1. INTRODUCTION

The growing field of formalin-fixed paraffin-embedded (FFPE) tissue proteomics holds promise for improving translational research. Worldwide archival tissue banks hold a significant number and variety of tissue samples, as well as a wealth of retrospective information regarding diagnosis, prognosis, and response to therapy. This makes them an important resource for protein biomarker discovery and validation. Direct tissue trypsinization (DT) and protein extraction followed by in solution digestion (ISD) or filter-aided sample preparation (FASP) are the most common workflows for shotgun LC-MS/MS analysis of FFPE samples. However, there is currently no consensus on the optimal protocol, and no studies critically comparing the performance of the three different methods with FFPE specimens have been reported so far. Liver tissue was chosen as a model in consideration of its high proteome complexity in terms of expressed proteins and metabolic pathways.

2. METHODS

**HUMAN LIVER TISSUE**

3 INDEPENDENT replicates per method

**3. RESULTS AND DISCUSSION**

DT

- lower reproducibility
- good preservation of high-MW proteins
- much lower keratin contamination
- higher abundance of non tryptic peptides

FASP and ISD

- depletion of high-MW proteins
- enrichment in hydrophobic and membrane proteins
- higher identification yields
- higher reproducibility

3.1. Reproducibility

**3.2. QUALITATIVE AND QUANTITATIVE COMPARISON**

**3.3. QUANTITATIVE PROTEIN DISTRIBUTION: SUBCELLULAR LOCALIZATION**

- Mean and SD value of NSAF percentage for three independent experimental replicates are shown. NSAF values were expressed as percentage of the annotated proteins.
- Asterisks indicate statistical significance according to Student’s t-test (p value < 0.05).
- A: statistically significant difference versus DT.
- B: versus FASP.
- C: versus ISD.
- D: versus all other methods.

**3.4. QUANTITATIVE PROTEIN DISTRIBUTION: PHYSICOCHEMICAL FEATURES**

- Quantitative protein distribution according to MW (A), pI (B), number of transmembrane domains (TMD, C) and hydrophobicity (GRAVY score, D). Mean and SD value of NSAF percentage for three independent experimental replicates are shown. NSAF values were expressed as percentage of all proteins.
- Asterisks indicate statistical significance according to Student’s t-test (p value < 0.05) as statistically significant difference versus DT, versus FASP, versus ISD and versus all other methods.

**3.5. NON-TRYPTIC AND FORMALDEHYDE-MODIFIED PEPTIDES**

**4. CONCLUSIONS**

These results highlight that diverse sample preparation strategies provide qualitatively and quantitatively different proteomic information, and present typical biases that should be taken into account when planning a shotgun proteomic investigation dealing with FFPE samples. In view of the considerable portion of unique identification provided by each method (particularly by DT and FASP), when a sufficient amount of tissue is available, a complementary, parallel use of different sample preparation strategies is suggested to increase proteome coverage, width and depth.

5. REFERENCES