BIOGNOSYS
NEXT GENERATION PROTEOMICS

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Optimizing Immunopeptide Analysis Sample Preparation in Needle Biopsy Size Tissue Samples with AFA® Technology and Spectronaut

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Introduction

Immunopeptides play an essential role in adaptive immunity by activating and ensuring T-cell specificity. Mass spectrometry is currently the only technology that can reliably measure and identify the immunopeptide profiles of biological samples at a large scale. However, studies are frequently limited by sample input and poor scalability. Here, we introduce a semi-automated workflow requiring low sample input to robustly identify immunopeptides from cultured cells and tissue samples and apply it to a cohort of colorectal cancer samples for immunopeptide profiling and neoantigen identification.

Methods

Native lysis with AFA-based ultrasonication and the immunoprecipitation workflow were optimized while ensuring scalability and reproducibility. 15 mg of fresh frozen tissue was processed for sequential class-I/class-II immunopeptide enrichment. FAIMS Data-Independent-Acquisition was performed and supported by a high-pH-reversed-phase FAIMS Data-Dependent-Acquisition library. Data analysis was performed with SpectroMine and Spectronaut (Biognosys) with 1% FDR at the PSM, peptide and protein group level. Whole genome sequencing on both tumor and associated normal tissue was used for high-confidence somatic variation calling (Indivumed) and neoantigen definition.

Results

With the optimized workflow, we can now identify over 2,800 class-I immunopeptides with 2.5 mg healthy lung tissue and >11,000 class-I and >10,000 class-II immunopeptides with 15 mg fresh frozen tissue. Overall, we have established a scalable, efficient pipeline for cell line and tissue immunopeptidomics for both class-I and II immunopeptides.

Conclusions

The workflow described here will demonstrate generation of high-quality identifications from minimal starting material and can be deployed to help shed light on immunopeptidomics heterogeneity through large-scale profiling of patients as it will be exemplified in the case of neoantigen identification in MSI-high colorectal cancer.

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