The temporal proteome response to severe traumatic injury in human leukocytes revealed by large-scale quantitative clinical proteomics using an ^{18}O-labeled reference

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Overview

- Inflammatory and immune response to severe trauma injury was studied by quantitative temporal profiling of the blood leukocyte proteomes including T-cells, monocytes, and neutrophils collected at different time points post-injury.
- ^{18}O-labeled reference based quantification enables large-scale population studies by providing comprehensive isotope labeled internal standards for all samples[1].
- Quantitative analyses were performed for ~1,600 monocyte and T-cell samples longitudinally collected from ~160 severe trauma patients.
- 532 and 312 proteins were observed with significant temporal post-injury response in monocytes and T-cells, respectively.
- The dataset revealed a number of important biological functions and pathways involved in post-trauma response.

Introduction

- The host response to injury is a collection of pathophysiological processes that critically depend upon the regulation of the innate immune system.
- Blood leukocytes such as T-cells, monocytes, and neutrophils (PMN) represent unique, easily accessible samples for studying inflammatory and immune response to injury.
- High-throughput quantitative clinical proteomics provides significant potential for gaining new insights into the host response to injury and for discovering novel protein targets for therapeutic interventions or for predicting clinical outcomes.
- ^{18}O-labeled reference based quantification provides robust reproducibility and an ideal solution for large-scale human population proteomics.

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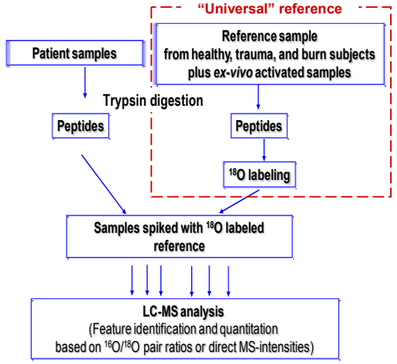
Methods

- **Leukocyte samples.** T-cells, and monocytes were isolated from whole blood using 8-mL CPT™ cell preparation tubes for proteome studies from each subject. Samples at 4-7 different time points following the injury were collected from each subject.
- **Sample preparation.** Cell pellet samples were processed using a trifluoroethanol (TFE)-based digestion protocol within a single tube without the need for clean-up.
- **^{18}O-labeled reference.** An ^{18}O-labeled reference peptide sample was generated by trypsin-catalyzed ^{16}O/^{18}O exchange reaction[2]. The labeled reference is prepared in sufficient quantity to cover >1500 samples for each cell type. The reference is then spiked into each clinical sample to enable ^{16}O/^{18}O based quantification.
- **LC-MS(/MS) analysis.** All samples analyzed on an LTQ-Orbitrap instrument equipped with automated 4-column LC system.
- **Data Analysis.** LC-MS data were analyzed using in-house software tools Decon2LS and VIPER, and peptides were identified using the accurate mass and time tag strategy. Quantitative data are analyzed with a downstream tool called DAnTE for normalization, data visualization, and statistical analyses[3].

Analytical approach:



Results

Reproducibility evaluation

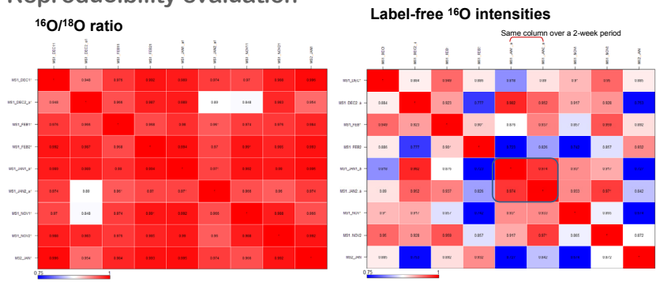


Figure 1. Pearson correlation plots for a sample analyzed over four months on different instruments.

Temporal proteome response to trauma

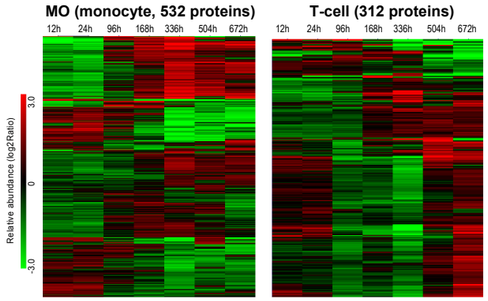


Figure 2. Heatmaps of proteins that show significant temporal changes following injury. Note that temporal responses from all 160 subjects were averaged. Proteins with significant changes were identified by ANOVA test with false discovery rate of <5% based on Q-value.

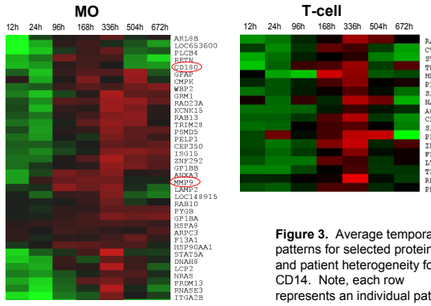
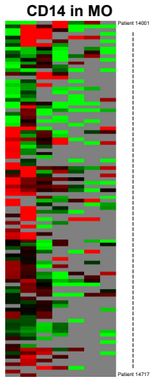


Figure 3. Average temporal patterns for selected proteins and patient heterogeneity for CD14. Note, each row represents an individual patient.



Biological functions and pathways

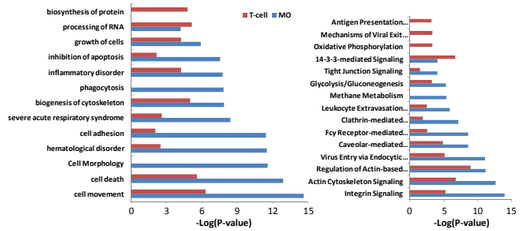


Figure 4. Top significant biological functions and pathways enriched in the trauma-responding proteins based on Ingenuity Pathway Analysis. Left: biological functions; right: canonical pathways.

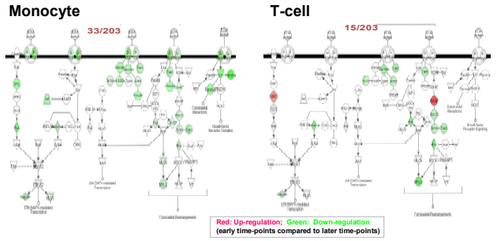
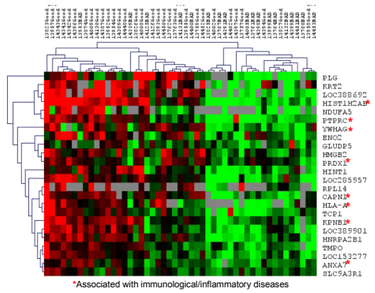


Figure 5. Proteins with significant abundance changes mapped to the integrin signaling pathway.

Patient classification

Candidate proteins predictive of multiple organ failure (MOF)

Higher Denver Score (~70%)



- **63 trauma subjects**
- **40 GOOD with Denver MOF score 0 or 1**
- **23 BAD with Denver MOF score >3**

Figure 6. Preliminary patient clustering based on 24 T-cell proteins (day 4).

*Associated with immunological/inflammatory diseases

Conclusions

- An overall picture of temporal proteome response to severe trauma was revealed for the T-cells and monocytes by identifying 532 monocyte proteins and 312 T-cell proteins with significant dynamic changes.
- Detailed protein abundance changes associated with different biological functions and canonical pathways were revealed. Many of these proteins may serve as novel protein targets for therapeutic interventions or for predicting clinical outcomes.
- Further preliminary analysis of T-cell proteins displaying differential abundances longitudinally revealed 24 proteins predictive of multiple organ failure based on early time point.
- ^{18}O-labeled 'universal' reference-based quantification provides robust reproducibility independent of instrument performance variations.
- The study represents a first-of-its-kind large-scale population proteomics application illustrating the potential for gaining a systems-level understanding of human disease.

Acknowledgements

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References

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