

Emerging and neglected tropical diseases: translational application of proteomics

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Abstract

The challenges of identifying and controlling emerging diseases impact individual health, as well as political, social and economic situations. In this review we discuss the role of proteomics for investigation of pathogen discovery, outbreak investigation, bio-defense, disease control, host–pathogen dynamics and vaccine development of emerging and neglected tropical diseases (NTDs). In the future the discipline of proteomics may help define multiple aspects of emerging and NTDs with respect to personalized medicine and public health.

Keywords: proteomics, infectious disease, emerging diseases, vaccine development, pathogen discovery, outbreaks, bioterrorism, biodefense

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Introduction

Emerging diseases are illnesses with increased incidence, resulting from newly identified pathogens, microbes that have crossed from an animal population to humans referred to as zoonoses or altered host–pathogen dynamics resulting in altered expression of a disease within a population. Conditions that influence emerging diseases include genetic mutations of the microbe, immune susceptibility of the host population, climate, inadequate infrastructure due to war or natural disaster, bioterrorism, climate change impacting disease vectors and human encroachment on wildlife habitats.^{1–6} Selected National Institute of Allergy and Infectious Disease (NIAID) Designated Emerging and Re-emerging Diseases are listed in Table 1.

Circumstances that lead to disease emergence are frequently found in the developing world and correlate with neglected tropical diseases (NTDs). The populations of all low income countries struggle with the impact of at least five concurrent NTDs.⁷ Subsequently, NTDs affect more than one billion people, primarily poor populations living in tropical and subtropical climates.^{7,8} The Global Burden of Disease (GBD) study determined trypanosomiasis, Chagas disease, schistosomiasis, leishmaniasis, lymphatic filariasis, onchocerciasis, intestinal nematode infections, Japanese encephalitis, dengue and leprosy accounted for an estimated 177,000 deaths worldwide in 2002, approximately 20 million disability adjusted life years (DALYs) and 1.3% of the GBD.⁹ Elevated rates of NTDs and their

subsequent economic impact often correlate to areas of political instability.¹⁰ Bioterrorism threats could potentially originate from these areas. Additionally, increased international travel directly impacts disease demonstrated in a predictive model of H1N1 spread.¹¹

Proteomics is the study of protein identity, sequence, post-translational modifications (PTMs), copy number, and interactions within a specific cell, tissue, organism or pathogen. Proteomics studies are conducted with the sophisticated mass spectrometers coupled to chemical and computational tools used for protein mining, PTM identifying, profiling and interactome mapping. It offers a novel and powerful approach to pathogen discovery, outbreak delineation, host pathogen dynamics, disease control and vaccine development.

Biological samples that can be evaluated include blood products, cerebrospinal fluid, urine, mucosal secretions and histopathology samples. Varied methods of simplifying protein or peptide mixtures prior to mass spectrometry include two-dimensional (2D) isoelectric focusing sodium dodecyl sulfate polyacrylamide gel electrophoresis (IEF-SDS PAGE), chromatography based separation of proteins or peptides, and a combination of these techniques. The scientific goal of the investigation, evaluation of changes in protein quantity, sequence, PTMs, is an additional factor in the choice of proteomic method.^{12–14} Three subgroups of proteomics have been described: differential proteomics, structural proteomics and functional proteomics.¹⁵

Table 1 Selected National Institute of Allergy and Infectious Disease (NIAID) Designated Emerging and Re-emerging Diseases⁷⁸

Group I Pathogens newly recognized in the past two decades	Group II Re-emerging pathogens	Group III Biodefense
<i>Acanthamebiasis</i>	Enterovirus 71	<i>Bacillus anthracis</i>
Australian bat lyssavirus	<i>Clostridium</i> <i>difficile</i>	(anthrax) <i>Clostridium</i>
<i>Babesia atypical</i>	Mumps virus	<i>botulinum</i> toxin
<i>Bartonella henselae</i>	Streptococcus,	(botulism)
Ehrlichiosis	Group A	<i>Yersinia pestis</i>
<i>Encephalitozoon</i> <i>cuniculi</i>	<i>Staphylococcus</i> <i>aureus</i>	(plague) <i>Variola major</i>
<i>Encephalitozoon</i> <i>hellem</i>		(smallpox)
<i>Enterocytozoon</i> <i>bieneusi</i>		<i>Francisella</i> <i>tularensis</i>
<i>Helicobacter pylori</i>		(tularemia)
Hendra or equine morbilli virus		Arenaviruses
Hepatitis C		Bunyaviruses
Hepatitis E		Hantaviruses
Human herpesvirus 8		Rift Valley Fever
Human herpesvirus 6		Lassa Fever
<i>Lyme borreliosis</i>		Dengue
Parvovirus B19		Ebola
		Marburg

Differential proteomics or protein profiling investigates changes in expression of proteins including translational modification and PTMs between healthy and diseased specimens with the expressed goal of determining therapeutic targets or biomarkers.¹⁵ Structural proteomics is a method of evaluating the 3D structures of proteins for the purpose of intelligent drug design. Functional proteomics then reveals protein interactions and related pathways.¹⁵

The translational application of proteomic analysis has the potential to significantly impact pathogen discovery, outbreak investigation, response to bioterrorism threats, disease control and vaccine development.

Pathogen discovery

Through comparison of two-dimensional differential gel electrophoresis (2D-DIGE) and a one-dimensional gel electrophoresis coupled to 1D chromatography followed by tandem mass spectrometry (GeLC-MS/MS), vaccinia proteins were identified that were only present in infected cells demonstrating proof of principle of pathogen discovery via proteomics.¹⁶ Recently, proteomics was applied to identifying emerging arboviral pathogens in clinical samples. Particularly, this is beneficial with new viruses that may be difficult to identify through traditional methods. While this is a promising development, limitations of the process include a minimum requirement of 10⁵ plaque forming units. Additionally, identification relies on full trypsin digestion and subsequent release of peptides. C, E1 and E2 structural proteins were detected, though E3 and 6K were not detected.¹⁷ The investigators noted this may be secondary to the location of 6K within the lipid bilayer of the virions.¹⁷

Outbreak investigation

Proteomics have been successfully applied to localized outbreaks of Staphylococcal food poisoning with reliable results.^{18,19} Those findings compliment the significance of proteomics investigations applied to severe acute respiratory syndrome (SARS) as an example of the application of proteomics to increase scientific knowledge and improve global response to emerging pathogens with pandemic potential.²⁰ The findings of the Structural Proteomics in Europe (SPINE) project, the National Institutes of Health NIAID center for Functional and Structural Proteomics for SARS-CoV-related proteins, and additional investigators demonstrate the role proteomics can play in aiding identification and response to emerging pathogens.^{21,22}

Initial evaluation establishing proof of principle that proteomics could be employed for early diagnosis of SARS in comparison to influenza and undifferentiated febrile patients demonstrated high sensitivity and specificity.²³ Samples from SARS patients were compared with control samples from patients with other respiratory infections and healthy individuals, demonstrating both up- and down-regulated protein expression of 12 distinct proteins.²⁴ Further, increased representation of serum amyloid A protein correlated with severity of disease based on examination of serial chest radiography.²⁴ In another study, 20 proteomic features were identified with differential expression in SARS patients versus the controls. Several of these proteomic features demonstrated correlation to prognosis, as well as viral load, including an N-terminal portion of the C3c α -chain, an internal fragment of the fibrinogen α -E chain and immunoglobulin κ light chain.²⁵ The findings of a third study confirmed an association with the C3c α -chain and fibrinogen with disease severity, as well as identified further markers of platelet factor 4 and beta-thromboglobulin.²⁶

More recently, the H1N1 pandemic demonstrated the individual health, economic and public health implications of emerging diseases.²⁷ Proteomic evaluation evidence suggested polymorphisms of the hemagglutinin, neuraminidase and nine additional proteins occur during adaption to the human host.²⁸ Additionally, there is proteomic evidence of increased pathogenicity of influenza A via both antigenicity and virulence.^{29,30} These findings could allow for early detection of emerging strains with pandemic potential allowing for improved response.

Bioterrorism and bio-defense

An important application of proteomics is in the bio-defense arena. Improved diagnostics, vaccines and counter-bioterrorism efforts can be enhanced via further definition of protein targets for vaccines and drug targets.³¹ Proteomic analysis of *Bacillus anthracis* via deletion of the pX01 plasmid revealed the function of proteins integral in metabolic processes, intra- and extracellular processes, processing of genetic information, virulence, and pathogenesis.³² Differential proteomic analysis of SCHU S4, a highly virulent strain of *Francisella tularensis*, demonstrated

increased and differential protein expression in comparison to three subspecies of *F. tularensis holarctica*.³³

Disease control

Interestingly, arboviral vectors also demonstrate host-pathogen alterations in protein expression. The host-pathogen dynamics of *Aedes aegypti* independently infected with Chikungunya or Dengue 2 viruses resulted in modulation of midgut proteins associated with antioxidant response, energy production and detoxification.³⁴ Preliminary findings suggest that proteins may be transferred from males to females during mating and subsequently impacting blood meal digestion in *Ae. aegypti*.³⁵ Taken in concert, these findings may represent possible pathways to dengue vector control in the future.

Host-pathogen dynamics

Leptospirosis is a zoonotic spirochete to which humans are exposed from contaminated soil and water, frequently associated with ecotourism and military maneuvers. Leptospirosis causes a varied disease spectrum in humans, from constitutional symptoms to Weil's disease with severe renal and hepatic failure which carries significant mortality.³⁶ Challenges to vaccine development include the significant genetic variability in the pathogenic serovars. Proteomic investigations employing both 2D-IEF SDS-PAGE and mass spectrometry have identified outer membrane proteins, flagellar, chemotactic and OMP like protein Loa22 as known or potential virulence factors, as such representing potential vaccine candidates.³⁷⁻⁴⁰ Differential protein expression in comparison of pathogenic versus non-pathogenic leptospirosis serovar proteomes may lend further evidence toward identification of immunogenic relevance.^{41,42}

Helminthic infections represent 85% of the NTD burden for the poorest 500 million people living in sub-Saharan Africa.⁸ Infection with helminth parasites results in both acute and chronic disease states which impact personal health and ability to effectively participate in society secondary to disability such as malnutrition, massive lymphedema, and blindness.⁴³ Preliminary proteomic evaluations indicate modified pathways and protein expression in significant helminthic pathogens, *Schistosoma japonicum*, *Schistosoma mansoni* and *Brugia malayi*.^{44,45}

Hepatitis E (HEV) is a non-enveloped, single-stranded RNA virus which causes significant morbidity in tropical and subtropical regions, with subsequent impact on travelers to endemic regions.⁴⁶ Additionally, hepatitis has severe consequences in pregnant women with the most critical sequelae resulting in death.⁴⁶⁻⁴⁹ Plasma and urine samples from HEV-infected patients were analyzed via 2D-DIGE, demonstrating greater than 30 proteins differentially expressed in acute HEV patients when compared with health controls.⁵⁰ These preliminary findings are intriguing as they illustrate the potential of proteomics employed for biomarker identification in diseased versus healthy individuals. Further investigations regarding the

expression of these proteins in the gamut of disease spectrum will aid in understanding the pathogenic importance.

Immunoinformatics is the study and identification of pathogen epitopes that interact with the host immune system, the immunome.⁵¹ To date, this field remains understudied and host-pathogen interactions are not well understood in many infectious diseases. Host biometric information, genetic analysis of host and pathogen polymorphisms, and PTMs all potentially impact disease progression, as well as response to treatment or vaccination. Proteomic evaluation in these areas could further the field of personalized medicine and vaccines, potentially prompting a paradigmatic shift in the approach to infectious disease.⁵²⁻⁵⁴

Forays into viral proteomics are endeavoring to bridge the basic science information available with practical clinical applications. Significant efforts at characterizing viruses and their respective host-pathogen interactions are advancing knowledge and developing standardized methods for data interpretation.^{55,56} Poxvirus proteomics and virus-host interactions, particularly vaccinia immunomodulators, have been extensively evaluated.^{57,58}

Retroviral proteomics analyses have demonstrated clues to host-pathogen interactions. Functional and structural proteomic evaluations of HIV indicate that greater than 3000 cellular proteins are differentially expressed in CD4 + CEM T cells.⁵⁹ Additionally, Tat, Nef and Gag genes are essential for establishing and maintaining HIV infection.⁵⁹ Proteomic analysis of human T-cell lymphotropic virus-infected cells demonstrates viral oncogene Tax related complexes involving 32 proteins including the SWI/SNF family, Baf 53, 57, 155 and BRG-1.⁵⁹

Vaccine development

Development of effective vaccines has both individual and public health implications. Efforts to identify appropriate candidates can be challenged by unpredictable host response to putative proteins. Reverse vaccinology is the concept of identifying an entire pathogen proteome for the purpose of identifying proteins with immunogenic potential. Proteomics allows for the identification of differentially expressed proteins in disease states, location within the pathogen with regard to antigenic potential secondary to surface exposure and the protein's respective function. With advanced knowledge to assist with selection of vaccine candidates, scientific and financial resources could be more efficiently focused. Proteomic characterization of *Chlamydia pneumoniae*, *Haemophilus influenza*, *Neisseria meningitidis*, *Helicobacter pylori*, *B. anthracis*, *Streptococcus agalactiae*, and differential protein expression of *Mycobacterium tuberculosis* and *M. bovis* have been completed in an effort toward disease pathogenesis and antigen discovery.^{51,53,60-71}

Relationship to metagenomics

Metagenomics is the sequencing of all genetic material in an environmental sample, allowing for pathogen discovery and

further understanding of microbial communities. Various methods including shotgun Sanger sequencing and parallel pyrosequencing have been employed with success for pathogen discovery, virus identification and analysis of the pandemic H1N1 influenza A.^{72–75} Unfortunately, genomics provides information regarding which genes are present, though it cannot determine if a gene is expressed and identify the gene function. An additional limitation to metagenomics is the inability to evaluate PTMs. Proteomics provides this additional information via profiling the protein expression under specific conditions, and allowing investigation into varied disease states and environments. The concept of metagenomics has been applied to proteomics in the context of human gut and salivary microbiomes.^{76,77} Used in conjunction, metagenomics and proteomics could provide extensive information regarding the constitution, function, quorum sensing, microbial interaction and the host–pathogen complex of environmental and community microbiomes.

Conclusion

Proteomics offers exciting possibilities in translational medicine. Pathogen discovery, outbreak investigation, response to bioterrorism threats, disease control and vaccine development are facets of emerging and NTDs which would benefit from further investigation of structural, differential and functional proteomics. While limitations to the data exist, with careful interpretation and application, paradigmatic shifts in personalized medicine and public health are possible.

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