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Topic: Modulation of Immune Cell Migration and Inflammatory Protein Profiles Using Tandem Mass Tags (TMT) Labeling

You are part of a research team in an inflammation biology lab aiming to decipher the protein network modifications that occur during immune cell migration under inflammatory conditions. A recent advanced proteomic protocol involving multiple-dimension liquid chromatography tandem mass spectrometry with extended multiplexing capability using Tandem Mass Tags (TMT) labeling offers an enhanced approach [1]. This technique can analyze up to 24 biological samples simultaneously, vastly improving the throughput and sensitivity of proteomic analyses. We were planning to focus on identifying novel proteins that contribute to the regulatory network of immune cell migration and determining how established inflammatory mediators are altered under these conditions.

Question:

How to identify new protein players involved in this process using an advanced Tandem Mass Tag (TMT) approach?

Broader Target:

By employing TMT labeling coupled with high-resolution mass spectrometry (LC-MS/MS), aim to delineate the comprehensive set of proteins that are involved in the orchestration of immune cell migration during inflammation. This study intends to uncover novel inflammatory mediators that could potentially be targets for therapeutic intervention in inflammatory diseases. Data generated will illuminate the complex biological pathways that are activated or suppressed during inflammation-driven immune cell migration, offering new insights into the mechanics of immune response regulation.

Method:

1. iTRAQ/TMT & LC-MS/MS

Procedure:

Sample preparation:

1. Cell culture and treatment (Assume we are studying the function in vitro):
    a. Culture immune cells (mice or human macrophages, neutrophils)
    b. Induce inflammation (TNF-alpha, LPS)
    c. Maintain control groups without inflammatory stimuli
2. Collect immune cells and extract proteins.
3. Protein Digestion (into peptides)
4. TMT labeling:
    a. Label proteins with TMT reagents

- e.g. 8-plex iTRAQ reagents, allowing the simultaneous analysis of up to 8 different samples. Each iTRAQ reagent consists of a reporter group, a balance group, and an amine-reactive group.

b. Labeling Reaction

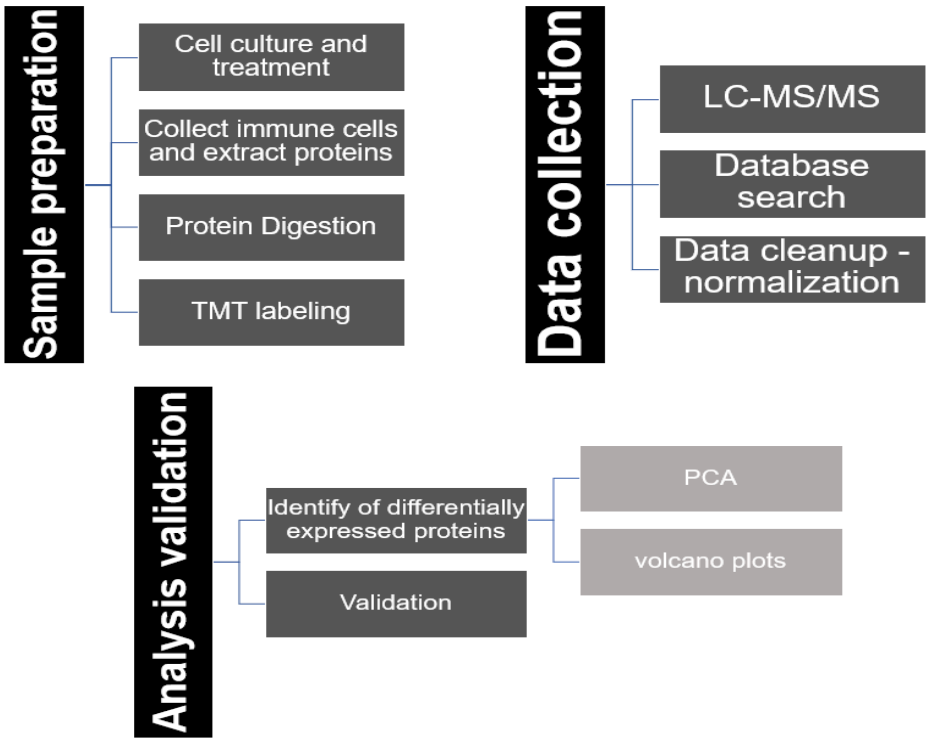
5. LC-MS/MS Analysis:
    a. Perform peptide separation using LC followed by analysis. And then optimize LC conditions to improve peptide separation and MS detection.

Data collection:

1. Database search:
    a. Use MS data to search UniProt using software like Mascot or SEQUEST. (In paper they used Proteome Discoverer[1])
2. Data cleanup: Apply normalization techniques to adjust for variations in sample loading and MS signal intensity.

Analysis validation:

1. ID of differentially expressed proteins:
    a. Use PCA to assess data quality and detect outliers;
    b. Use volcano plots and heatmap to display the results of differential expression analyses and identify proteins that show significant changes under different conditions.
2. Validation: Use western blotting to validated proteins identified as significantly differentially expressed to confirm their biological relevance.



Expected Result:

In the presented study, we use Tandem Mass Tag (TMT) labeling coupled with liquid chromatography tandem mass spectrometry (LC-MS/MS) to explore protein network alterations in immune cells during inflammatory response. We anticipate this comprehensive analysis will facilitate the identification of novel proteins integral to immune cell activation, migration, and regulatory mechanisms under inflammatory conditions.

Quantitatively, our approach is expected to deliver detailed expression levels of key inflammatory mediators and other proteins, including data on post-translational modifications which may influence protein function during inflammation.

In terms of data representation, heatmaps and volcano plots will visually articulate the clustering and significance of protein expression differences between experimental groups, providing an intuitive understanding of the impact of inflammatory stimuli on cellular proteomics. The study incorporates robust statistical methods such as principal component analysis (PCA) to ensure the validity and quality of the proteomic data, fostering confidence in the differential expression findings.

The validation of these findings will be pursued through complementary techniques such as Western blotting to confirm the biological relevance of key identified proteins. Beyond mere identification, this validation aims to elucidate potential biomarkers or therapeutic targets that could be crucial in controlling inflammatory processes. The data generated from this study will not only enrich our understanding of the proteomic adjustments in inflammation but will also be curated into a robust dataset. This dataset, inclusive of all identified proteins and their respective quantifications, will be shared via the ProteomeXchange repository.

Reference:

[1] Plubell DL, Wilmarth PA, Zhao Y, Fenton AM, Minnier J, Reddy AP, Klimek J, Yang X, David LL, Pamir N. Extended Multiplexing of Tandem Mass Tags (TMT) Labeling Reveals Age and High Fat Diet Specific Proteome Changes in Mouse Epididymal Adipose Tissue. Mol Cell Proteomics. 2017 May;16(5):873-890. doi: 10.1074/mcp.M116.065524. Epub 2017 Mar 21. PMID: 28325852; PMCID: PMC5417827.

[2] Leschevin, M., Marcelo, P., Ismael, M.,San-Clemente, H., Jamet, E, Rayon, C, & Pageau, K. (2021). A Tandem Mass Tags (TMTs) labeling approach highlights differences between the shoot proteome of two Arabidopsis thaliana ecotypes, Col-0 and Ws.Proteomics,21,e2000293. https://doi.org/10.1002/pmic.202000293.