In silico peptide based vaccine design against non-structural protein 3 of hepatitis C

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Abstract

Designing drug against Hepatitis C Virus (HCV) is not effective, as people who are most recently infected with such infection do not show any symptoms and when the condition become fatal after time of exposure from the time of infection, the liver becomes scarred, known as liver Cirrhosis which is incurable. Designing of peptide based vaccine may overcome this problem as they are the preferred candidate for designing epitopes because of their high activity and specificity. The prediction of epitopes in NS3 protein provides a suitable primary immunodiagnostic antigen for the detection of the HCV. In our study, one epitope i.e. LLGTIVTSL was selected on the basis of half-life of dissociation, isoelectric point and binding score between predicted epitopes and MHC. The 3D structure of the epitopes was modeled using homology modeling by Swiss Model. The predicted epitope LLGTIVTSL was identified to be a highly conserved, immunogenic and potential vaccine candidate, capable for generating both CD8+ and CD4+ responses.

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Keywords: Epitopes, HCV, NS3, Vaccine

1. Introduction

Hepatitis C virus was identified in 1989 through expression cloning of immunoreactive cDNA derived from the infectious non-A and non-B Hepatitis agent which had already being recognized as the major cause of transfusion-acquired hepatitis. It typically causes persistent hepatotropic infection, although it is the major challenge to detect viral antigens reliably in infected liver tissues. Flavivirus belongs to the family Flaviviridae and genus HCV having positive single stranded RNA as a genomic material. Before the era of Molecular Biology, members of the family Flaviviridae had been previously classified as Togaviridae. The HCV genome is an uncapped 9.6-kb RNA containing highly structured 5’ and 3’ ends. The 5’ non coding region is a well conserved, 341 nucleotide sequence element that folds into a complex structure containing four major domains. Flavivirus encodes one large open reading frame containing a 5’ type cap and conserved RNA structures at both the 5’ and 3’ untranslated regions that are important for viral genome translation and replication [1]. The genomic RNA is translated into a single polyprotein precursor consisting of three structural Capsid (C), perinuclear membrane (prM), and Envelop (E) protein and seven non-structural NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5 proteins arranged in the order C-prM-E-NS1-NS2a-NS2b-NS3-NS4a-NS4b-NS5. Only the structural proteins become part of the mature, infectious virion, whereas the non-structural proteins are involved in the polyprotein processing, viral RNA synthesis and virus morphogenesis. Non-structural protein 3 is a multi-functional protein with an N-terminal protease domain (NS3pro) that is responsible for proteolytic processing of the viral polyprotein, and a C-terminal region that contains an RNA triphosphatase, RNA helicase and RNA-stimulated NTPase domain are essential for RNA replication. The serine protease domain of NS3 is a key player in the replicative cycle of Flavivirus. The data shows approximately 66% population of Northern India is found to be infected with NS3a [2]. In one study, data showed that NS3a also infects the Hepatitis C in Punjab-Pakistan also to a greater extent [3]. In the Southern part of India the data shows that 60% of population is found to be infected by NS1 and 40% of population is found to be infected with NS3 [4]. Therefore it can be concluded from these data that, although NS3 is somewhere affecting with greater extent and somewhere with less extent it also affects the population of other Asian countries but NS3 is the most common viral protein which infects the population, so it is the subject of choice to study and to design the novel vaccine for human welfare.

In this in silico study, we designed a 9-mer peptide from the uncharacterized non-structural 3 protein having subtype 3a (NS3a) of the Hepatitis C virus by the CPHmodel-3.2 Server, an online tool for homology modeling and the final structure

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IN SILICO PEPTIDE BASED VACCINE DESIGN AGAINST NON-STRUCTURAL PROTEIN 5 OF HEPATITIS C VIRUS

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Abstract

Objective: Hepatitis C virus (HCV) is the cause of hepatitis C in human. A hepatitis C infection does not show any noticeable symptoms in the very early stages of the infection, but a chronic infection can ultimately lead to cirrhosis. The chronic condition results in liver failure or cancer. Protein-Protein interactions play a vital role in the pathogenesis of any pathogen. Protein-Protein interactions maps designed and created in this research provide accurate and valuable resource for better understanding of the pathogenicity pathways of Hepatitis C Virus. The objective of the study was to predict the epitope against non-structural protein NS (5a) of Hepatitis C Virus which could be used as suitable vaccine candidate against Hepatitis C virus infections.

Methods: A specific protein-protein interaction is selected on the basis of its significance in the pathway leading to replication of Hepatitis C genome i.e. Interaction between Hepatitis C Nonstructural protein 5A (NS5A) with s3 domain of E2 tyrosine protein kinase. Epitopes was predicted and screened by using various bioinformatics tools. Each of the predicted structure was docked with MHC Class I and class II molecules using PatchDock and FireDock.

Results: The MVGLNSYRI epitope was selected on the basis of half life of dissociation and binding score. The average score of half-life of disassociation (t1/2) for MVGLNSYRI was 20 hrs, which is the greater than the other epitopes. Structure based modeling of epitopes was done and further the energy was optimized. Then after that the binding score was calculated, which was again best in case of MVGLNSYRI.

Conclusion: These findings conclude that the designed protein-protein interaction maps and predicted epitopes can be of great use in the wet laboratory formulations of vaccines against Hepatitis C Virus.

Keywords: Bioinformatics, Protein-protein interaction, HCV, Vaccine, 3D structure.

Introduction

Hepatitis C virus (HCV) is the cause of hepatitis C in humans. A hepatitis C infection does not show any noticeable symptoms in the very early stages of the infection, but a chronic infection can ultimately lead to cirrhosis. The chronic condition results in liver failure or cancer. Hepatitis C spread mostly through blood to blood contact, unsterilized equipment used for intravenous drug transfer. The number of people affected by hepatitis C infection worldwide is approximately 200 million per annum which is responsible for hundreds of thousands of deaths each year.

Hepatitis C virus infects only humans and chimpanzees. The knowledge about hepatitis C infection was first reported in the year 1989[1]. It belongs to the genus Hepacivirus and is a member of family flaviviridae [2]. It is an enveloped positive sense single stranded RNA virus with approximately 55-65 nm. Despite the discovery of HCV over 15 years ago, knowledge of the HCV lifecycle has been limited by inability to grow the virus in cell culture, as well as by the lack of small-animal models of HCV infection [3].

HCV has an RNA genome which consists of 9600 nucleotide bases. It consists of UTRs at 5’ and 3’ ends of the RNA. The genome contains a non-coding region (5’-3’) and a coding region. HCV genome comes with a high genetic variability caused due to the mutations that happens frequently during the viral replication. These mutations vary in different genomic regions of HCV [4]. Structural proteins of HCV are core protein (E1 and E2) which are primarily present in the coding region of the genome and non-structural proteins (NS2, NS3, NS4, NS4A, NS4B, NS5, NS5A, NS5B) [2].

The proteins encoded by Hepatitis C genome (structural and non-structural proteins) are primarily responsible for the pathogenesis of Hepatitis C virus. The Hepatitis C viral proteins interact with the proteins present in the human host cells and generate certain signaling pathways which in turn regulate the replication of Hepatitis C genome and its survival in human cells. The non-structural NS1 protein is a hydrophobic transmembrane protein which basically form hydrophilic pores and there by regulating the permeability of the membrane for the propagation of viral assembly and release of viral particles to increase infectivity, NS2 protein works as to attract the envelope proteins to the assembly site and favours viral assembly, NS3 has helicase as well as protease activity. It plays a central role in the process of viral replication. It has its major role in unwinding of viral RNA alone and in complex with NS4A, NS4A functions as cofactor for proper working of NS3 protein for increased enzymatic activities. NS4A also forms complex with NS4B and NS5A to facilitate viral replication on the endoplasmic reticulum membrane, NS4B is associated with NS5B so as to modify its polymerase activity, thus showing its role in carcinogenesis. NS4B favours viral replication as it is involved in the formation of the membranous structure which acts as a platform for viral replication to happen, NS5A is necessary for the viral replication as well as viral assembly, NS5B works as RNA dependent RNA polymerase [5].

The overall pathogenicity of Hepatitis C virus depends upon the interactions between the proteins coded by its genome and their interactions with the human proteins which together result in manipulations to the originally occurring cellular processes and the pathways, thereby increasing pathogenicity. Thus in order to further known the pathogenicity pathways, various protein-protein interactions can be extracted and used in the construction of pathways. Protein–protein interaction maps provide information about the proteins which are highly involved in the viral replication and life cycle. Thus, maps provide a more easy and illustrative way of acquiring knowledge about important interactions between different proteins which are involved in the viral replication.

By studying the interaction map for Hepatitis C protein NS5A various assertions can be made supporting the fact that it is the

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Original Article
IN SILICO PEPTIDE BASED VACCINE AGAINST HEPATITIS C VIRUS

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Abstract

Hepatitis C is a severe disease caused by Hepatitis C virus which leads to human fatality and affected 180 million people across the globe. Its chronic infection leads to liver damage and malignant hepatoma. Till now there is no vaccine in the market for this virus. The objective of the study was to predict the best epitope using Bioinformatics tools for designing a vaccine against HCV. Here T-cell epitope was considered since it can recognize only antigen that processes to generate peptide by antigen presenting cell. For selecting the best T cell epitope, the binding energy with the MHC molecule must be high, must have a protease cleavage site, conserved site, motif, good binder with hydrophobic binding pocket and half-life of dissociation must be high. By considering above criteria suitable bioinformatics tools were used to predict the epitopes from NS3, NS5A and NS5B of 3α and 3β genotype. A total of 600 epitopes from different tools for each protein were predicted and from there only 11 efficient epitopes was virtually screened out using protein-protein interaction between MHC-I and MHC-II molecules and their energy. IMYAPTIWV peptide of NS5A protein was found to be the best epitope. The selected epitope for T-cell can further be used for future work in a wet laboratory for the development of vaccine against HCV.

Keywords- Docking, Epitopes, HCV, In-Silico study, Interaction, Prediction

I. INTRODUCTION

Hepatitis C virus (HCV) affects more than 180 million people worldwide that is a severe worldwide health crisis and the majority of the patient are acute hepatitis C developing chronic HCV infection, which eventually lead to permanent liver failure and malignant hepatoma. [1, 2]. Hepatitis C virus species belongs to the family Flaviridae and genus to Hepacivirus. [3]. Its open reading frame consists of 9600 nucleotides long and that translates a large polyprotein of 3010aa. Further the polyprotein is processed postranslational by cellular and virally encoded proteases to create the mature structural and non-structural proteins. Viral maturation and replication required NS3 serine-like protease and the RNA-dependent RNA polymerase. Hepatitis C virus proteins consist of structural protein and non-structural proteins. Structural proteins consist of core protein, E1 and E2 protein, while non-structural protein consist of NS2, NS3, NS4A, NS4B, NS5A, NS5B and P7 protein and frame-shift protein. [4]. The replicon acquired adaptive mutations due to an unknown mechanism. These mutations are generally found in NS3, NS5A and NS5B proteins of Hepatitis C Virus [5]. Adaptive mutation in the NS5A region creates interferon resistance. The highly adapted replicon contained 3 adaptive mutations (two in NS3 and one in NS5A). These adaptive mutations strongly increase RNA replication [6]. NS3 protein consists of protease which processed the cleavage between NS3/NS4A, NS4A/ NS4B, NS4B/NS5A and NS5A/NS5B [7]. NS5A take significant part in viral replication and its complexes with the help of other viral protein. NS5A contain interferon-alpha sensitive-determining region (ISDR). [8, 9]. NS5B protein is of 65kDa in size, which is responsible for the synthesis of both negative strand RNA in immediate template and the positive strand genomic RNA. [10,11].

Here T-cell epitope was considered since it can recognize only antigen that processes to generate peptide by antigen presenting cell. The T cell is under adaptive immunity, which can display antigenic specificity, diversity, immunologic memory and self and non-self-recognition. In adaptive immune system, T cell plays a numerous role in the direct killing of malignant and virus-infected cells, it also contributes to the generation and maturation of humoral immune responses. T cells have three major groups based on their function: cytotoxic T cells, helper T cell, and regulatory T cells. T-cell recognizes only antigen that is processed to generate peptides that interact specifically with MHC–I or MHC-II molecules. T-cell possesses an antigen specific and clonally restricted receptor that has the ability to interact with MHC molecule and so it is needed to consider this interaction while viewing T-cell epitope. MHC-I is recognized by CD8+ and CD4+ T cells, while MHC–II molecules recognized by CD4+ T cells. CD8+ cytotoxic T cell releases perforin, granzymes, and granulysin, whereas CD4+ T helper cells secrete cytokines that act on B cells, eosinophils, macrophages, and neutrophils to clear pathogens. [12]

T cell epitope are presented on the surfaced antigen presenting cell where they bound to MHC-I molecules. T-cell epitope is short peptide sequences which presented to major histocompatibility complex. Peptide between 8 to 11 amino acids in length are presented to MHC class I molecule and for MHC class II molecules 13 to 17 amino acids long peptide are presented to it. [13]
BIOINFORMATICS TOOLS FOR CONFORMATIONAL B-CELL AND T-CELL EPITOPE PREDICTION: POTENTIAL VACCINE CANDIDATE

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ABSTRACT

Exploration of sequencing approaches has provided an advanced notion in peptide based vaccination in which isolated protein sequence, adequate for inducing a precise immune response, have been figured out and used to attain peptide based vaccine formulations; substituting the ones constituted by whole pathogen-formulations. In present study, immunoinformatics approaches play a central role to scrutinize multiple genomes and proteomes to choose the capable epitopes. Description of epitopes which incite both humoral as well as cell mediating responses is an important concept in the field of immunology. This review paper gives a epitomize view of advanced immunological tools to aid the design and development of vaccine candidates. To vivify the development of better servers and to gem out potential immune stimulators, some assuring directions are also extensively discussed. Within this review paper, we collected the advance bioinformatics tools and resources for B-cell and T-cell epitope prediction. Here, Hepatitis C virus has been seized as an example for development of vaccine candidate against hepatitis.

KEYWORDS: Bioinformatics, Epitope, Immunodiagnostics, Peptide-based Vaccine, Prediction tools

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Bioinformatics Techniques used in Hepatitis C Virus Research

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Hepatitis C is widely spread and induces life threatening situations. Researchers from various fields have developed vaccine, but they are not that effective because of the variation in genotype of Hepatitis virus and also not much affordable. In-silico approach is of greater importance in designing and testing the model vaccine. In this study, investigation has been done for the available Bioinformatics tools and methodologies used in HCV research. Different types of tools and databases commonly used by researchers were reviewed to get an overall picture of bioinformatics techniques, computational biology tools and databases used in Hepatitis C Virus research. Exclusive study has also done to figure out different statistical methods used by different research groups. This paper will provide an up-to-date picture of computation approaches used for exploring Hepatitis C treatment.

Keywords: liver disease, In-silico, tools, techniques, database, vaccine.

The causative agent of Hepatitis C, a life threatening disease is Hepatitis C virus. Characteristically it affects the liver and the individual can develop acute and chronic infections. The infection starts with slight infirmity for few weeks to major lifetime illness. HCV infection occurs via the blood transfusion of the infected patient to the normal individual, using non sterilized medical equipment, using same syringe or needle for more than one individual. Globally 130–150 million people develop chronic hepatitis C infection. Out of those, considerable numbers of chronically infected individuals develop liver cirrhosis and cancer. Numbers of individual who lose their lives from this infection are close to 500,000 per year. Among the HCV infected population antiviral medicines can neutralise the effect of 90% population which leads to minimized chances of death from liver cirrhosis and cancer but access to diagnosis and treatment protocols are very poor. Research to avail first vaccine globally is still in process. Hepatitis C virus came into picture in 1989 by expression cloning of immunoreactive cDNA isolated from infectious non-A and non-B Hepatitis agent. HCV comes under the flaviviridae family and genus Hepacivirus. Its genetic material comprises of positive single-stranded RNA. The genome size of HCV is 9.6 kb RNA having highly structured 5' and 3' ends (Fig. 1). The 5' end is 341 nucleotides long conserved non coding region which contains four major domains when folded into complex structure. Flavivirus codes for a long open reading frame having 5' cap and conserved RNA structures at both the 5' and 3' untranslated regions essential for replication and translation of viral genome. The structural HCV proteins are currently designated as core, E1, E2 and p7. Additionally, the non-structural proteins are NS2, NS3, NS4A, NS4B, NS5A and NS5B. The genomic RNA is translated into a single polyprotein precursor consisting of three structural Capsid (C),
In silico identification of vaccine candidate from various screening methods against hepatitis C virus

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Abstract: Hepatitis C virus (HCV) being an infectious disease is prevalent in most parts of the world. Till date, no vaccine is being developed in the market for HCV. This paper focuses mainly on developing a peptide-based vaccine against HCV. The purpose for this study is taken to determine the suitable epitope with the help of Bioinformatics tools developed for designing a vaccine against infectious diseases such as HCV. In present work, T-cell epitope is taken into consideration, as it recognises the antigen that helps to generate peptide with the help of antigen presenting cell. With respect to T-cell epitope selection, high binding energy must be required for binding major histocompatibility complex molecule. Moreover, T-cell epitope were considered on the basis of conserved site, protease cleavage site, motif, as well as an excellent hydrophobic binding pocket with a high half-life of dissociation. In consideration to the mentioned criteria, the required bioinformatics tools are used which are designed to predict the epitopes from different envelope and non-structural proteins of HCV virus. On an average, 1,000 epitopes from various databases and tools were extracted, from which 11 adept epitopes were withdrawn virtually with a base of binding energy using MHC I and II molecule protein interaction. The best epitope predicted during study was IMYAPTIWV peptide of NS5A protein. The T-cell predicted epitope can be further used for later chore in vaccine discovery for HCV.

Keywords: docking; epitopes; HCV; in silico study; interaction; peptide based vaccine.