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NEXT GENERATION PROTEOMICS

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Reproducible Quantification of more than 10,000 Class 1 Immunopeptides from Little Tissue Enabled by an Extensively-Optimized, High-Throughput Pipeline

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Introduction

Human leukocyte antigen-associated peptides, known as immunopeptides, play an essential role in adaptive immunity by activating and ensuring the specificity of T-cells. The identification and quantification of the immunopeptidome bear the potential to enable personalized treatments, especially in cancers, vaccines, infectious, and autoimmune diseases. Mass spectrometry is currently the only technology that can reliably measure and identify immunopeptide profiles of biological samples on a large scale. However, the usually high sample input amount and poor scalability are limiting. Here, we introduce a semi-automated workflow to robustly identify 10,000+ unique immunopeptides from low amounts of cultured cells and tissue samples by systematically optimizing each step of the sample preparation and acquisition. Notably, Data Independent Acquisition and ion mobility significantly increased sensitivity.

Methods

Cell lines were directly lysed in Biognosys' immunopeptide lysis buffer and native tissue lysis was supported by adapted focused acoustics assisted ultrasonication. Washing and elution of the magnetic-bead assisted immunoprecipitation of class 1 and subsequently class 2 immunopeptides were fully automated. Upon immunopeptide isolation and injection preparation, data was acquired with FAIMS- Data-Dependent-Acquisition (DDA) and Data-Independent-Acquisition (DIA) and subsequently processed with SpectroMine and Spectronaut (Biognosys).

For the sensitivity and reproducibility assessment, increasing amounts of cells (between 0.5 and 50 million of HeLa and JY) and healthy human lung tissue (between 5 and 135 mg) were processed and analyzed by FAIMS-DIA. The quality of the identifications was assessed by means of the peptide length distribution, binding affinity prediction, and anchor positions.

Preliminary Data

Firstly, we optimized the acquisition method. The use of FAIMS based ion mobility led to a 74%-sensitivity increase and DIA to a further 80%-sensitivity boost, compared to DDA. We found the

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acquisition method optimization to lead to the most significant gains in sensitivity, likely due to the immunopeptides' physicochemical properties.

Secondly, we optimized the native lysis and immunoprecipitation workflow while ensuring scalability and reproducibility. Under non-denaturing conditions, short lysis times and mild conditions efficiently lead to more identifications than aggressive approaches. Leveraging the magnetic properties of the beads, 1,000 samples can be processed within a week by a single operator. Technical replicates are highly reproducible (Szymkiewicz–Simpson coefficient >=80%) and identifications of good quality. For class 1 immunopeptides, >60% of the peptides identified are 9-mers, >80% predicted strong binders, and the expected amino acids are enriched at the anchor positions. For class 2, >50% of the peptides identified are 14-to-16-mers, and >50% are predicted strong binders. Overall, the pipeline is scalable, highly reproducible, and results in high-quality identifications.

Thirdly, we quantified the sensitivity of the pipeline by processing decreasing amounts of starting material (using both fresh frozen tissue and cell lines). The more starting material we processed, the more immunopeptides we identified, but plateauing around 45 mg. For example, processing as little as 3x45 mg healthy lung tissue led to the identification of 11,147 unique immunopeptides originating from 4,642 proteins. When processing 10 mg, we could identify 50% of the immunopeptides, showing limited sample input compatibility.

Overall, we established a scalable, efficient pipeline for cell line and tissues immunopeptidomics for class I and II that generates high-quality identifications and that only requires small amounts of input material and is ready to shed light into immunopeptidomics heterogeneity through large-scale profiling of patients. We are applying this pipeline to a cancer cohort.

Novelty aspect

We present a semi-automated workflow requiring a low sample input to identify high quality immunopeptides suited to profile biological conditions.