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Definitions

- **Proteomics**: The study of all proteins in a cell, tissue, or organism.

- **Liquid Chromatography-Mass Spectrometry (LC-MS)**: A technique used to identify and measure the amount of proteins in a sample.

- **Tissue Storage Method (TSM)**: Ways to store tissue samples, such as freezing (FR) or using formalin-fixed paraffin embedding (FFPE).

- **Tissue Extraction Method (TEM)**: Techniques to prepare tissue samples for analysis, like using surfactants (MAX, TX.MAX, SDS.MAX).

- **Missing Values (MVs)**: Data points that are not recorded or are absent in the dataset.

Key Findings

- FFPE is better than frozen tissue storage for proteomics analysis.

- A one-step extraction method (MAX) is more effective than two-step methods.

- Imputing missing values is better than excluding incomplete data.

- Different data transformation and normalization methods impact the analysis results.

Introduction

This study aims to standardize the workflow for proteomics using liquid chromatography-mass spectrometry (LC-MS). Proteomics helps us understand the proteins in a sample, but there are challenges like missing data and variability in sample preparation. The researchers studied different methods of storing and preparing tissue samples to find the best approaches for accurate proteomics analysis.

Main Content

Background

Proteomics is a powerful tool for studying proteins, but variability in sample handling can affect the results. This study evaluates different methods for storing and extracting proteins from tissues to improve consistency in proteomics research.

Methods

- **Participants**: Human kidney tissues from deceased donors not suitable for transplantation.

- **Tissue Storage Methods (TSMs)**:

- o **Frozen (FR)**
- o **Formalin-Fixed Paraffin Embedded (FFPE)**
- **Tissue Extraction Methods (TEMs)**:
  - o **MAX**: A single-step method using a surfactant.
  - o **TX.MAX**: A two-step method with Triton X-100 followed by MAX.
  - o **SDS.MAX**: A two-step method with SDS followed by MAX.
- **Analysis**:
  - o **Data Transformation and Normalization**:
    - Logarithmic transformation
    - Quantile normalization
    - Variance stabilizing normalization
  - o **Statistical Methods**:
    - Excluding missing values
    - Including missing values
    - Imputing missing values

Results

- **Comparison of Methods**:
  - o FFPE storage showed lower variability compared to frozen storage.
  - o The one-step MAX extraction method had lower variability than the two-step methods.
  - o Imputation of missing values provided more reliable data than excluding them.
- **Statistical Analysis**:
  - o The imputation method reduced variability in the data.
  - o Quantile normalization and variance stabilizing normalization were more effective than simple logarithmic transformation.

Conclusion

The study concludes that FFPE is the preferred tissue storage method for proteomics. The one-step MAX extraction method is more effective than two-step methods. Imputing missing values is the best approach for handling incomplete data. These findings help improve the consistency and reliability of proteomics research using LC-MS. Researchers should consider these methods when designing experiments to ensure accurate and reproducible results.

Word Count: 433