

Research Overview

Omics-Based Systems Vaccinology for Vaccine Target Identification

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ABSTRACT Omics technologies include genomics, transcriptomics, proteomics, metabolomics, and immunomics. These technologies have been used in vaccine research, which can be summarized using the term “vaccinomics.” These omics technologies combined with advanced bioinformatics analysis form the core of “systems vaccinology.” Omics technologies provide powerful methods in vaccine target identification. The genomics-based reverse vaccinology starts with predicting vaccine protein candidates through in silico bioinformatics analysis of genome sequences. The VIOLIN Vaxign vaccine design program (<http://www.violinet.org/vaxign>) is the first web-based vaccine target prediction software based on the reverse vaccinology strategy. Systematic transcriptomics and proteomics analyses facilitate rational vaccine target identification by detecting genome-wide gene expression profiles. Immunomics is the study of the set of antigens recognized by host immune systems and has also been used for efficient vaccine target prediction. With the large amount of omics data available, it is necessary to integrate various vaccine data using ontologies, including the Gene Ontology (GO) and Vaccine Ontology (VO), for more efficient vaccine target prediction and assessment. All these omics technologies combined with advanced bioinformatics analysis methods for a systems biology-based vaccine target prediction strategy. This article reviews the various omics technologies and how they can be used in vaccine target identification. Drug Dev Res 73 : 559–568, 2012. © 2012 Wiley Periodicals, Inc.

Key words: omics; vaccine; microarray; genomics; transcriptomics; proteomics

INTRODUCTION

Vaccination is one of the most effective tools to prevent against infectious diseases, cancer, allergy, and autoimmune diseases. Infectious diseases are a major source of mortality, contributing to 26% of global

human mortality in 2001 [Becker et al., 2006]. Cancer, allergy, and autoimmune diseases also cause significant mortality and morbidity in human and animal victims. Vaccine immunization induces strong host immune responses to the administered antigen and provides a

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long-term protection against a disease. However, an effective and safe vaccine for many deadly diseases, including acquired immunodeficiency syndrome, tuberculosis, and malaria, still does not exist. New approaches toward efficient vaccine target identification and vaccine development are desired.

A new era of vaccine research began in 1995 when the complete genome of *Haemophilus influenzae* (a pathogenic bacterium) was published [Fleischmann et al., 1995]. Since then, thousands of pathogen genomes have been sequenced. Various host (such as human and mouse) genomes are also available. With the availability of large amounts of genome sequences, high-throughput-omics technologies—genomics, transcriptomics, proteomics, metabolomics, immunomics, and other omics approaches—have been invented. Advance bioinformatics approaches have also been developed to support the analysis of large amounts of omics data at differing levels, ranging from gene annotation, data normalization, significant gene expression detection, function enrichment, to pathway analysis. These omic and bioinformatic technologies enable the testing and screening of millions of possible vaccine candidates and vaccine-induced host immune targets in real time.

This article reviews how omics technologies combined with advanced bioinformatics data analyses have been used in vaccine target identifications.

GENOMICS-BASED REVERSE VACCINOLOGY FOR VACCINE TARGET IDENTIFICATION

Reverse vaccinology is an emerging and revolutionary vaccine development approach that starts with the prediction of vaccine targets by bioinformatics analysis of genome sequences. Predicted proteins are selected based on desirable attributes. Reverse vaccinology was first applied to the development of a vaccine against serogroup B *Neisseria meningitidis* (MenB), the major cause of sepsis and meningitis in children and young adults [Pizza et al., 2000]. The complete MenB genome was screened using bioinformatics algorithms for open reading frames coding for putative surface-exposed or secreted proteins, which are susceptible to antibody recognition and therefore the most suitable vaccine candidates. Out of approximately 600 novel vaccine candidates, 350 were expressed in *Escherichia coli*, and 28 were found to elicit protective antibody response. It took less than 18 months to identify more vaccine candidates in MenB than had been discovered over the previous 40 years by conventional methods [Pizza et al., 2000]. Derived from the first reverse vaccinology attempt, Bexsero, a multicomponent, broad-coverage MenB vaccine, was developed. Following the

generation of comprehensive clinical and epidemiological data on Bexsero, Novartis submitted a Marketing Authorization Application in late 2010 to the European Medicines Agency for the use of Bexsero in humans [Althoff and Gassenbach, 2010]. This milestone occurred approximately 10 years after the first reverse vaccinology publication, representing a huge success in vaccine research and development (R&D).

Besides identifying secreted or outer membrane proteins, many more reverse vaccinology criteria have been developed since its first application report in 2000 described above. For example, when an outer membrane protein contains more than one transmembrane helix, the recombinant protein is often difficult to clone and purify [Pizza et al., 2000]. Therefore, the number of transmembrane domains of a protein can be used as a filtering criterion. Another criterion is the selection of bacterial adhesins that are responsible for adherence, colonization, and invasion of microbes to host cells [Sachdeva et al., 2005]. With the availability of multiple genomes sequenced for pathogens, it is also possible to run comparative genomics analyses to identify vaccine targets shared by many pathogenic organisms. These conserved proteins can be used to induce protection against multiple pathogenic strains. It is also important to compare sequence similarities between vaccine protein candidates and host proteome. A pathogen protein homologous to a host protein may induce an autoimmune disease or immune tolerance. The traditional immunoinformatics approaches for prediction immune epitopes can also be used for screening protective antigens [He et al., 2010b].

Vaxign (<http://www.violinet.org/vaxign>) is the first web-based vaccine design program utilizing the reverse vaccinology strategy [Xiang and He, 2009a; He et al., 2010b]. Predicted features in the Vaxign pipeline include protein subcellular location, transmembrane helices, adhesin probability, conservation among pathogen genomes, conservation to human and/or mouse proteins, sequence exclusion from genome(s) of non-pathogenic strain(s), and epitope binding to major histocompatibility complex (MHC) class I and class II. Vaxign has been demonstrated to successfully predict vaccine targets for *Brucella* spp. [Xiang and He, 2009a; He and Xiang, 2010], uropathogenic *E. coli* [He et al., 2010b], and human herpesvirus 1 virus [He and Xiang, 2011]. Currently, more than 200 genomes have been precomputed using the Vaxign pipeline and available for query in the Vaxign website. Vaxign also performs dynamic vaccine target prediction based on input sequences.

The concept of reverse vaccinology has been successfully applied to many other pathogens (Table 1).

TABLE 1. Omics Studies for Vaccine Target Identification

#	Pathogen	Genomics RV			DNA microarray		Proteomics		Immunomics	
Gram-negative bacteria										
1	<i>Bordetella pertussis</i> (pertussis)	[Bart et al., 2010]								[Vidakowics et al., 2007; Alindis et al., 2009; Zhu et al., 2010]
2	<i>Brucella</i> spp. (brucellosis)	[Crasia et al., 2008; Xiang and He, 2009; He and Xiang, 2010]			[Viadas et al., 2009]					
3	<i>Burkholderia</i> spp. (glanders, melioidosis)	[Chong et al., 2006]								[Felgner et al., 2009; Mariappan et al., 2010]
4	<i>Chlamydia pneumoniae</i> (pneumonia)	[Montigiani et al., 2002]								[Vandahl et al., 2001; Montigiani et al., 2002]
5	<i>Chlamydomphila</i> spp. (cervicitis)	[Heinz et al., 2009]								[Sharma et al., 2006; Molina et al., 2010; Cruz-Fisher et al., 2011]
6	<i>Clostridium difficile</i>	[Stabler et al., 2009]			[Scaria et al., 2011]					[Wright et al., 2005, 2008]
7	Enterotoxigenic <i>Escherichia coli</i>	[Welch et al., 2002; Durant et al., 2007]			[Snyder et al., 2004]					[Sommer et al., 2010]
8	<i>Helicobacter pylori</i> (ulcer)	[Chakravarti et al., 2000; Dutta et al., 2006]								[Alteri and Mobley, 2007; Walters and Mobley, 2009]
9	<i>Legionella pneumophila</i> (pneumonia)	[D'Auria et al., 2010]								[Chakravarti et al., 2000; Kornilovska et al., 2002; Meinke et al., 2009]
10	<i>Neisseria meningitidis</i> serogroup B	[Pizza et al., 2000; Giuliani et al., 2006]			[Grifantini et al., 2002; Dietrich et al., 2003]					[Gaika et al., 2008; Khemiri et al., 2008]
11	<i>Porphyromonas gingivalis</i>	[Ross et al., 2001]								[Bernardini et al., 2007]
12	<i>Pseudomonas aeruginosa</i>									[Montor et al., 2009]
13	<i>Salmonella</i> spp. (typhoid fever)									[Charles et al., 2009]
14	<i>Shigella dysenteriae</i> (dysentery)									[Pieper et al., 2009]
15	<i>Shigella flexneri</i> (dysentery)									[Peng et al., 2004; Ying et al., 2005a,b]
16	<i>Vibrio cholerae</i>				[Larocque et al., 2005; Osorio et al., 2005]					[Hang et al., 2003; LaRocque et al., 2008]
17	<i>Yersinia pestis</i>	[Yen et al., 2008]								[Jabbour et al., 2010]
Gram-positive bacteria										
1	<i>Bacillus anthracis</i>	[Ariel et al., 2002; Read et al., 2003]			[Bergman et al., 2007]					[Chitlaru et al., 2004]
2	<i>Listeria monocytogenes</i> (listeriosis)	[Reis et al., 2010]								[Trost et al., 2005]
3	<i>Mycobacterium tuberculosis</i>	[Cockle et al., 2002]			[Betts, 2002; Stewart et al., 2002]					[Betts, 2002; Sartain et al., 2006; Giri et al., 2010; Li et al., 2010]
4	<i>Neisseria meningitidis</i> (serogroup B)	[Pizza et al., 2000; Tettelin et al., 2000; Giuliani et al., 2006]			[Grifantini et al., 2002]					[Steller et al., 2005; Bernardini et al., 2007]
5	<i>Staphylococcus aureus</i> (bacteremia)	[Stranger-Jones et al., 2006; McCarthy and Lindsay, 2010]								[Eitz et al., 2002; Yvyvyska et al., 2002]
6	<i>Staphylococcus epidermidis</i> (endocarditis)	[Beres et al., 2004]			[Yao et al., 2005]					[Yang et al., 2006]
7	<i>Streptococcus pyogenes</i> (GAS) (pneumonia)	[Tettelin et al., 2002; Maione et al., 2005]								[Rodriguez-Ortega et al., 2006; Bombaci et al., 2009; Fritzer et al., 2010]
8	<i>Streptococcus agalactiae</i> (GBS) (pneumonia)	[Wizemann et al., 2001; Hiller et al., 2007]			[Song et al., 2009]					[Doro et al., 2009]
9	<i>Streptococcus pneumoniae</i> (pneumonia)	[Beres et al., 2004]								[Giefing et al., 2008; Morsczech et al., 2008]
10	<i>Streptococcus pyogenes</i> (GAS)	[Liu et al., 2009]								[Rodriguez-Ortega et al., 2006]
11	<i>Streptococcus sanguinis</i> (endocarditis)									[Ge et al., 2010]
12	<i>Streptococcus suis</i> (meningitis)									[Baums and Valentin-Weigand, 2009]
Viruses										
1	Dengue virus (Dengue fever)									[Kanlaya et al., 2009]
2	HIV (AIDS)									[Munday et al., 2010]
3	Measles-mumps-rubella									[Bartlam et al., 2007; Zhang et al., 2010]
4	RSV (respiratory tract infections)									[Pastorino et al., 2009]
5	SARS virus (SARS)									[Thomas et al., 2006; Hernaez et al., 2010; Mochon et al., 2010; Sorigo et al., 2010]
6	West Nile virus (West Nile fever)									[Asif et al., 2006; Gautam et al., 2008]
Fungi										
1	<i>Candida albicans</i> (Candidiasis)									[Doolan et al., 2008; Crompton et al., 2010; Singh et al., 2010]
2	<i>Aspergillus fumigatus</i> (Aspergillosis)									
Parasites										
1	<i>Plasmodium falciparum</i> (malaria)									

AIDS, acquired immunodeficiency syndrome; GAS, group A *Streptococcus*; GBS, group B *Streptococcus*; HIV, human immunodeficiency virus; RSV, reverse vaccinology; SARS, severe acute respiratory syndrome.

Other features may also be considered for vaccine target prediction, for example, the application of possible three-dimensional (3D) structure in epitope prediction and antigen discovery [Mora et al., 2006; Serruto and Rappuoli, 2006]. It is also possible to predict vaccine targets based on other omics technologies, which will be introduced below.

TRANSCRIPTOMICS-BASED DATA ANALYSIS FOR VACCINE TARGET IDENTIFICATION

High-throughput transcriptomics analysis of gene expression using DNA microarray or RNA-seq next-generation sequencing technologies has revolutionized the way of studying genes that are involved in microbial pathogenesis. These assay systems are able to measure the expression pattern of thousands of genes in parallel, permitting the generation of large amounts of gene expression data. DNA microarrays can be hybridized with complementary DNA (cDNA) prepared from messenger RNA isolated from microorganisms grown in vitro or in vivo under different growth conditions. RNA-seq, also called “Whole Transcriptome Shotgun Sequencing,” is a technique that uses high-throughput next-generation sequencing technologies to sequence cDNA in order to get information about a sample’s RNA contents. The next-generation sequencing has deep coverage and base-level resolution and does not require the prior knowledge of the genome sequence [Pinto et al., 2011]. The genes that are differentially transcribed in response to alternation in environmental variables in wild type or gene mutant microbes can be measured. It is important to find out what genes are expressed during host infection. Those genes that are expressed during disease represent most likely protective vaccine targets.

Many examples exist. For instance, the sexual stages of malarial parasites are essential for transmission of malaria by the mosquito and can be targeted for rational development of malaria vaccines. To better understand how genes participate in the sexual development process, Young et al. utilized microarrays to profile the transcriptomes of *Plasmodium falciparum* gametocytes at sexual stages [Young et al., 2005]. A 246-gene cluster associated with sexual development was identified using an ontology-based pattern identification algorithm. Some of the genes in the cluster can be potentially used for malaria vaccine development. More examples can be found in Table 1.

One challenge in vaccine target identification is the difficulty in experimental verification. The gold standard in vaccine target validation is a vaccination-challenge experiment, which tests if a vaccine immunization is able to induce protection against challenge of

an infection with virulent pathogen in vivo. However, such experiments are often expensive and difficult to perform, especially for deadly pathogens (e.g., human immunodeficiency virus [HIV] and *Mycobacterium tuberculosis*) that do not have ideal small animal models that mimic human pathogenesis mechanisms. Therefore, it is important to identify an immune response that correlates well with protection. For many diseases, an ideal immune response that correlates with protection has not been found. Omics approaches can be used to identify host gene signatures that correlate and even predict protective immunity. For example, to identify early gene signatures induced in humans vaccinated with the attenuated yellow fever vaccine YF17D, two studies have examined total peripheral-blood mononuclear cells from human volunteers at different time points following vaccination with YF17D [Gaucher et al., 2008; Querec et al., 2009]. Microarrays were used to determine early effects (3 and 7 days postvaccination) of the vaccination on gene expression. A group of transcription factors, including IRF7, STAT2, and ETS2, functions as key regulators of the early immune response to YF17D vaccination [Gaucher et al., 2008]. A list of gene signatures (e.g., EIF2AK4 and TNFRSF17), which correlate with the magnitude of antigen-specific CD8⁺ T-cell responses and antibody titers, has also been identified and verified [Querec et al., 2009]. These gene signature profiles may serve as correlates of protection and be used in high-throughput screening of vaccine targets.

PROTEOMICS-BASED DATA ANALYSIS FOR VACCINE TARGET IDENTIFICATION

The availability of complete genome sequences allows the identification of all possible protein products. The advances in protein separation and mass spectrometry technologies make it possible to identify total protein components of a given cellular population or a subset of proteins from a particular cell compartment (e.g., outer membrane) under any specific growth condition. The combination of proteomics with serological analysis forms a new valuable approach called serological proteome analysis [Serruto and Rappuoli, 2006]. These proteomics approaches have been used in vaccine target predictions (Table 1).

Bioinformatics analysis of high-throughput gene expression results is a key to make novel discoveries. Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product. The gene expression process occurs in the transcription level as well as the protein expression level. Therefore, omics gene expression data analysis covers the transcriptiomic and proteomic data analy-

ses. The bioinformatics methods used for both transcriptomic and proteomic gene expression are similar and include the following steps: (i) data preprocessing, including data quality controls and normalization; (ii) identification of significantly regulated genes using statistics methods; (iii) clustering, classification, and pattern discovery analyses; and (iv) inference of biological pathways and networks [Liang and Kelemen, 2006; Hendrickson et al., 2008]. The detailed methods have been surveyed and described in previous report [He et al., 2010a].

IMMUNOMICS-BASED DATA ANALYSIS FOR VACCINE TARGET IDENTIFICATION

Immunomics is the study of the set of antigens, especially T- and B-cell epitopes, which are recognized by host immune systems including human or animal hosts.

As comprehensively reviewed in a previous article [He et al., 2010a], many immunoinformatics algorithms have been invented to predict T- and B-cell immune epitopes. T-cell epitopes are bound in a linear form to MHC class I or class II molecules. T-cell epitopes can be predicted with high accuracy [He et al., 2010a]. B-cell epitopes can be linear or nonlinear (or called conformational). It remains a huge challenge to computationally predict B-cell immune epitopes. Currently, the best accuracy of predicting linear B-cell epitopes is approximately 60–70%. Over 90% of B-cell epitopes are nonlinear and require the knowledge of native 3D protein structure. There has not been proper approach achieving high performance in nonlinear B-cell epitope prediction.

Many examples of proteomics-based vaccine target identification studies are summarized in Table 1. It is noted that computational epitope prediction-based immunomics methods have often been integrated with other omics technologies. For example, using DNA microarray data, Sturniolo et al., [1999] developed a matrix-based computational algorithm to successfully predict a list of immunogenic epitope peptides uniquely associated with colon cancer. These peptides are likely vaccine targets for development of a colon cancer vaccine.

ONTOLOGY-BASED INTEGRATED OMICS DATA ANALYSIS FOR VACCINE TARGET IDENTIFICATION

Different omics technologies, in combination with advanced bioinformatics analyses, can be integrated to more effectively support vaccine target identification. Without effective integration of various omics data, it is

difficult to provide comprehensive prediction of vaccine targets. The data integration problem can be addressed by the use of a biomedical formal ontology, i.e., a consensus-based controlled vocabulary of terms and relations, with associated definitions that are logically formulated in such a way as to promote automated reasoning. The Gene Ontology (GO) database (<http://www.geneontology.org>) has been widely used [Ashburner et al., 2000] in three categories: biological process, molecular function, and cellular component. GO has been widely used in various data integration and omics data analysis studies. As of July, 2012, over 4000 papers in PubMed cited the GO.

The community-based Vaccine Ontology (VO; <http://www.violinet.org/vaccineontology>) was developed to support vaccine data standardization, integration, and computer-assisted reasoning. VO contains more than 3000 terms, including more than 2000 vaccines that are licensed, in clinical trial, or proven effective in animals. These vaccines are targeted for over 20 animal species (e.g., human, cattle, and fish) against over 100 pathogens. Each vaccine is classified in VO using a logically defined, structured ontological hierarchy. As a vaccine knowledge base, VO also stores terms related to other vaccine-associated information, including different vaccine components (e.g., protective antigens and vaccine adjuvants) and vaccine-induced immune responses. Semantic relations between these terms are also included in VO to represent existing knowledge. These representations, written in the Web Ontology Language, can be parseable and readable by computer programs. VO has been used to model the meta-analysis of vaccine protection investigation [Brinkman et al., 2010; Todd et al., 2012]. VO can also be used to improve the indexing and literature mining of vaccine articles for analysis of *Brucella* gene-vaccine interaction networks [Xiang and He, 2009b; Hur et al., 2010] and discovery of IFN- γ and vaccine-associated gene networks [Ozgun et al., 2010]. The VO-based literature mining of all PubMed literature provides a novel method to predict vaccine targets [Hur et al., 2011].

DISCUSSION

Omics technologies combined with bioinformatics data analysis form the core parts of the “systems vaccinology,” a term derived from “systems biology” that is applied to the field of vaccinology. Systems vaccinology studies scientific questions in the field of vaccine and vaccination in a systems biology way. These omics and bioinformatics-based systems vaccinology methods have greatly supported the prediction and identification of vaccine targets.

Besides the omics technologies introduced above, many other vaccine target identification methods have been developed. For example, analysis of manually curated vaccine target data available in existing databases provides a powerful way for vaccine target prediction. As part of the VIOLIN vaccine database and analysis system [Xiang et al., 2008], Protegen is a web-based database that contains over 600 protective antigen information [Yang et al., 2011]. These antigens are also collected in the VO. Protective antigens are targeted by host acquired immunity and able to induce protection against infectious diseases. To identify features enriched in protective protein antigens, 201 protective protein antigens from Gram-negative bacteria and 69 protective protein antigens from Gram-positive bacteria collected in Protegen were analyzed [He and Xiang, 2012]. Of the protective antigens in Gram-positive bacteria, 64% are extracellular or cell wall proteins and 48% of protective antigens in Gram-negative bacteria belong to extracellular or outer membrane proteins. Over 40% protective bacterial antigens are adhesins or adhesin-like proteins. Many conserved motifs, including Autotransporter and TonB domains, are enriched in protective bacterial antigens. A predictive method based on the support vector machine (SVM) algorithm has a performance of 92% true positive rate of sequence-based protection. However, this SVM-based method has poor performance in differentiating true negative from false negative results [He and Xiang, 2012]. Overall, this study is pioneer in identifying specific patterns in protective antigens and computationally predicting protective antigens.

Many challenges also exist in the area of omics-based data analysis for vaccine target identification. Particularly, new vaccines are still needed to fight against infections with many deadly pathogens, such as HIV, *M. tuberculosis*, and *P. falciparum*. Although huge amounts of financial support have been invested and intensive research has been conducted, there still have not been safe and effective vaccines available for tackling these problems. Novel methods and ideas derived from the area of omics-based systems biology may provide hope toward better identification of new vaccine targets to support vaccine development.

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