Development of Biomarkers for Improved Diagnosis of Active Tuberculosis

Sybille Hunt, Amirkhani A, Cole R, Harcourt R, Hsu M, Marlborough D, Pedersen S, Qiu J, Ranaweera S, Remediakis C, Richards E, Seroa L, Sharp-Paul I, Sloane A, Vizgoft J, Harry J, Lindner R. Proteome Systems Ltd, Sydney, NSW, Australia.

proteome systems

Introduction

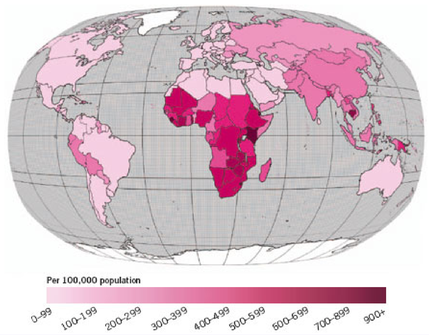
Proteome Systems is a life science company generating innovative new diagnostics using proteomics

Why Proteomics?

Protein changes that occur as a consequence of disease progression simultaneously measure the endpoint for changes in gene expression, post-translational modifications and alterations in biochemical pathways. These proteins have potential application as diagnostics markers for disease or targets for new therapeutics

Applying proteomics to global health problems:

Infectious diseases result from infection by a human pathogen and this invariably leads to pathogen proteins being released into the infected subject's body fluids such as sputum or blood. The presence of pathogen proteins in clinical samples is therefore a direct indication of infection. This makes infectious diseases a perfect target for applying proteomics to identify proteins that can be used in a diagnostic test for these diseases



Tuberculosis - a global pandemic

- One third of the world's population is infected with TB
- 2 million people die & 8 million are newly infected each year
- TB is the leading killer of people co-infected with HIV/AIDS
- Rapid re-emergence in the developed world is due to increase in travel, HIV and immigration
- Extensively drug-resistant (XDR)TB strains have emerged in recent years that are virtually untreatable & in response WHO has announced a 2 year US$2.15 B plan to tackle XDR-TB
- "XDR-TB is a threat to the security and stability of global health"

Reference: WHO June 2007

A need for improved TB diagnosis

- Current TB diagnosis relies on an insensitive 100-year-old technique of smear microscopy
- Hence millions of cases are left undiagnosed and untreated
- BCG, the only vaccine against TB offers some protection but is not very effective against adult pulmonary TB, the most contagious form of the disease
- The TB diagnostic market lacks a simple, rapid and cost-effective test to accurately identify active TB
- Rapid assays developed to date rely on the detection of a human response to TB infection and can be complicated by the presence of HIV infection

Proteome Systems' Target

To develop a cost effective antigen-based diagnostic test for active TB infection with point-of-care utility that:

- Will not be compromised by the immune status of an individual e.g. HIV co-infection
- Will detect pulmonary (sputum) and extra-pulmonary (serum) active TB
- Will have potential for monitoring treatment response

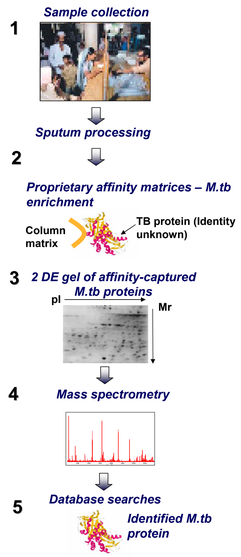
Proteome Systems' strategy for detection of TB biomarkers

- Apply a proteomic approach
- Focus on human clinical samples for biomarker discovery to ensure detection of proteins expressed *in vivo*
- Apply proprietary platforms for discovery and validation of new biomarkers of disease
- Employ robust processes to select biomarkers as diagnostic targets

This presentation describes the strategy we used in:

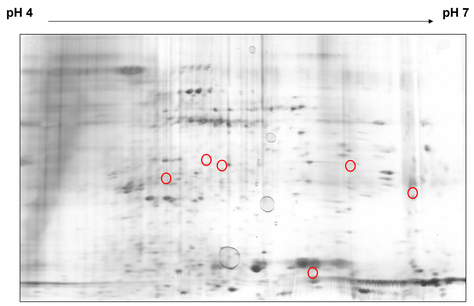
1. The discovery of *M.tb* protein biomarkers in human clinical samples and
2. The validation of the biomarkers and development of sensitive assays for screening for the biomarkers. Our ultimate target is to incorporate these markers into a diagnostic test format to facilitate improved diagnosis of this disease

Discovery of endogenous *M.tb* proteins in clinical samples



1. Subjects recruited from clinics in South Africa and China; 51 sputum and 23 plasma samples collected for proteomic discovery
2. Proprietary enrichment strategies make use of the biology of the disease to minimise sample complexity allowing for detection of low abundant *M.tb* proteins in sputum and plasma
3. Captured proteins are arrayed on 2D SDS PAGE gels and silver stained
4. >70 *M.tb* proteins were identified by Peptide Mass Fingerprinting

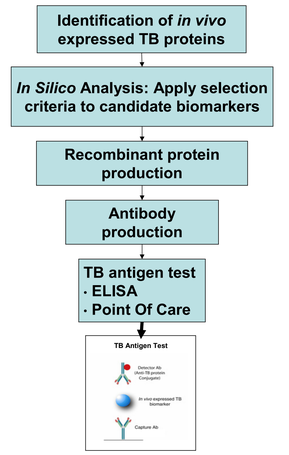
Representative 2-DE gel of enriched sputum



- 6 spots identified as *M.tb* proteins (red circles)
- 12 were pathogen proteins – this indicates that the technology can be applied to other infectious diseases

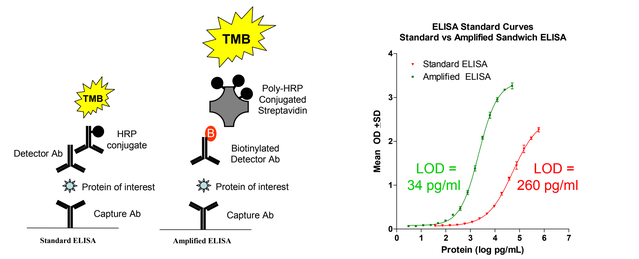
For a positive MS ID, we strictly adhered to the guidelines suggested by Carr *et al*, Mol Cell Prot 2004, 3: 531-533 and Wilkins *et al,* Proteomics, 2006, 6: 4-8

Strategy for TB diagnostic test development



1. We ranked the >70 identified *M.tb* proteins for test development based on:
   - Frequency of identification
   - Confidence of identification
2. *In silico* validation, e.g.
   - Homology to other pathogens
   - Prior art
3. Antibody production:
   - To increase our likelihood of success, we worked with multiple antibody groups to produce poly and monoclonal antibodies

Challenges for developing a diagnostic test



1. **Validation of reagents**
   - Confirmed reagent specificity in lab and clinical strains of *M.tb*
2. **Sensitivity**
   - Need assays with low limit of detection (LOD)
   - Developed amplified sandwich ELISAs (Figure above)
   - Amplification led to a 5-10 fold improvement in LOD
3. **Sample preparation**
   - Need sample preparation compatible with a Point Of Care format

Conclusion

- We have discovered >70 *M.tb* proteins from human clinical samples
- Using stringent selection criteria we have prioritised a subset of these proteins for antibody production and developed sensitive ELISAs for detection of *M.tb* proteins in clinical samples