

Designing an Immunoinformatics Vaccine Based on Epitopes Against Human Papillomavirus: One of the Novel Methods to Replace Laboratory Animals

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Executive Summary

The human papillomavirus (HPV) is the most common sexually transmitted viral infection, with low-risk types causing genital warts and high-risk types, particularly HPV 16, 18, 31 and 45 leading to cervical cancer. This study designed a vaccine targeting the E6 and E7 oncoproteins of HPV 16, 18, 31, and 45, utilizing a TLR4 agonist as an adjuvant. Simulations indicate that this vaccine can have preventive and therapeutic effects against HPV infection and cervical cancer. This new vaccine may aid in the design of vaccines against other viral sexually transmitted diseases.

Keywords: Immunoinformatics, human papillomavirus, HPV, multi-epitope vaccine, TLR4, TLR9

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Introduction

HPV is an oncogene (carcinogenic) DNA virus that belongs to the papillomavirus family.¹ HPV is the most common viral sexually transmitted disease (STD) worldwide.² Direct skin-to-skin contact with an infected person, which is typically expected during sexual activity, leads to HPV transmission.³ Several types of HPV have been identified. HPV is generally classified into two types: low-risk and high-risk.⁴ Low-risk types usually cause genital warts in both sexes, while high-risk types lead to various types of malignancies, especially cervical cancer in women, which is very important.⁵ The incubation period after first exposure to HPV types that cause genital warts is about 14 days to 8 months, but most genital warts appear up to 3 months after sexual activity.⁶ About a third of genital warts disappear on their own without the need for treatment.⁷ E6 and E7 proteins affect cell cycle regulation.⁸ Research has shown that the E6 and E7 proteins in HPV types 16, 18, 31, and 45 play a more prominent role in causing cervical cancer.⁹ Ultimately, the goal of this study

is to design an effective vaccine against human papillomavirus and to apply this vaccine for the prevention and treatment of infections and associated cancers, particularly cervical cancer.

Materials and Methods

This article discusses the methods for retrieving protein sequences of HPV and predicting immune epitopes for the design of a multi-epitope vaccine. Initially, the amino acid sequences of E6 and E7 proteins from various HPV types (16, 18, 31, and 45) were extracted from the Uniprot and NCBI databases and evaluated using BLASTp to prevent unwanted autoimmune reactions. Subsequently, the identified epitopes for MHC-I and MHC-II were predicted using the prediction tools available in the Immune Epitope Database (IEDB) and NetMHCpan. To assess the potential to stimulate the immune system, CTL and HTL epitopes with a minimum length of 9 and 15 amino acids, respectively, were examined. Additionally, evaluations of

antigenicity, allergenicity, and toxicity of the epitopes were conducted using the VaxiJen, AllerTOP, and ToxinPred servers to select non-allergenic and non-toxic epitopes. Finally, through molecular dynamics (MD) simulations and codon optimization, the final protein sequence of the vaccine was designed and prepared for in silico cloning. This sequence was converted into a DNA sequence to facilitate its placement in a plasmid. Various tools such as ExPasy Translate and Jcat were utilized to aid in the transcription and translation processes. This study contributes to the design of an effective and safe vaccine to combat HPV infections.

Results

In this study, we performed a comprehensive evaluation of selected epitopes to predict their potential for eliciting immune responses against Human Papillomavirus (HPV). The epitopes specific to MHC-I/CTL were derived from a range of HPV proteins, including HPV-16 E6, E7, HPV-18 E6, E7, HPV-31 E6, E7, and HPV-45 E6, E7. These were identified using prediction tools from the Immune Epitope Database (IEDB) and NetMHCpan-4.1. Additionally, MHC-II/HTL epitopes were predicted using the NetMHCIIpan-4.1 and IEDB servers, while LBL epitopes were identified through ABCpred and IEDB. To enhance the reliability and efficacy of the vaccine, selected CTL, HTL, and LBL epitopes were merged from conserved regions, ensuring a streamlined vaccine sequence. The antigenicity, allergenicity, and toxicity of these selected epitopes were rigorously assessed using online tools such as ToxinPred, AllerTOP, and VaxiJen v2.0. The results indicated that none of the selected epitopes exhibited allergenic or toxic properties, with the highest scoring epitopes chosen for inclusion in the final vaccine design. The final multi-epitope vaccine sequence comprises 284 amino acid residues, refined from an initial pool of 24 epitopes down to 11 regional epitopes after eliminating overlapping sequences. To further enhance the vaccine's performance, an adjuvant (RS09) was strategically added to the N-terminus, along with linkers (GPGPG and EAAAK) to facilitate the connectivity of the epitopes. A His-tag was also incorporated at the C-terminus to serve as a distinguishing marker between cloned and non-cloned plasmids. The designed vaccine demonstrated promising properties, achieving a high antigenicity score of 0.7917, confirming its suitability for inducing an immune response. Additionally, the vaccine was assessed for allergenicity and toxicity, revealing it to be non-allergenic and non-toxic. The structural analysis of the vaccine indicated stability in both secondary and tertiary structures, with a solubility score of 0.857658, suggesting that the vaccine is well-suited for further development and has the potential to

effectively combat HPV infections. Overall, these findings underscore the vaccine's promise as a viable candidate for HPV prevention and treatment.

Discussion and Conclusion

The widespread prevalence of Human Papillomavirus (HPV) infection has become one of the major challenges for global health organizations. The sexual transmission of this virus has led to infections in both women and men across various age groups, particularly among adolescents and young adults. Consequently, prevention and treatment programs for HPV and its associated cancers, especially cervical cancer, have been prioritized in the policies of countries worldwide, with significant funding allocated for research in this area. Advances in bioinformatics and available tools help us study pathogenic factors and identify target antigens for innovative vaccine design. Additionally, the immunoinformatics approach enables us to effectively identify the best epitopes for developing multi-epitope vaccines against pathogens and cancers.¹⁰ Many studies have been conducted to develop an HPV vaccine using immunoinformatics strategies. Various antigens from the human papillomavirus have been examined to predict immunogenic epitopes. Each study has produced distinct constructs due to the use of different immunoinformatics tools, linkers, and other variables. Therefore, the constructs from each immunoinformatics study open new avenues for designing innovative and unique vaccines. In this study, several bioinformatics computational tools and immunoinformatics techniques were used to design a chimeric vaccine, focusing on four high-risk HPV types (16, 18, 31, and 45) that play a significant role in causing cervical cancer.¹¹ On the other hand, the E6 and E7 proteins have shown undeniable oncogenic activities in cervical cancer.⁸ Our multivalent vaccine is designed based on the oncoproteins E6 and E7. Additionally, unlike other studies, this study focused not only on HPV types 16 and 18 but also on types of HPV that have received less attention from researchers, including HPV 31 and 45. However, most studies have only focused on HPV 16 or 18, which has negatively impacted the advancement and diversity of new and innovative vaccines. For example, Muhammad Shahab and his colleagues focused solely on the L1 protein of human papillomavirus type 16.¹² Furthermore, unlike other research, we targeted the E series proteins to simultaneously prevent HPV infection and the subsequent cervical cancer. Targeting the L antigen alone is not effective for treating cervical cancer. For example, a long peptide vaccine was designed by Alexandru Tîrziu and his colleagues against the L1 antigen of human papillomavirus types 16 and 18.¹³ To date, three HPV vaccines have been approved by the US Food and

Drug Administration (FDA), including the bivalent Cervarix vaccine (2006)¹⁴, the quadrivalent Gardasil vaccine (2009)¹⁵, and the ninevalent Gardasil vaccine (2014).¹⁶ Sarvarix, Gardasil 4-valent, and Gardasil 9-valent specifically target the L1 protein of HPV types 16/18, 6/11/16/18, and 6/11/16/18/31/33/45/52/58, respectively.¹⁶⁻¹⁸ Our designed vaccine targets four types of HPV (16, 18, 31, and 45) and is superior to the bivalent Cervarix. This vaccine targets the oncogenic proteins E6 and E7, while Gardasil only acts against the L1 antigen and cannot effectively treat HPV infections and cervical cancer. Our vaccine has both preventive and therapeutic properties and is resistant to HPV mutations. Additionally, the removal of overlapping epitopes facilitates the vaccine's manufacturing process and improves its quality. Immunogenicity simulations show that even with a single dose, higher levels of antibodies and immune cells are produced. This vaccine supports the global population and shows better results compared to similar studies.¹⁹ We used TLR4 and TLR9 to evaluate the molecular binding of the ligand to the receptor. TLR4 and TLR9 play an undeniable role in the tumorigenesis of cervical cancer in HPV-positive cells, while all similar studies have only docked a single receptor. Therefore, our chimeric vaccine has multiple advantages over other HPV vaccines.

Conflicts of Interest Disclosures

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Consent For Publication

Not applicable

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