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Using In Silico Methods to Search the Helicobacter Pylori Genome for Potential Vaccine Candidates

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ABSTRACT

Pathogen genome sequences that are publicly available have yielded a wealth of information that can be applied to the development of new drugs and vaccines. As a result, vaccination candidates that are proteins or peptides with the ability to trigger a mucosal immune response are of considerable interest. The insilico method of vaccine creation has emerged as a result of recent breakthroughs in bioinformatics, genetics, and proteomics. To find promising vaccine candidates, this research takes a "reverse vaccinology" tack by utilising whole-genome sequencing and recent bioinformatics developments. Using a multi-step computational screen, we evaluated in silico the entirely sequenced genome of Helicobacter pylori 26695 (NC 000915), which consists of 1576 electronically annotated ORFs. Selection criteria included cellular location, sequence similarity to known virulence factors, and size, among others. Based on these criteria, 316 open reading frames (ORFs) encoding both established and putative proteins were chosen as promising vaccine candidates.

Keywords: In-Silico, reverse vaccinology, vaccine candidates, virulence factors

INTRODUCTION

More than half of the world's population has a chronic infection with Helicobacter pylori, a gram-negative, microphilic, spiral-shaped bacteria that is a leading cause of gastritis, peptic ulcer disease, and an early risk factor for stomach cancer . Helicobacter pylori relies on well-known bacterial gene products for its colonisation and pathogenicity [1]. While medications that reduce stomach acid have a minimal risk of side effects, some people taking antibiotics may acquire a temporary Candida infection. Shorter treatments have lower cure rates, but lengthier ones haven't been found to improve them. When it comes to fighting off H. pylori, the human immune system shows remarkable variety[2]. Human and animal research demonstrate that combating H. pylori requires a significant energy investment from the immune system. However, the infection usually remains even after antibiotic therapy, and the immune response produced against the organism does not contribute to

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clearance or prevent reinfection. The biggest challenge for vaccination researchers is finding a technique to expose antigens to the host immune system that would trigger protective or therapeutic immune responses in the stomach mucosa [3].

Antigens, adjuvants, and delivery systems have all been screened using purely empirical methods because of the lack of knowledge surrounding the mechanisms by which H pylori evades immunity and the involvement of T and B cells in effector responses. The UreA and UreB urease genes from H. pylori have been expressed in a recombinant vaccine created by modifying Salmonella typhi. There were no adverse reactions to this vaccination, but there was also no protection against urease. It was also common practise to use an adjuvant with a vaccination consisting of inactivated whole-cell killants. Formalin-killed H. pylori cells were used in the experimental vaccination, along with different concentrations of the adjuvant LTR192G. Unfortunately, in healthy people, the vaccination increased IFN- levels while doing little to increase them in sick people [4]. H. pylori-specific antibody-secreting cells were generated in gastric tissues of uninfected volunteers after oral administration of this vaccination, with a particularly strong response in the duodenum. By comparing the genomes of a bacterium and its host, scientists may get insight into how the two organisms' biological processes are intertwined and perhaps identify novel therapeutic targets. The strategy rests on the premise that the target is a vital part of the pathogen's metabolic process and is necessary for its survival.

It is expected that the computational genomics technique would hasten the drug development process by avoiding the dead ends and toxicity of traditional methods. Antibiotic-resistant clinical strains are a growing problem, although screening against such novel targets for functional inhibitors could lead to the development of new therapeutic drugs active against bacteria [5]. Unfortunately, there is currently no treatment for H. pylori infection that is both effective and safe for routine use. Development of medications with a particular interaction with the pathogen is likely to result from the discovery of non-human homologs in the important genes of H. pylori and the subsequent screening of the proteome to uncover the equivalent protein product. The surface proteins and their non-human homologs would make great vaccine targets. Vaccines targeting these surface proteins might theoretically render the virus harmless. The study's goal is to identify potential vaccine candidates in Helicobacter pylori by computational analysis of the bacterium's DNA. By doing a BLAST comparison of the open reading frames (ORFs) of the bacteria and choosing the proteins that have no homology to host protein and function as virulence factors, we may identify and study these pathogens. The BLAST analysis was used to determine proteins likely to be found in the outer membrane and then the ORFs were screened using psortb and sosui servers.

PROCEDURE

To find vaccine candidates in the H. pylori genome, scientists have employed many databases and programmes available online, including: • BLAST – to find proteins that lack homology to those found in humans, which may then be used as a basis for further selection. Using PSORTB, we can determine where in the cell the proteins are found; using SOSUI, we can determine whether or not the protein is a membrane protein or a soluble protein.

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Methodology

To find promising vaccine candidates, researchers used an in silico selection process based on a logical approach. One, proteins that have a high degree of sequence similarity with previously identified virulence factors may play an important role in the pathogenesis of H.pylori and might be used as targets for a vaccine to prevent the spread of the bacteria. Antigens are proteins that may be recognised by the immune system and neutralised by it [6].

The 1576 electronically annotated open reading frames (ORFs) in the fully sequenced genome of Helicobacter pylori 26695 (NC 000915) were screened using specific selection/filtering criteria to identify a small collection of proteins that might serve as prospective vaccine candidates.

Helicobacter pylori 26695 (NC 000915) open reading frames (1576 of them) were retrieved from the TIGR CMR database (Comprehensive Microbial Resource) (www.tigr.org). Proteins with established or proposed function (group 1), hypothetical proteins (group 2), and conserved hypothetical proteins (group 3) were the first classifications applied to the 1576 ORFs (group 3).

Similarity checks were performed on all of the downloaded sequences at once. Blast analysis against the NCBI's non-redundant database was used to look for proteins in both groups 2 and 3. Proteins were chosen for their lack of homology to proteins found in humans, as well as their classification as transmembrane proteins, membrane proteins, adhesions/flagellar proteins, secretory proteins, lipoproteins, regulatory proteins, multidrug resistance proteins, hypothetical proteins, or proteins with more than one function. Psortb, an online localization prediction tool, is used to check the chosen proteins for their sub-cellular localization. Proteins from the outer membrane, which are found outside of the cell, were chosen. The sosui membrane protein prediction service was used to check for the existence of transmembrane helix in proteins whose sub- cellular localization was predicted as unknown. Proteins with longer than 100 amino acid sequences were prioritised by the psortb server and the sosui server, and these proteins were shown to be promising candidates for use in vaccines[7].

RESULTS

Choosing people via a process of emulation

After an initial sequence similarity screening on groups 2 and 3, the number of hypothetical and conserved hypothetical proteins was decreased to 166 and 48, respectively; these proteins are categorised into 11 groups, as shown in tables 1 and 2 for hypothetical and conserved hypothetical proteins.

Selection based on localization

The proteins in group 1 are selected based on their site of location in which the membrane proteins and extracellular proteins are selected[8].

DISCUSSION

Based on the selection criteria, the in silico method has yielded a total of 385 proteins. The chosen proteins are completely unlike to those found in the host bacterium. Targeting is simplified since only proteins found in the cell's membrane, outside the cell, or released from the cell were

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considered.

Choosing amongst candidates by comparing their sequences
Homologous proteins were selected for the blast analysis as membrane proteins, lipoproteins, transmembrane proteins, adhesions/flagellar proteins, secreted proteins, regulatory proteins, outer membrane proteins, integral membrane proteins, multi drug resistance proteins, proteins with multiple functions, and strictly hypothetical proteins, even though they share no homology with the host proteins[9].

Membrane proteins, including those found in the outer and inner membranes. Proteins like these are found on the cell membrane of the pathogen. Protein vaccines work by directing the host immune system to attack the pathogen's membrane and phagocytes [10].
Adherens/flagellar proteins

Since adhesions are surface proteins that help in the attachment of the pathogen, adhesions/flagellar proteins are an immunologically important group. Mucosal adhesions are powerful antigens that stimulate the production of secretory IgA antibodies.

Secreted Proteins
Since blocking protein secretion prevents the transport of virulence components out of the pathogen, secretory proteins are an attractive target in vaccine development. E. Lipoproteins The outer membrane is mostly composed of lipoproteins. "F" for "selection based on localization of the proteins" A greater proportion of membrane proteins than soluble proteins are subject to the host immune response [11]. Therefore, membrane proteins are chosen as potential vaccine candidates by using psortb and sosui analysis.

CONCLUSION

As a first step in the process of discovering vaccine targets, the in silico method helps to restrict the field of potential vaccine candidates. The usefulness of the outcome depends heavily on the selection criteria used and the level of strictness permitted in the selection process. Whole sequencing data for many diseases is now publicly available, which has sparked new lines of inquiry into vaccine development. However, information from genomes alone is not sufficient to reliably foretell whether or not a protein would be immunogenic in vivo or make a good vaccine candidate. This means that in addition to suitable animal models, genomic, proteomic, genetic, biochemical, and bioinformatic techniques should be used to evaluate vaccine candidates chosen using an in silco strategy. The final pool of proteins might be narrowed down from 316 to less than 200 using techniques like motif analysis and other forms of specificity filtering.

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