Combining Time Series and Feature Correlation Analysis Linked Two Biologically Important Pathways in CD8+ T-cell Activation

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Introduction

Metabolic processes are subject to constant dynamics. Therefore, the investigation of dynamic changes is of high importance in Metabolomics, Lipidomics, Pharma and Phenomics. Analysis of metabolic dynamics enables, for example, the detection of relevant markers for immune cells responses to pathogen attacks that would otherwise remain undiscovered. Cytotoxic (CD8+) T-cells play a central role in immune response. During activation, T-cells undergo a complex transition and major metabolic changes are triggered.

To better understand the dynamics in CD8+ T-cell activation, detailed investigation of global metabolic changes paired with metabolite identification (ID) is required. Here, we combined metabolite ID by matching internal standards with time series and feature correlation analysis using interactive visualizations to detect relevant markers with similar molecular dynamics. This allowed us to ultimately connect two biologically important pathways in T-cell activation.

Methods

- Isolation of primary murine naive CD8+ T-cells from spleens and in vitro activation with cytokines.
- Samples were taken every 12 h until 96 h.
- LC-QTOF-MS acquisition on a Bruker Impact II system (QTOF-MS) with an Agilent 1290 II UHPLC.
- Data processing, metabolite ID, time series and correlation analysis with MetaboScape 2021b
- Internal MS/MS libraries and reference retention times of standards were matched in MetaboScape.

Results and Discussion

A) Polyamine pathway time series

- MS/MS library search and comparison of retention times to single standards enabled the detection of **spermidine** and **spermine** as well as their precursor **arginine**.
- Interactive time series plots were used to investigate changes in signal intensities
- These showed that spermidine and spermine increased in peak intensity whereas their precursor arginine remained almost constant over time.

B) Correlation between spermine and SAH

- Peak intensity-based similarity search of spermine using feature correlation in MetaboScape pointed to several features with similar dynamic changes.
- One of these features had been annotated as **S-adenosyl homocysteine (SAH)**, which is related to S-Adenosyl methionine (SAM).
- SAM and its derivatives play important biological roles, for example, in RNA modification.

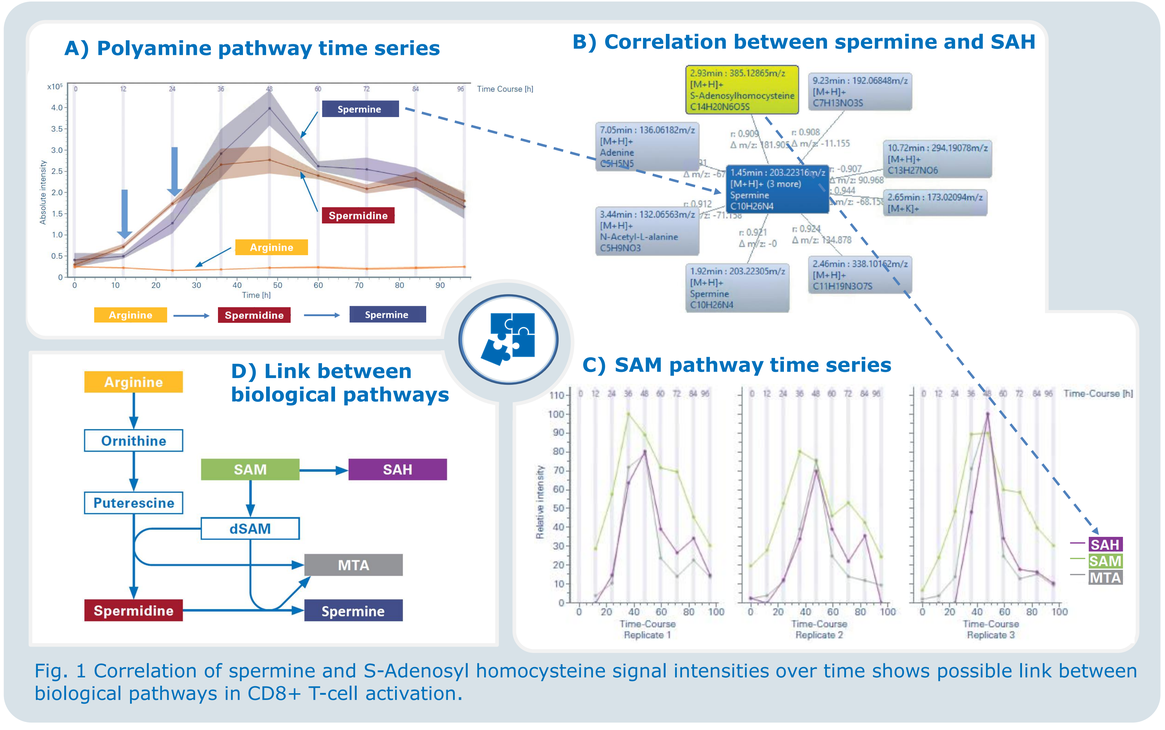

Fig. 1 Correlation of spermine and S-Adenosyl homocysteine signal intensities over time shows possible link between biological pathways in CD8+ T-cell activation.

C) SAM pathway time series

- By searching the feature table explicitly for SAH derivates, SAM and 5'-methylthioadenosine (MTA) could be annotated. These are directly involved in polyamine biosynthesis.
- Time series analysis of the 3 SAM related compounds differ strongly in signal intensities (data not shown), but relative intensities showed similar dynamic changes.
- Similar dynamic patterns of the 3 SAM derivates could be confirmed by three biological replicates separately.

D) Link between biological pathways

- With time series and correlation analysis we were able to identify initially unassigned metabolites that would otherwise have remained hidden.
- This enabled to establish a possible mechanistic link between immune cell function and RNA modification in CD8+ T-cell activation.

Summary

Combination of time series and feature correlation analysis linked polyamine pathway and S-adenosyl-methionine pathway in CD8+ T-cell activation.

References

1. Edwards-Hicks, J., Mitterer, M., Pearce, E. L. & Buescher, J. M. Metabolic dynamics of in vitro CD8+ T cell activation. Metabolites 11, 1–17 (2021).

Data availability

Download raw data here

Conclusions

- Three members of the **polyamine pathway** could be identified with internal standards
- The precursor arginine remained rather constant, whereas its intermediate spermidine and its product spermine increased in peak intensity over time
- Feature correlation of spermine analysis pointed to metabolites of **S-adenosyl-methionine metabolism**
- In this way, **time series** and **feature correlation** analysis enabled to establish a possible mechanistic link between **immune cell function** and **RNA modification**

Metabolomics