Quantitative Targeted Proteomics of Mycobacterium Tuberculosis Disease Markers

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Outline

• Introduction: Goals of the program

• Development of SRM resource: Compendium of MS observable peptides and Mtb SRMAAtlas: Unified transition resource

• Biomarker discovery
  – Identification
  – Validation

• Conclusions
Objective One: Mtb Target Prioritization

- Published MtbSecretome
- MTB Latent and active cultures
- 2D gels
- LC-MS/MS
- QC cultures

Objective Two and Three:

- SRM assay development
- MtbAtlas
- SRM LOD/LOQ
- Spiked MTB

Objective Four

LOD/LOQ of Mtb-SRM in spiked human biofluid

Objective Five

Mtb SRM assay deployment in Human samples
Human and MTB SRMAtlas

**Human**
- 20,333 proteins (20,277 2010 version)
- 32,562 proteins incl. isoforms
- 658,684 tryptic peptides (any length)
- 480,284 tryptic peptides (7-30aa)
- 439,213 proteotypic peptides (proteotypic)

**MTB**
- 4,012 proteins (Tuberculist v2.3)
- 3,972 distinct protein sequences
- 80,371 tryptic peptides (any length)
- 54,760 distinct peptides (7-30aa)
- 52,707 peptides (7-30aa, SSR 4-60)
- 52,273 peptides (proteotypic)

But where can we get this information?
Are all proteins available for analysis?
Developing SRM Assays

1. Develop target lists
2. Synthesize peptides
3. Create fragmentation spectra using quadrupole technology
4. Optimize fragmentation
5. Select new peptides and repeat process

Query SRMAAtlas for 6 peptides per protein

SRM transitions

APLAAGTWR 471.76, 774.43 471.76, 661.34

Download data to program instrument

Deploy, verify and validate SRM assays

Highly curated “Gold Standard” SRM transitions

Qtof Qtrap
**Mtb Proteome SRM Assay Availability**

97.6% of *M. tuberculosis* (H37Rv) proteome

Protein coverage by observed peptides in MTB:
- PeptideAtlas 2011-09
- SRMAtlas 2012-10

Unmapped proteins: > Rv3367 GTDGNPG
The Mtb Proteome Library: A Resource of Assays to Quantify the Complete Proteome of Mycobacterium tuberculosis

Schubert et al.

Cell Host & Microbe, Volume 13, Issue 5, 602-612, 15 May 2013
Biomarker Discovery Stages

Stage I Qualitative profiling
- Proteotypicity/selectivity
- Observability
- Inter-peptide signal interferences

Stage II Quantitative profiling
- Detection limit
- Linearity of response
- Technical reproducibility

Stage III Biomarker discovery and validation
- Detection in sample matrix
- Peptide interferences
- Dynamic range
- Chemical stability
Overall MtB Target List Overlap

Total of 505 targets selected
Proteomic profiling of TB patient samples

Patient samples

Proteins

Tryptic digestion

Clean-up:

Solid phase extraction (SPE)

Sep-Pak tC18 column

Peptides

OGE

Clean-up: SPE

tC18 elution plate

Fractionation

Data analysis

SRM MS analysis of 24 fractions

Patient samples

Sputum

Urine

Plasma
ANALYTICAL CHALLENGES
Sample matrix signal response

a) Neat solution titration series

- 0.39 fmoles = 0.64 × LOQ
- 1.56 fmoles = 2.58 × LOQ
- 6.25 fmoles
- 25.00 fmoles
- 100.00 fmoles
- 400.00 fmoles

b) Matrix-matched titration series (SPEVLLGSAR)

- 3.13 fmoles = 0.73 × LOQ
- 6.25 fmoles = 1.45 × LOQ
- 12.50 fmoles
- 25.00 fmoles
- 50.00 fmoles
- 100.00 fmoles

(n=62)

<table>
<thead>
<tr>
<th>LOD_{median}</th>
<th>Calibration Curve (Neat Solution)</th>
<th>Calibration Curve (Urine Matrix)</th>
<th>MMu/NS ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.776</td>
<td>2.012</td>
<td>2.59</td>
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79.2% peptides demonstrated quantitative in neat solution remains quantitative in human sample matrix.
Interference removal of Mtb target protein SRM

Body Fluid Matrix (urine)

Observed

Corrected
Conclusions

- **Quantitative profiling** applied to biomarker discovery
- Developed sample processing for **sputum, plasma and urine**
- Analysed sputum samples after OGE fractionation, plasma and urine with minimal fractionation
- SRM analysis and biomarker verification in progress...
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www.srmatlas.org