

Review Article

Reverse Vaccinology Approach Against Viruses: A Review

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Summary

Vaccine design is a complex process. However, progresses in bioinformatics will probably make vaccine design and drug development easy. Vaccine design using reverse vaccinology approach is a quick as well as inexpensive than conventional vaccinology. Therefore, the objective of this manuscript is to review the reverse vaccinology approach against viral vaccine development. Reverse vaccinology against viruses is based on the information contained in the database found online from which protein sequences can be obtained without the need to grow the organism. Using this information, it is possible to select, identify, predict and analyze the target information according to the desire. That desirable information to construct vaccine is epitopes that are the immunologically active region of viruses. Since new, emerging diseases need fast measurement, reverse vaccinology approach utilizes bioinformatic tools like VaxiJen2.0 (which is able to calculate antigenic proteins), major histocompatibility complex class I and II prediction tools that helps to predict epitopes from the information contained in the protein data bank found online without the need to cultivate the virus which is a tiresome process. Therefore, based on this concept to construct vaccine against viruses reverse vaccinology approach should be followed.

Keywords: Bioinformatics tools; Conventional Vaccinology; Database; Epitopes; Reverse Vaccinology

Abbreviations: RV: Reverse Vaccinology; WHO: World Health Organization; MERS-COV: Middle East Respiratory Syndrome Corona Virus; FASTA: Fast Adaptive Shrinkage Threshold Algorithm; MEV: Multiepitope based Vaccine; RSV: Respiratory Syncytial Virus; SARS-COV: Severe Acute Respiratory Syndrome Corona Virus; HCOV: Human Corona Virus; MHC: Major Histocompatibility Complex; IEDB: Immune Epitope Data Base; DENV: Dengue Virus; HPV: Human Papilloma Virus; NCBI: National Center for Biotechnology Information; PyMOL: Python language Molecule; RasMOL: Raster of a Molecule; PSIPRED: PSI-blast based Secondary Structure Prediction; PEP-FOLD: Peptide Fold; HLA: Human Leukocytes Antigen; PDBQT: Protein Data Bank, partial charge (Q), & Atom Type (T); PDB: Protein data bank; TLR₄: Toll-like Receptor 4; CTL: Cytotoxic T Lymphocyte; HTL: Helper T Lymphocyte; BCL: B cell Lymphocyte; PADRE: Pedigree-Aware Distant-Relationship Estimation.

Introduction

Vaccines have transformed public health, particularly since national programmes for immunization first became properly established and coordinated in the 1960s [1]. A vaccine is a biological product that can be used to safely induce an immune response that confers protection against infection and/or disease on subsequent exposure to a pathogen [2]. Vaccines exploit the extraordinary ability of the highly evolved human immune system to respond to, and remember, encounters with pathogen antigens. However, for much of history, vaccines have been de-

veloped through empirical research without the involvement of immunologists [3]. Vaccine design is a complex process; however, progresses in bioinformatics will probably make vaccine design and drug development easy [4]. The design of vaccines can be divided into two broad categories: the traditional and the modern approach [5]. Traditional methods may take decades to unravel pathogens and antigens, diseases and immunity. However, modern approaches can be very fast, allowing identifying new vaccines for testing in only a few years [6]. Due to limita-

tions of the conventional technology [7], modern technologies have come into existence, from which Reverse Vaccinology (RV) is one of the new technologies for vaccine development which represents a genome-based approach to vaccine development and developed for the first time in early 1990's by Rappuoli [8] to identify meningococcal protein vaccine candidates in Group B meningococcus (MenB) [9].

In reverse vaccinology, various tools of in silico biology are used to discover the novel antigens by studying the genetic makeup of a pathogen and the genes that could lead to good epitopes are determined. This method is a quick easy and cost-effective way to design vaccine [10]. It is a process of vaccine development where the novel antigens are identified by analyzing the genomic information of a virus or other organism [11]. RV offers two main advantages compared to traditional vaccine development approaches: identification of candidate antigens without the need to grow the pathogen and identification of any antigen independently by its purified quantity to be suitable for vaccine testing [12].

Therefore, the objective of this review is to highlight the reverse vaccinology approach against viral vaccine development.

Basic Concept of Vaccination

The history of vaccine production started with Edward Jenner's and Louis Pasteur's innovation and immunization practices, making the development of vaccination a necessary practice for improving wealth [13]. The World Health Organization (WHO) has divided vaccines into three major categories: i) traditional, ii) innovator: new vaccines as RV; and iii) targeted. Traditional vaccines lead global market volume, and innovator vaccines drive global market value [14].

Vaccines, like natural infections, act by initiating an innate immune response, which in turn activates an antigen-specific adaptive immune response. Innate immunity is the first line of defence against pathogens that have entered the body. Adaptive immunity provides a second line of defence, generally at a later stage of infection, characterized by an extraordinarily diverse set of lymphocytes and antibodies able to recognize and eliminate virtually all known pathogens [15]. Vaccine design has made significant advances in the last century, evolving from serendipity to a more rational design due to advances in understanding immunological mechanisms and technology [16].

Vaccines can be produced using different processes. Vaccines may contain live attenuated pathogens (usually viruses), inactivated whole pathogens, toxoids (an inactivated form of the toxin produced by bacteria that causes the disease), or parts of the pathogens (e.g. natural or recombinant proteins, polysaccharides, conjugated polysaccharide or virus-like particles).

Reverse Vaccinology

Reverse Vaccinology refers to the concept of using genomic knowledge, without the actual cultivation of pathogen to determine the immunologically active components [17]. Pioneered by Dr. Rino Rappuoli, RV is an emerging vaccine development strategy that initiates a vaccine development from genome sequence bioinformatics analysis. It was first applied to development of a vaccine against serogroup B *Neisseria meningitidis* (MenB), the major cause of sepsis and meningitis in children and young adults [18].

Retrieval of Viral Protein Sequences

As suggested by [19], the generated genomic information is used to screen the inclusive set of potential proteins encoded by pathogens for the search of vaccine candidates. The availability of genomic information of the pathogen under study and, even the human or animal cell genome is an important pre-requirement for using RV. If the genome sequence is obtained, it is possible to identify all likely proteins that could be expressed [16,20].

During the study by [21] on Rational design of multi epitope-based subunit vaccine by exploring *Middle East Respiratory Syndrome Corona Virus (MERS-COV)* proteome, Amino-acid sequence of structural (spike (AKL59401), membrane (AKL59407), Envelope (AKL59406) and nucleocapsid (AKL59408)) and non-structural proteins (A0A2P1ITC7) /1ab(A0A140AYZ4), NS3/3B/3C/3D/4A/4B/5, ORF3 (K9N796) /4a (K9N4V0) /4b (K9N643) /5(K9N7D2) /8 (A0A0U2GQ91)) [22,23] were obtained from Uniprot database in the fast adaptive shrinkage threshold algorithm (FASTA) format [24,25].

Epitope Selection

Epitopes are the immunologically active region of organisms which are recognized as foreign by the host responses and thus provide an excellent means for the production of an efficient vaccine because of their specificity towards a particular organism. An effective vaccine could be designed by the epitope prediction tools as the epitope stimulates immune reactions from both B cells and T cells (Kreiter *et al.*, 2015). [26] suggested that epitopes selection is one of the critical steps for immunoinformatics study and during epitope selection we should select epitopes that are multi-specific and broad-based.

The research report by [27] summarized the general work flow with a set of immunoinformatics tools to design a *Multi-epitope Based Vaccine (MEV)* against Respiratory Syncytial Virus (RSV) (Figure 1). A schematic representation of methodology and tools used in the present study for new vaccine development). Epitopes with the following characteristics are generally preferred to design a subunit vaccine: (a) highly antigenic, (b) immunogenic, (c) non-allergenic, (d) non-toxic, and (e) with significant population coverage [28- 30].

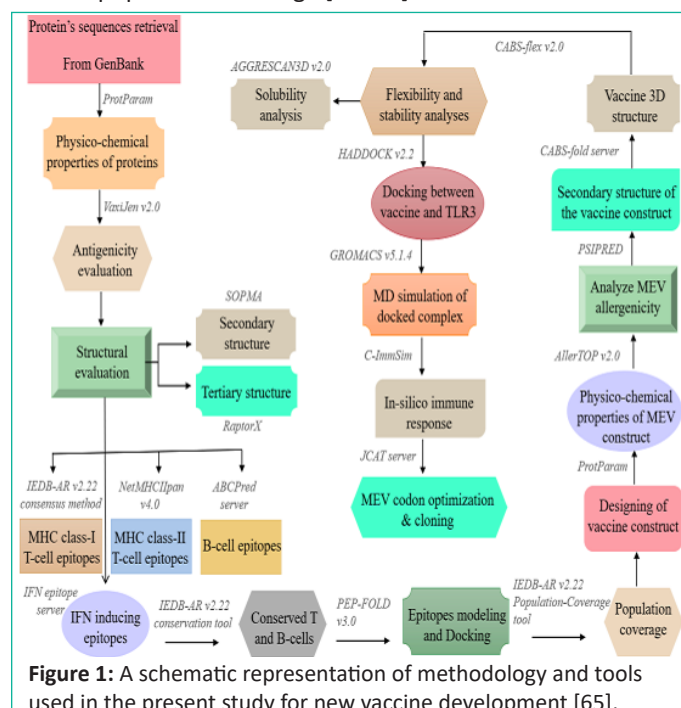


Figure 1: A schematic representation of methodology and tools used in the present study for new vaccine development [65].

Research done on viruses (Chikungunya and Mayaro) showed that to assess the best possible antigen, all the retrieved structural polyproteins of both of the viruses were subjected to computational antigenicity investigation in widely applied server known as VaxiJen2.0 (<http://ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html>) which is able to calculate antigenic proteins more than 80% accurately [31]. The research report of [32] indicated the role of the Vaxign analysis framework [33,34] to compare the full proteomes of seven human coronavirus strains Severe acute respiratory syndrome 2 (SARS-CoV-2); SARS-CoV; MERS-CoV; Human coronavirus 229E (HCoV-229E); HCoV-OC43; HCoV-NL63 and HCoV-HKU1 [35].

Prediction of B-Cell and T-Cell Epitopes

The identification of immunodominant B- and T-cell epitopes that trigger protective immune responses in the host is critical for effective vaccine design [36,37]. The retrieved Homologous sequences of the antigenic proteins (*i.e.*, capsid protein VP1 and protein VP2) of the Norovirus were used to perform multiple sequence alignment using Clustal Omega [38] and further for the prediction of T-cell epitope, Major Histocompatibility Complex I (MHC-I) (<http://tools.iedb.org/mhci/>) and MHC-II prediction tools (<http://tools.iedb.org/mhcii/>) of immune epitope data base (IEDB) were used [39].

As a result, Epitopes that will bind with a higher number of MHC alleles are considered to be more immunogenic (Hajighahramani *et al.*, 2017). As [40] confirmed, those epitopes that bind with the alleles involved in MERS-COV protection were chosen. B cell epitope prediction is concerned in finding the potential antigen that would interact with B lymphocytes and initiate an immune response [41]. As research done by [42] on vaccines against Dengue Virus 1 (DENV-1) and Human Papillomavirus 16 (HPV-16) indicated that the B cell lymphocytic epitopes were selected based on their lengths (the sequences with over ten amino acids in length were selected) and obtained using BepiPred linear epitope prediction method [43].

Antigenicity, Allergenicity and Toxicity Prediction of the Predicted Epitopes

Several bioinformatics studies perform toxicity or allergenicity prediction on peptide candidates to rule out adverse effects in the resulting candidate vaccine [44,45]. In the research of [46] for vaccine development against Chikungunya and Mayaro viruses indicated that the need of the three web servers. The VaxiJen, and ToxinPred web servers were applied consecutively to find out the most appropriate antigenic epitopes, the allergenic and toxic activity of the short-listed B cell and T-cell epitopes respectively [31,47,48]. As the research work of [42] showed, the epitopes that showed high antigenicity, non-allergenicity, non-toxicity, were considered as the best selected epitopes for further analysis and vaccine construction.

Conservancy Analysis of the Selected Epitopes

Conservation analysis is a common method for predicting residues that are functionally important in protein sequences [49]. When designing a vaccine, epitopes of that remain conserved across various strains, are given much priority than genomic regions that are highly variable among the strains since the conserved epitopes of protein(s) provide broader protection across various strains and sometimes even species [50].

The conservancy analysis of the selected epitopes can be performed via the epitope conservancy analysis tool of IEDB

server [51]. As the study done by [42] revealed, for the conservancy analysis of the DENV-1 epitopes, the envelope protein E from other types of Dengue viruses, DENV- 2, 3 and 4 (UniProt accession numbers: Q8BE39, Q66394, Q7TGD1, Q7TGC7, respectively), were used for comparison that gave 100% conserved sequences which were used for vaccine construction, so the constructed vaccines might also confer immunity towards the DENV serotypes- 2, 3 and 4 along with DENV-1 [52].

Cluster Analysis of the Major Histocompatibility Complex Alleles

Cluster analysis of the Major Histocompatibility Complex (MHC) alleles helps to identify the alleles of the MHC class-I and class-II molecules that have similar binding specificities [53]. In the research work of [42] done on RV approach against DENV-1 and HPV-16 indicated, the cluster analysis of the possible MHC class-I and MHC class-II alleles that may interact with the predicted epitopes were carried out by the online tool MHC cluster 2.0 that can generate the clusters of the alleles in a phylogenetic manner.

Generation of the 3D Structures of the Selected Epitopes

Commonly used software to view 3D structure data are Cn3D available from the national center for biotechnology information (NCBI) website, and Python Language Molecule (PyMOL) and Raster of a Molecule (RasMOL) which are powerful molecular graphics visualization tools with capabilities to highlight segments of user's interest. Nowadays, numerous tools are available in the new bioinformatics era, and more tools and servers are constantly being added. It is up to the user to make the best use of the available data, information and analytics to derive the end results desired [54,55] used the tools *PSI-blast based secondary structure Prediction* (PSIPRED) v4.01 and RaptorX-Property [56] to predict the secondary structure of the proposed vaccine and to demonstrate a graphical presentation of it. PSIPRED, a highly accurate predictive secondary structure tool, is a sequence profile-based fold recognition system [55].

As [57] revealed, the 3D structures of the selected best epitopes to identify potential vaccine candidates against SARS-CoV-2 were generated using online 3D generating tool Peptide Fold 3 (PEP-FOLD3) (<http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/>) [58].

Molecular Docking of the Selected Epitopes

Molecular docking is an in-silico approach which aims to evaluate the binding affinity between a receptor molecule and a ligand [59]. All together for the appropriate elicitation of immune response, the interaction amongst the antigenic molecule and immune receptor molecule is essential [60]. As the research finding by [27] on RV approach against RSV showed, molecular docking of the epitopes binding to specific human leukocytes antigen (HLA) alleles was performed to evaluate their binding effectiveness [58].

Reverse vaccinology approach to design a novel multi-epitope subunit vaccine against *avian influenza A* (H7N9) virus by [61] used AutoDOCKVina program to conduct docking based on parameters all the analyses were done at 1.00- °A spacing, and the exhaustiveness parameter was kept at 8.00 while the number of outputs was set at 10. All the output *Protein Data Bank, Partial Charge (Q), & Atom Type (T)* (PDBQT) files were converted in Protein Data Bank (PDB) format using OpenBabel (version 2.3.1). The best output was selected on the basis of

lower binding energy. The docking interaction was visualized with the python molecular (PyMOL) graphics system, version 1.5.0.4 (<https://www.pymol.org/>) [41].

Another research revealed that Toll-like receptor 4 (TLR4) was chosen as the receptor and found from the research col-laboratory for structural bioinformatics protein data bank (RCSB PDB) database (<https://www.rcsb.org/>) (PDB ID: 4G8A) [63] while the vaccine model was used as a ligand. Then, using the ClusPro 2.0 server (<https://cluspro.bu.edu/login.php>) the binding affinity between the multi-epitope vaccine and the TLR4 receptor was identified via molecular docking [64].

Vaccine Construction

Peptides are the promising candidates as immunotherapeutics, but are less immunogenic when used alone in vaccine design. The peptides need potent immunostimulatory adjuvants to effectively activate the innate and adaptive immunity [66]. As the research work of [67] showed, for vaccine construction, the best selected *Cytotoxic T Lymphocyte* (CTL); Helper T Lymphocyte (HTL) and B cell Lymphocyte (BCL) epitopes were combined and joined together by suitable linkers [68].

[10] constructed a vaccine against Ebola Virus in sequential manner: first the adjuvant sequence was conjugated with the Pedigree-Aware Distant-Relationship Estimation (PADRE) sequence by EAAAK linker, then the PADRE sequence was added to the CTL epitopes by GGS linkers, the CTL epitopes were also conjugated with each other by the GGS linkers. Next, the HTL epitopes were conjugated by GPGPG linkers and the BCL epitopes were linked by KK linkers. The research conducted by [61] on RV approach to design vaccine against *Avian Influenza A* (H7N9) virus revealed three vaccine sequence were constructed named V1, V2 and V3, each associated with different adjuvants including beta defensin (a 45 mer peptide), 237 L7/L12 ribosomal protein and HABA protein (*M. tuberculosis*, accession number: AGV15514.1) [68].

Conclusion and Recommendation

Using a reverse vaccinology approach, it is possible to develop effective vaccines against viral diseases in a matter of years and at a low cost without the requirement to culture the organism. Reverse vaccinology against viral diseases employs the entire proteome of the virus by getting it from an online database (e.g., the Uniprot database in the FASTA format). After obtaining the necessary data, epitope selection, prediction, and analysis can be carried out using several bioinformatics tools (which are also publicly available online) in order to produce the right vaccine.

Based on the above conclusion the following recommendations are forwarded:

- Reverse vaccinology approach should be used to design vaccines that are effective against viruses for new, emerging viral diseases that need immediate measurement;
- Due to its rapidity reverse vaccinology approach should be used to overcome challenges of viruses that show genetic diversity; and

Flexible analysis that cannot be performed using conventional methods should be conducted using reverse vaccinology approach.

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