcription activator and regulator (Oliveira et al. 2006), E4 (Yajid et al. 2017); (Doorbar 2013) and E5 modulates the productive phase of HPV life cycle (Müller et al. 2015). E5, E6 and E7 are considered as the major hallmarks of HPV infection, these three are also essential for the development of HPV positive carcinoma (Estêvão et al. 2019). E6 and E7

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modulates cell cycle control and contributes to viral genome maintenance (Schiffman et al. 2016). E6 inhibits the p53 and causes loss in cell cycle regulation, thus overriding the cell cycle process. E7 mediates the inhibition of retinoblastoma protein and results in unrestrained cell proliferation (Pal and Kundu 2020), being the major onco-players in the process of HPV mediated cervical cancer, while E6 and E7 represents the most effective targets for immunotherapeutic (Pal and Kundu 2020).

Late region of HPV viruses consist of capsid coding protein regions; L1 major capsid protein, and L2 minor capsid protein. Through L1 protein virus initiates its interaction with host cells, gets attached to Heparan Sulfate Proteo-Glycans (HSPG) receptors of the cell (Posner and Peterson 2013). L1 interaction with HSPG, followed by conformational changes and cleavage of L2 by cellular furin (Bronnimann et al. 2016), thus entering the virus into host cell (Richards et al. 2006). LCR of HPV have multiple functions in regulating the viral transcription (Fang et al. 2020), comprises about 10% of complete genome. Some specific LCR mutations results in the development of cervical cancer (Xi et al. 2017).

It has already been established that persistent infection with hr-HPVs is associated with cancerous lesions, invasion and cancer (Radley et al. 2016). In the majority of HPV infected persons, the infection is cleared by the immune system (Gillison et al. 2000); however, the viral infection can continue to persist and subsequently results into cancer at the site of infection (Frazer 2009). Although such persistent is relatively low, the prevalence of HPV associated cancers is significantly high among the general population worldwide. Prophylactic vaccines against some predominant HPVs have also been developed; Cervarix (GlaxoSmithKline) for HPV 16 and 18, and Gardasil (Merck & Co.) for HPV 16,18,6, and 11 (Ribeiro-Muller et al. 2013). Despite this, developed prophylactic vaccines provide no therapeutic benefit and are only generates the antibodies against the L1 capsid protein of HPVs (Schiller et al. 2012). However, these come under preventive measures for cervical cancer and should be administrated before adolescent age (LaVigne and Leitao 2019); (Kaarthigeyan 2012). Moreover, high prevalence and mortality rate due to HPV infection indicating a serious concern to develop effective treatment strategies to control HPV infection and cease the development of cervical cancer.

Therapeutic vaccine development is one of potential treatment method that has now been explored to treat and clear the existing HPV infection, therapeutic vaccines differ from prophylactic vaccines (Zur Hausen 2002), as they aimed to stimulates cell mediated immunity rather than neutralising antibodies, thus targeting and killing the infected cells. Peptide based therapeutic vaccines have the advantages of stability, safety and their feasibility of largescale production (Hung et al. 2008). Several studies showed the constant expression of HPV oncoproteins i.e. E6 and E7 proteins in cervical cancer cases, but not in normal tissues. Due to this E6 and E7 protein of HPV makes them ideal target for the therapeutic vaccine development. Peptide based vaccines are categorized into; specific epitope/short peptides and synthetic long peptides (SLPs), in this study we predicted specific epitope/short peptides as these short peptides binds exogenously to the major histocompatibility complexes (MHCs) (van der Burg et al. 2006). This research paper covers prediction of all the potent epitopes against one of the second most prevalent hr-HPV (hr-HPV-18) worldwide. As several attempts by many researchers have been made to identify potent vaccine target against hr-HPV-16 (Kumar, et al. 2015a,b,c); (Bahmani et al. 2020); (Nakagawa et al. 2004), not much efforts have been made to predict epitopes against hr-HPV-18-E2, E4, E5, E6, E7, L1, and L2 proteins altogether.

Methods

Hr-HPV-18 Protein Sequence Retrieval

The complete hr-HPV-18 genome ID was NC_001357.1 and the complete sequence was retrieved from the NCBI (https://www.ncbi.nlm.nih.gov/nuccore/NC_001357) database.

Protein Sequence Analysis

The number of amino acids, molecular weight of proteins, isoelectric points, percentage of strongly basic, acidic, hydrophobic, polar amino acids, in the hr-HPV-18 E2, E4, E5, E6, E7, L1, and L2 proteins was calculated using Protparam (http://web.expasy.org/protparam/) software.

Secondary Structure Prediction

An online server, PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/) was used to analyze the secondary structure of hr-HPV-18; E2, E4, E5, E6, E7, L1 and L2 proteins, respectively.

MHC-I T-Cell Epitopes Prediction

Prediction of hr-HPV-18 proteins Major Histocompatibility Complex I (MHC-I) T cell epitopes was done using Immune Epitope Database Analysis (IEDB) (http://tools.iedb.org/mhci/). The frequently expressed HLAs were selected for the analysis. A method specific IC50 value was selected along with a low Percentile Rank (PR) value, as a lower IC50 or PR means high affinity. In this study predictions

were performed against 9 mer, peptides with PR < 0.5, and Immunogenicity Score (IS) was set at < 1 for research.

MHC-II T-Cell Epitopes Prediction

The MHC-II T cell epitope binding prediction was done using IEDB MHC-II binding predictor tool (http://tools.iedb.org/mhcii/). The prediction for 15 mer achieved by Consensus (smm/nn/sturniolo)/(comb.lib./smm/nn), and NetMHCIIpan.

B-cell Epitopes Prediction

The ABCpred (ABCpred submission page (osdd.net)) software was used to identify B-cell epitopes against hr-HPV-18 proteins, default parameters with an 0.8 threshold value were used for the prediction method.

Visualization of the 3D Structure of Predicted Epitopes

The 3D structure of HPV-18 E2, E4, E5, E6, E7, L1, and L2 proteins were retrieved from the RCSB PDB data base (E2 PDB ID: IF9F; E4 PDB ID:6ZFG; E5 PDB ID: 2R5I; E6 PDB ID: 2I0I; E7 PDB ID: 6IWD; L1 PDB ID: 5W1X; L2 PDB ID: 1QQH and the predicted epitope were marked on the 3D structure using visualization chimera software.

Results

Structure Analysis

The amino acid compoisition of each protein (hr-HPV-18 E2, E4, E5, E6, E7, L1 and L2 proteins) and individual detailed results of them are given in the form of supporting information Excel files (S1 File). Threonine (Thr/T) and Leucine (Leu/L) were the most frequent amino acids found in hr-HPV-18 proteins.

Epitope Prediction for MHC-I Alleles

MHCs are highly polymorphic; they have different alleles within the population with a diverse peptide binding specificity. In alleles which are majorly expressed in the humans were selected in this study from the dbMHC database. MHC-I alleles were HLA-A*02:11, HLA-A*03:01, HLA-A*11:01, HLA-A*24:02, HLA-A*26:01; HLA-B*40:06, HLA-B*08:01, HLA-B*35:03, HLA-B*44:03, HLA-B*51:01; HLA-C*04:01, HLA-C*07:01, HLA-C*07:02, 14:02, and 15:07. In the present study a total of 143 epitopes were predicted against the MHC-I; all the predicted epitopes having a length of 9mer, with less than 1 percentile value were chosen. Moreover, all the peptides had an immunogenicity score higher than 0.5 and percentile value < 1. A maximum number of potent peptides found against HLA-B* 51:01, and HLA-A*11:01 followed by the other HLAs. The list of potent predicted epitopes, against MHC-I, with high immunogenicity score are given in Table 1. The detailed results of all the epitopes against MHC-I are given in supporting information, as excel files (S2 File).

Epitope Prediction for MHC-II Alleles

For MHC-II the HLA reference list from the dbMHC database were chosen and alleles were HLA-DRB1*01:01, HLA-DaRB1*03:01, HLA-DRB1*04:01, HLA-DRB1*04:05, HLA-DRB1*07:01, HLA-DRB1*08:02, HLA-DRB1*09:01, HLA-DRB1*11:01, HLA-DRB1*12:01, HLA-DRB1*13:02, HLA-DRB1*15:01, HLA-DRB3*01:01, HLA-DRB3*02:02, HLA-DRB4*01:01, HLA-DRB5*01:01, HLA-DQA1*05:01/DQB1*02:01, HLA-DQA1*05:01/DQB1*03:01, HLA-DQA1*03:01/DQB1*03:02, HLA-DQA1*04:01/DQB1*04:02, HLA-DQA1*01:01/DQB1*05:01, HLA-DQA1*01:02/DQB1*06:02, HLA-DPA1*02:01/DPB1*01:01, HLA-DPA1*01:03/DPB1*02:01, HLA-DPA1*01:03/DPB1*04:01, HLA-DPA1*03:01/DPB1*04:02, HLA-DPA1*02:01/DPB1*05:01, and HLA-DPA1*02:01/DPB1*14:01. A total 222 potent epitopes were found against the MHC-II. The parameter for peptide selection were chosen at default 15 mer length by the IEDB software. Peptides with percentile rank < 1 were selected, the highest number of peptides found against the HLA-DRB1*04:01, HLA-DRB1*04:05, HLA-DPA1*01:03/DPB1*04:01, HLA-DRB5*01:01; peptides 21, 21, 19 and 18 respectively. The list of potent predicted epitopes, against MHC-II, with high immunogenicity score are given in Table 2. The detailed results of all the epitopes against MHC-II are given in supporting information, as excel files (S3 File).

B-Cell Epitope Analysis

The B-cell epitopes for E2, E4, E5, E6, E7, L1, and L2 proteins were predicted using ABCpred with a threshold value 0.80 and other default parameters. Total 94, 16 amino acid long B-cell epitopes were predicated against hr-HPV-18 proteins (E2 = 24, E4 = 05, E5 = 02, E6 = 04, E7 = 01, L1 = 30 and L2 = 28). The list of potent predicted epitopes, with high immunogenicity score are given in Table 3. The detailed results of all the epitopes against B-cell are given in supporting information, as excel files (S4 File). The epitopes were marked on the 3D structure of protein (in Fig. 1).

Table 1 The predicted potential MHC-I epitopes in Hr-HPV-18

Sl.no	Protein region	Epitope	Start	End	Length	Immunogenicity score	Percentile rank
2	HPV-18 E2	STVSVGTAK	230	238	9	0.948363	0.01
		DSVQILVGY	354	362	9	0.944315	0.01
		TPSPYSSTV	224	232	9	0.942037	0.01
		SPYSSTVSV	226	234	9	0.937738	0.01
		NTTPIIHLK	285	293	9	0.908606	0.02
		GYNTFYIEF	168	176	9	0.892103	0.03
		YVAWDSVYY	135	143	9	0.840453	0.03
		RYKTEDWTL	90	98	9	0.867001	0.04
		HYRDISSTW	312	320	9	0.84558	0.04
		YYMTDAGTW	142	150	9	0.841327	0.04
12	HPV-18 E4	SYSTPPHRI	20	28	9	0.942668	0.01
		TRYPLLSLL	10	18	9	0.728053	0.01
		SIVDLSTHF	58	66	9	0.685812	0.06
		CAVPVTTRY	4	12	9	0.684205	0.06
		DGNSVVVTL	78	86	9	0.658506	0.09
17	HPV-18 E5	VPLLPSVCM	20	28	9	0.78659	0.05
		MLLLHIHAI	61	69	9	0.73391	0.05
19	HPV-18 E6	SVYGDTLEK	84	92	9	0.98913	0.01
		VYGDTLEKL	85	93	9	0.9184	0.02
		VYCKTVLEL	33	41	9	0.8948	0.03
		NPAEKLRHL	113	121	9	0.84636	0.03
		DPTRRPYKL	6	14	9	0.80712	0.03
		DFYSRIREL	70	78	9	0.75091	0.05
		FEDPTRRPY	4	12	9	0.82963	0.06
		AFKDLFVVY	48	56	9	0.7399	0.07
27	HPV-18 E7	ATLQDIVLH	6	14	9	0.53839	0.26
28	HPV-18 L1	IYNPETQRL	151	159	9	0.974224	0.01
		DVMSYIHSM	448	456	9	0.954823	0.01
		FPIFLQMAL	56	64	9	0.963233	0.01
		LPDPNKFGL	138	146	9	0.953865	0.01
		EEYDLQFIF	430	438	9	0.973715	0.01
		LPPPSVARV	74	82	9	0.946367	0.01
		VPLDICQSI	281	289	9	0.928381	0.01
		FYHAGSSRL	95	103	9	0.978774	0.01
		IYNPETQRL	151	159	9	0.95655	0.01
		LYHPRPLPL	21	29	9	0.948142	0.01
38	HPV-18 L2	STTSFAFFK	365	373	9	0.962559	0.01
		APSPEYIEL	327	335	9	0.951818	0.01
		TPLPTVRRV	213	221	9	0.949236	0.01
		TRPSSLITY	243	251	9	0.865615	0.01
		YYLWPLYYF	435	443	9	0.937947	0.02
		EFLTRPSSL	240	248	9	0.857795	0.02
		CPPDVVPKV	27	35	9	0.891908	0.02
		AFEPVDTTL	255	263	9	0.832221	0.02
		EPVDTTLTF	257	265	9	0.886599	0.03
		SYSNVTVPL	383	391	9	0.884489	0.03

Table 2 The predicted potential MHC-II epitopes in Hr-HPV-18

Sl.no	Protein region	Epitope	Start	End	Length	Percentile rank
48	HPV-18 E2	QRTKFLNTVAIPDSV	342	356	15	0.61
49	HPV-18 E4	TTRYPLLSLLNSYST	9	23	15	0.16
		TRYPLLSLLNSYSTP	10	24	15	0.17
		VTTRYPLLSLLNSYS	8	22	15	0.17
		RYPLLSLLNSYSTPP	11	25	15	0.21
		YPLLSLLNSYSTPPH	12	26	15	0.23
54	HPV-18 E5	CMCAYAWVLVFVYIV	27	41	15	0.12
		MCAYAWVLVFVYIVV	28	42	15	0.12
		YAWVLVFVYIVVITS	31	45	15	0.23
		AWVLVFVYIVVITSP	32	46	15	0.23
		WVLVFVYIVVITSPA	33	47	15	0.23
		VLVFVYIVVITSPAT	34	48	15	0.23
		LVFVYIVVITSPATA	35	49	15	0.23
		WVLVFVYIVVITSPA	33	47	15	0.24
		VLVFVYIVVITSPAT	34	48	15	0.15
		LVFVYIVVITSPATA	35	49	15	0.15
64	HPV-18 E6	NEKRRFHNIAGHYRG	122	136	15	0.16
		EKRRFHNIAGHYRGQ	123	137	15	0.16
		KRRFHNIAGHYRGQC	124	138	15	0.16
		RRFHNIAGHYRGQCH	125	139	15	0.16
68	HPV-18 E7	LRAFQQLFLNTLSFV	83	97	15	0.28
69	HPV-18 L1	PTSIFYHAGSSRLLT	91	105	15	0.07
		TSIFYHAGSSRLLTV	92	106	15	0.07
		SIFYHAGSSRLLTVG	93	107	15	0.07
		TPTSIFYHAGSSRLL	90	104	15	0.09
		IFYHAGSSRLLTVGN	94	108	15	0.09
		YPLGRKFLVQAGLRR	524	538	15	0.13
		PLGRKFLVQAGLRRK	525	539	15	0.13
		LGRKFLVQAGLRRKP	526	540	15	0.13
		GRKFLVQAGLRRKPT	527	541	15	0.13
		RKFLVQAGLRRKPTI	528	542	15	0.13
79	HPV-18 L2	IHGTHYYLWPLYYFI	430	444	15	0.08
		FAFFKYSPTISSASS	369	383	15	0.12
		YLWPLYYFIPKKRKR	436	450	15	0.14
		LWPLYYFIPKKRKRV	437	451	15	0.13
		HGTHYYLWPLYYFIP	431	445	15	0.15
		GTHYYLWPLYYFIPK	432	446	15	0.16
		PLYYFIPKKRKRVPY	439	453	15	0.19
		WPLYYFIPKKRKRVP	438	452	15	0.19
		SFAFFKYSPTISSAS	368	382	15	0.2
		WPLYYFIPKKRKRVP	438	452	15	0.13

Discussion

HPV is the most commonly sexually transmitted infection in the men and women worldwide (de Martel et al. 2017). HPV has an intra epithelial infection cycle; particularly infects mucosal and cutaneous layers (Bansal et al. 2016). Persistent infection with HPV leads to cervical cancer development (Tornesello and Buonaguro 2020).

As of now, two vaccines are approved by the FDA for the prevention of HPV associated cervical cancers(Fontecha et al. 2015). GlaxoSmithKline's Cervarix® contains HPV 16, 18 virus like particles (VLPs), and Merck's Gardasil® contains HPV 6, 12 VLPs along with HPV 16, 18 VLPs (Wang and Roden 2013), according to some reports these two prophylactic vaccines are highly efficient in preventing HPV infection (Schiller and Lowy 2018). However, none

Table 3 The predicted potential B CELL epitopes in Hr-HPV-18

Sl. No	Protein region	Epitope	Start position	Immunogenicity Score
89	HPV-18 E2	QDKIIDHYENDSKDID	16	0.93
		TFYIEFKSECEKYGNT	171	0.92
		SSTWHWTGAGNEKTGI	371	0.91
		TGTWEVHFGNNVIDCN	186	0.91
		KGGQTVQVYFDGNKDN	116	0.91
		DWTLQDTCEELWNTEP	95	0.90
		TWDKTATCVSHRGLYY	149	0.88
		TVTYHSETQRTKFLNT	334	0.87
		YHSETQRTKFLNTVAIPDSV	337	0.87
		GQTSAATRPGHCGLAE	241	0.86
99	HPV-18 E4	PWAPQRPTARRRLLHD	33	0.93
		LNSYSTPPHRIPAPCP	18	0.91
		PPHRIPAPCPWAPQRP	24	0.90
		AVPVTTRYPLLSLLNS	5	0.87
		LHLQATTKDGNSVVVT	70	0.81
104	HPV-18 E5	CFCVCMYVCCHVPLLP	9	0.86
		PATAFTVYVFCFLLPM	46	0.83
106	HPV-18 E6	HNIAGHYRGQCHSCCN	128	0.89
		VGGEDGEGI	480	0.86
		TRRPYKLPDLCTELNT	8	0.82
		HKCIDFYSRIRELRHY	66	0.82
110	HPV-18 E7	KATLQDIVLHLEPQNE	5	0.82
		SFVCPWCAS	95	0.94
		IPTKENNTG	953	0.823
113	HPV-18 L1	KPTIGPRKRSAPSATT	539	0.93
		CQSICKYPDYLQMSAD	286	0.92
		KGTASKSRPLSQGDCPPLE	171	0.91
		KFLVQAGLRRKPTIGP	529	0.90
		GTACKSRPLSQGDCPP	233	0.90
		ASTQSPVPGQYDATKFK	346	0.90
		SSILEDWNNKDPYDKLKF	397	0.8 7
		AVVNTDDYVTRTSIFYHAGS	20	0.87
		AITCQKDAAPAENKDP	487	0.87
122	HPV-18 L2	GTQIGARVHFYHDISP	310	0.94
		FEPVDTTLTFDPRSDV	256	0.92
		DISPIAPSPEYIELQP	322	0.91
		SGTCPPDVVPKVEGTT	24	0.91
		EEPISSTPLPTVRRVA	207	0.91
		MVSHRAARRKRASVTD	1	0.91
		GRTGYIPLGGRSNTVV	67	0.89
		TGSGTGGRTGYIPLGG	61	0.89
		VWPIVSPTAPASTQYI	413	0.89
		SPTISSASSYSNVTVP	375	0.88

of these vaccines were effective against pre-existing HPV infection or cancers associated with HPV infection. Furthermore, HPV prevalence and mortality is still high in several developed and developing countries, given this rationale, so far, several therapeutic vaccines for HPV infection or cancer clearance have been developed (Namvar, Panahi et al. 2020a; b); (Namvar et al. 2020a,b) but, many of them induced an inadequate immune response. Given this limitation there

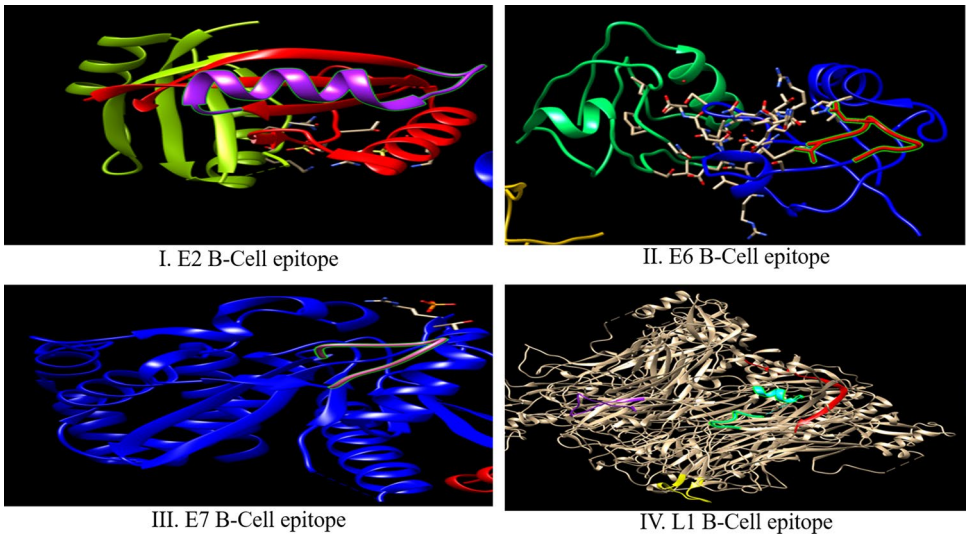

Fig. 1 3D view of predicted B-cell epitope on the E2 E6, E7, and L1 peptide construct; I. Representing structure of E2 B cell epitope "YHSETQRTKFLNTVAIPDSV" with purple colour, II. Representing structure of E6 B cell epitope "VGGEDGEGI" with red colour, III. Representing structure of E7 B cell epitope "SFVCPWCAS" with pale purple, and IV. Representing structure of L1 B cell epitope "KGTASKSRPLSQGDCPPLE" "ASTQSPVPGQYDATKFK" "SSILEDWNNKDPYDKLKF" "AVVNTDDYVTRTSIFYHAGS" with purple, cyan, yellow and red colours respectively at structural level using Chimera software (Color figure online)

is an urgent need to develop an effective therapeutic vaccine against HPV associated cervical cancers (Frazer and Chandra 2019). Additionally, for cervical cancer, no FDA-approved immunotherapy is present till date. Immunotherapy to treat cervical cancer or HPV infection can be achieved by development of DNA or peptide based vaccines against the viral genome proteins (Panahi et al. 2018). Peptide based vaccines depends on the recognition of highly immunogenic epitopes as they rely on usage of short peptide fragments to engineer the induction of highly targeted immune responses (Li et al. 2014) restricted to a specific MHC. There are several studies focused on the immune-therapeutic target for the HPV-16 (Kumar et al. 2015a,b,c; Kumar et al. 2015a,b,c; Kumar et al. 2015a,b,c; Yufeng Yao 2013), however few studies have been done on the 2nd most common HPV type (i.e., HPV-18) along with HPV-16 causing cervical cancer (Basit Jabbar et al. 2018).

The aim of the study was the prediction of T and B cell epitopes from E2, E4, E5, E6, E7, L1, and L2 oncoproteins of hr-HPV-18. E2, E4, E5, E6 and E7 are crucial for transcription activation, cell cycle deregulation/transformation, tumor pathogenesis, and virus replication. L1 and L2 are responsible for recognition, attachment and entry into host cell, later they help in viral self-assembly and proliferation (Pinidis et al. 2016), Therefore, the early and late HPV regions are considered as the ideal targets for therapeutic vaccine generation.

Hr-HPV-18 is the second most carcinogenic and prevalent type HPV worldwide (Burni et al. 2019). However it does not show symptoms (Geng et al. 1999) like hr-HPV-16. Currently, people with hr-HPV-18 infections show normal cytology or precancerous lesions, hence, the infection remains undetectable and causes the progression of infection into cervical cancer. Hr-HPV-18 is known to be present in a higher proportion of cervical adenocarcinomas (ADC) ~ 37% than cervical squamous cell carcinomas (SCC) ~ 12% (Li et al. 2011). As of now the factors that favor the hr-HPV-18 pathogenies are poorly understood. Thus, in this study, hr-HPV-18 is targeted for the epitope prediction. hr-HPV-18 protein sequences were obtained from NCBI GenBank database, and the majorly expressed alleles for MHC-I and II within the Indian population were chosen for the study. There are various studies which showed the importance of amino acid residues for the functions of proteins (Kumar et al. 2015b). So, amino acid composition was also identified for hr-HPV-18, and Threonine (Thr/T) and Leucine (Leu/L) were the most frequent amino acids. The potent epitopes against antigenic proteins can be identified using bioinformatic software and can be used as immune therapeutic targets against HPV infection (De Groot et al. 2010). Adaptive immunity is accelerated by lymphocytes, precisely by B- and T-cells (Sanchez-Trincado et al. 2017).

B- and T-cells particularly recognizes the molecular components known as antigens. A specific region of the antigen known as epitope is recognized by the immune cells to elicit the immunogenic response (Jespersen et al. 2019). Potent peptides against the antigenic epitopes of hr-HPV-16 E5 and E6 have also been identified (Kumar, Singh, et al. 2015a; b, c) (Kumar et al. 2015a), and shown an promising role in terms of vaccine development. In this study,

IEDB software was used to predict the potent immunogenic epitopes for MHC I and II alleles for hr-HPV-18 oncoproteins. We have screened 143 potent epitopes for MHC I, 222 epitopes for MHC II on the basis of percentile rank and immunogenicity score. In future these identified epitopes further can be study in vitro and in vivo for the development of effective immunotherapeutic targets. The epitopes having a high immunogenicity score (> 0.9); STVSVGTAK, DSVQILVGY, TPSPYSSTV, SPYSSTVSV of E2, SYSTPPHRI of E4, MLLLHIHAI of E5, SVYGDTLEK, SVYGDTLEK of E6, ATLQDIVLH of E7, LPPPSVARV, IYNPETQRL, DVMSYIHSM, FPIFLQMAL of L1, and CPPDVVPKV, STTSFAFFK, APSPEYIEL of L2 against MHC-I; TTRYPLLSLLNSYST, TRYPLLSLLNSYSTP, VTTRYPLLSLLNSYS of E4, CMCAYAWVLVFVYIV, MCAYAWVLVFVYIVV, YAWVLVFVYIVVITS, AWVLVFVYIVVITSP of E5, NEKRRFHNIAGHYRG, EKRRFHNIAGHYRGQ of E6, LRAFQQLFLNTLSFV of E7, PTSIFYHAGSSRLLT, TSIFYHAGSSRLLTV, SIFYHAGSSRLLTVG, TPTSIFYHAGSSRLL of L1, and IHGTHYYLWPLYYFI, FAFFKYSPTISSASS of L2 against MHC-II. The potent B cell epitope predicted were YHSETQRTKFLNTVAIPDSV of E2, VGGEDGEGI of E6, SFVCPWCAS, IPTKENNTG of E7, and KGTASKSRPLSQGDCPPLE, ASTQSPVPGQYDATKFK, of L1 can be considered as potential candidates for therapeutic vaccines development. In-silico analysis or experimental evidence are someway limited to the identification of epitopes against oncoproteins E6 and E7 only. However, among our MHC-I predicted epitopes MLLLHIHAI of E5, and ATLQDIVLH of E7 were also reported in a comprehensive in silico study of hr-HPV-18 (Panahi et al. 2018). In another similar study LPPPSVARV epitope of L1, and CPPDVVPKV epitope against L2 were predicted and analyzed for development of cross-subtype prophylactic vaccine development (Namvar et al. 2019). Moreover, predictive analysis of the thorough biological information characteristics of the T-cell and B-cell dominant epitopes will give rise to the further wet laboratory experiments and development of efficient HPV therapeutic vaccines against hr-HPV-18.

Conclusion

In the present study, this is first time that in a laborious in silico study epitope prediction of E2, E4, E5, E6, E7, L1, and L2 proteins of 2nd most common type was targeted i.e., hr-HP-18 have been investigated altogether. For the development of an effective vaccine both prophylactic and therapeutic vaccine candidates need to be identified. Considering the role of these protein in viral replication, maintenance, oncogenicity, and virus assembly, HPV-18 proteins are good candidates for antigenicity and immunogenicity. In the present study, we have targeted the late region of the HPV-18 (L1 & L2) responsible for the viral entry and structure also an ideal target for the prophylactic vaccine, and Early protein E6, E7 main oncoproteins for the therapeutic target. These predicted targets need to be further validated by in-vitro & in vivo study will be warranted.

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Declarations

Conflict of interest The authors declare no potential conflict of interest.

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