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IN SILICO VACCINE DESIGN AGAINST THE TARGET L1 BINDING PROTEIN OF HUMAN PAPILLOMAVIRUS, AN ETIOLOGICAL AGENT OF CERVICAL CANCER, USING BIOINFORMATICS TOOLS

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ABSTRACT: Human Papilloma Virus (HPV) is the smallest virus in the world. HPV is a non-enveloped double-stranded circular DNA virus, which can widely infect human skin and reproductive tract and respiratory epithelium. HPV also has a close relationship with benign and malignant tumors. There is no universal HPV treatment. Although there is a vaccine called Gardasil that can prevent some diseases caused by HPV, it is not recommended for everyone and does not eliminate the chance of getting other types of HPV. Thus, the aim of the present study is to design a subunit vaccine against HPV using bioinformatics approaches. In order to achieve our objective, we have used B-cell and T-cell epitope prediction methods. The possible vaccine target was proposed by using the conserved sequence among the chosen L1 binding protein from twenty different sequences. Structure prediction of this sequence by PSIPRED revealed that one helix is present in the sequence. The use of immune-informatics has greatly revolutionized the field of vaccine research, discovery and development. It demands the proper computational investigations of every possible antigenic candidate. The present study finds that L141 protein of HPV can be also an effective candidate for the development of preventive measures against the drastic diseases caused by the virus by blocking its resistance efficiency. In fact *in silico* approach for vaccine target prediction are definitely reducing manpower, time and cost in relation to searching a lead antigenic molecule against the L1 protein.

INTRODUCTION: Cancer refers to a class of diseases in which a cell or a group of cells divide and replicate uncontrollably, intrude into adjacent cells and tissues (invasion) and ultimately spread (metastasis) to other parts of the body from the location at which they originate [1]. There are several types of cervical cancer, classified on the basis of where they develop in the cervix.

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Cancer that develops in the ecto-cervix is called squamous cell carcinoma, and around 80-90% of cervical cancer cases (more than 90% in India) are of this type [2]. Cancer that develops in the endo-cervix is called adenocarcinoma.

In addition, a small percentage of cervical cancer cases are mixed versions of the above two, and are called adenosquamous carcinomas or mixed carcinomas. There are also some very rare types of cervical cancer, such as small cell carcinoma, neuroendocrine carcinoma etc. [3]. Carcinoma of the cervix is the second most frequent cancer amongst women worldwide with half a million new cases and nearly 300,000 deaths every year.

An estimated 2, 05,496 new cases and 1, 19,097 deaths are due to 29% and 30% respectively of the global burden of cervical cancer cases and mortality. In India, current estimates indicate that every year 1, 34,420 women are diagnosed with cervical cancer and 72,825 die from the disease. Cervical cancer ranks as the 1st most frequent cancer among women in India, between 15 and 44 years of age [4].

A number of anecdotal reports of malignant conversion of genital warts (*Condylomata acuminata*) had appeared in the medical literature during the preceding 100 years and resulted in speculation on a possible causal role of HPV infection in cervical cancer that led to initial attempts to characterize the viral DNA in genital warts [5, 6]. These and other studies led to the early discovery of the heterogeneity of the HPV family [7, 8], which currently numbers more than 100 fully sequenced genotypes [9].

The new studies revealed that more than hundred genotypes have been identified. HPV types 16, 18, 31, 33, 35, 52b and 58 are sexually transmitted and infect the cervix. Papillomaviruses are small, non-enveloped, epitheliotropic, double-stranded DNA viruses that infect mucosal and cutaneous epithelia in a wide variety of higher vertebrates in a species-specific manner and induce cellular proliferation. The recent demonstration of the efficacy of virus-like particles in the prevention of persistent infection of HPV 16 in early precursor lesions of cervical cancer [10, 11] has had a considerable impact on the development of prophylactic vaccines. In addition, the potential contribution to carcinogenesis [12] of certain types of cutaneous HPV that prevent apoptosis in cells damaged by ultraviolet light [13, 14] and / or target tumour-suppressor genes [15] has been hypothesized.

Moreover, new perspectives have emerged for the prevention of these infections by the application of HPV testing technologies and vaccines. During the viral life cycle, E6 and E7 proteins of HPV facilitate the state maintenance of viral episomes and stimulate differentiating cells to re-enter the S phase. The L1 and L2 proteins assemble in capsomers, which form icosahedral capsids around the viral genome during the generation of progeny virions [16].

To date two prophylactic vaccines have been developed and used in large multicentric trials[17-19]. One of the vaccines known as Gardasil (a quadrivalent vaccine, produced by Merck and Co.) [20] protects against HPV types 6, 11, 16 and 18, and another Cervarix (a bivalent vaccine, produced by Glaxo Smith Kline) protects against types 16 and 18 [21]. Computational methods used in vaccine design have been changing drastically in recent years.

Classical immunological research results could be recorded by pen and pencil or in a spreadsheet, but new experimental high throughput methods such as sequencing, DNA arrays, and proteomics have generated a wealth of data that are not efficiently handled and mined by these approaches. This has fueled the rapid growth of the field of Immunological Bioinformatics (or Immuno-informatics) that addresses how to handle these large amounts of data in the field of immunology and vaccine design. Although available vaccines can prevent some diseases caused by HPV, it is not recommended for everyone and does not eliminate the chance of getting other types of HPV. Thus, the aim of the present study is to design a subunit vaccine against HPV using bioinformatics approaches.

The L1 protein that surrounds the DNA and makes up the capsid of the human papillomavirus is the focus of the HPV vaccine. The vaccine is used to elicit a response from the body to protect from infection. L1 spontaneously self-assembles into pentameric capsomers. Purified capsomers can go on to form capsids, which are stabilized by disulfide bonds between neighboring L1 molecules. L1 capsids assembled in vitro are the basis of prophylactic vaccines against several HPV types.

MATERIALS & METHODS:

Retrieval of target sequence: The L1 protein sequence of HPV is retrieved from Protein Sequence Database of NCBI. FASTA file was prepared of the chosen 20 sequences.

Conserved sequence prediction: Multiple sequence alignment and conserved sequence prediction were conducted using BioEdit.

B cell epitope prediction: B-cell epitopes are antigenic determinants on the surface of pathogens that interact with B-cell receptors. Sequence-based tool used for the analysis of continuous B-cell epitopes include BepiPred method.

T-cell epitope prediction: T-cell epitope are antigenic determinants recognized by T-cell receptors. As T-cell is MHC restricted for activation hence MHC- binding regions of antigen have to find out first. We use Epijen for the prediction of T-cell epitope.

MHC-I & MHC- II prediction: The prediction of promiscuous MHC class-I and class-II binding peptides was done by using ProPred 1 [22].

Structural predictions: PSIPRED is a web based interface used to determine structural & the statistical properties of the amino acids. It determines that the target sequence is a helix, strand or coil.

RESULTS: Twenty sequences of the L1 protein of HPV were fetched from the site of NCBI and their FASTA file was prepared to find out the conserved sequences using Bioedit.

Conserved sequence finding: Only one conserved region was found from Position 1 to 47 having Segment Length: 47

1FVTVVDTTRSTNMSLCAAISTSEPTYKNTNF KEYLRHGEEYDLQFIF 47

B-cell Prediction Results: B-cell epitope were predicted by BepiPred Linear Epitope Prediction Method.

Sequence:

1 FVTVVDTTRS TNMSLCAAIS TSEPTYKNTN FKEYLRHGEE YDLQFIF 47

DISCUSSION: In biology, conserved sequences are similar or identical sequences that may occur within nucleic acid proteins or polymeric carbohydrate within different organisms. In the case of cross species conservation indicates that a particular sequence may have been maintained by evolution despite specification. Sequence similarities serve as evidence for structural and functional conservation as well as the evolutionary relationship between the sequences [23]. Compared to other papillomavirus genes, the amino acid sequences of most portions of L1 are well-conserved between types.

However, the surface loops of L1 can differ substantially, even for different members of a particular papillomavirus species. This probably reflects a mechanism for evasion of neutralizing antibody responses elicited by previous papillomavirus infections. During the analysis of conservation among a sequence of amino acid of protein L1, a sequence of 47 amino acids length was found to be conserved. Further study of this conserved region was done regarding its antigeniticy and ability to evoke the immune system. B-cell epitope are vital in designing peptide based vaccines. Mature B-cell display membrane bound immunoglobulin molecules, which serve as receptors for epitopes present on antigen. Interaction between antigen and membrane bound antibody on mature B-cells induce the activation, differentiation of B-cells clones of corresponding specificity and secretion of specific antibodies. Secreted antibodies are the major effector molecules of humoral immunity.

Antibodies are particularly effective in protecting against infection if they are localized at the site of viral entry into the body. Most viruses express a surface receptor molecule which enables them to initiate infection by binding specific host cell membrane molecules. If antibody to the viral receptor is produced it can block infection all together by preventing the binding of viral particle to host cells. Secretory IgA in mucous secretion play an important role in host defense against viruses by blocking viral attachment to mucosal epithelial cell.

Viral neutralization by antibody sometimes involves a mechanism that operates after viral attachment to host cell. In some case, antibodies may block viral penetration by binding to epitopes that are necessary to mediate fusion of the viral envelope with the plasma membrane [24].

During B-cell epitope prediction of conserved sequences using BepiPred method (combination of HMM and Parker) [25] 3 B-cell epitopes were found on a conserved region at position 7-10 (TTRS), 20-30 (STSEPTYKNTN) and 40 (E).

The binding capacity of this conserved sequence was also assessed for its ability to bind with MHC-I using the ProPed-I server. As per the results of ProPred, 39 sequences were found to bind with MHC-I alleles. During this analysis, total 48 alleles of MHC-I were tested by ProPred for binding. Varied peptides score was revealed with the binding regions viz. Allele HLA-A1 has predicted binder at peptide positions 20 and 36; HLA-A*0201 has at position 26; A*0205 at 2 & 26; A*1101 at 27 & 23; A24 at 32, 26, 6 & 36; A3 at 27 & 23; A*3101 at 27 & 23; A*3302 at 27 & 2; A68.1 at 27, 18 & 2; A20 cattle at 25, 27 & 18; A2.1 at 26 & 10; B14 at 34 & 7; B*2702 at 34, 7, 38 & 26; B*2705 at 34, 7, 26 & 6; B*3501 at 22, 6, 17 & 26; B*3701 at 37, 4 & 38; B*3801 at 36, 22 & 26; B*3901 at 34 & 10; B*3902 at 26 & 34; B40 at 38, 37, 4 & 21; B*4403 at 38, 37, 21 & 22; B*5101 at 22, 16 & 10; B*5102 at 16, 10 & 22; B*5103 at 16 & 10; B*5201 at 38, 37, 36 & 22; B*5301 at 25, 22, 38 & 4; B*5401 at 25, 30 & 33; B*51 at 25, 22, 4 & 34; B*5801 at 8, 20, 36 & 9; B60 at 6, 37, 26 & 4; B61 at 37, 4, 38 & 21; B62 at 17 & 22; B7 at 6 & 26; B*0702 at 26, 22, 8 & 6; B8 at 6, 22 & 4; Cw*0301 at 10, 38 & 20; Cw*0401 at 22, 32, 6 & 26; Cw*0602 at 26, 10, 34 & 37; Cw*0702 at 32, 25, 17 & 38.

The higher the score of any peptide frame, the greater the probability of its binding to given MHC molecules. Highest peptide score and binding were founded with allele HLA-B*2705 with the binding part of protein as LRHGEEYDL. This prediction is helpful for designing proper vaccine candidate.

During the analysis of MHC-II binding, total 51 alleles were analyzed for their binding efficiency with conserved regions of protein L1. Both class I and class II MHC gene are highly polymorphic that is within species each gene exists in many different forms for alleles. The binding of multiple epitope peptides with MHC is important of generating cell mediated immunity [24].

Among 51 alleles analyzed the conserved region of protein L1 showed binding affinity with 12 alleles (DRB1_0102, DRB1_0402, DRB1_0404, DRB1_0405, DRB1_0408, DRB1_0410, DRB1_0423, DRB1_0801, DRB1_0806, DRB1_0817, DRB5-0101 and DRB5_0105) with one or more than one paratope found in each.

During the last step of study, this protein sequence was also analyzed for its binding affinity with T-cell receptors using the EpiJen server, the multi-epitope peptide showed T-cell binding region at following positions in allele HLA-A*0101- 26 and 18, HLA-A*0201- 7 and 1, HLA-A*0202- 7, HLA-A*0203- 7 and 38, HLA-A*0206- 7 and 18, HLA-A*0301- 7 and 18, HLA-A*1101- 7 and 18, HLA-A*24- 33 and 11, HLA-A*3101- 28 and 1, HLA-A*6801- 28 and 1, HLA-A*6802- 1 and 7, HLA-B*07- 35 and 7, HLA-B*27- 35, HLA-B*3501- 9, HLA-B*40- 39 and 38, HLA-B*44- 39 and 38, HLA-B*51- 11 and 38, and HLA-B*53- 38 and 39.

Riemer *et al* (2010) observed that HPV-16 is the causative agent of 50% of cervical cancers and many other HPV-associated tumors [26]. The transforming potential/tumor maintenance capacity of this high risk HPV is mediated by two viral oncoproteins, E6 and E7, making them attractive targets for therapeutic vaccines. Of 21 E6 and E7 peptides computed to bind HLA-A*0201, 10 were confirmed through TAP-deficient T2 cell HLA stabilization assay. Those scoring positive were investigated to ascertain which were naturally processed and presented by surface HLA molecules for CTL recognition.

Because IFNγ ELISpot frequencies from healthy HPV-exposed blood donors against HLA-A*0201-binding peptides were unable to identify specificities for tumor targeting, their physical presence among peptides eluted from HPV-16-transformed epithelial tumor HLA-A*0201 immunoprecipitates was analyzed by MS^3 Poisson detection mass spectrometry. Only one epitope ($E7_{11–19}$) highly conserved among HPV-16 strains was detected. This 9-mer serves to direct cytolysis by T cell lines, whereas a related 10-mer ($E7_{11–20}$), previously used as a vaccine candidate, was neither detected by MS^3 on HPV-transformed tumor cells nor effectively recognized by 9-mer specific CTL. These data underscore the importance of precisely defining CTL epitopes on tumor cells and offer a paradigm for T cell-based vaccine design.

Structure prediction of this sequence by PSIPRED revealed that one helix is present in the sequence. This data can be very helpful for generating antigenic candidate by wet lab researchers. The use of immune-informatics has greatly revolutionized the field of vaccine research, discovery and

development. It demands the proper computational investigations of every possible antigenic candidate. The present study finds that the L1 protein of HPV can be also an effective candidate for the development of preventive measures against the drastic disease caused by the virus by blocking its resistance efficiency. In fact, *in silico* approach for vaccine target prediction are definitely reducing manpower, time and cost in relation to searching a lead antigenic molecule against the L1 protein.

Conflict of interest: There are no financial competing interests (political, personal, religious, ideological, academic, intellectual, commercial or any others) to declare in relation to this manuscript.

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