MS-based Immunopeptidomics Analysis with PEAKS Studio X

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Abstract

Workflows for immunopeptidomics are different from ones for more established shotgun proteomics. There are inherent challenges with the abundance and natural complexity when doing mass spectrometry analysis for the exploration of human immunopeptidome (e.g. HLA class-I and class-II peptides). A new approach harnessed by PEAKS Studio X was integrated with PEAKS' *de novo* sequencing and database searching workflow for HLA peptide identifications, which increased peptide sequencing coverage and confidence. In addition, by introducing a feature-based identification method, PEAKS Studio X can also resolve the identification challenge of co-eluting peptides. Comparing with PEAKS Studio 8.5 and MaxQuant, PEAKS Studio X results show an increase of 13% and 73% more peptides identified, respectively.

Introduction

Identification of tumor antigens is needed for development of effective cancer immunotherapy. Ideal sources for such antigens are the pools of peptides presented by human leukocyte antigen (HLA) molecules on the tumor cells exclusively. Some of these tumor-specific antigens can induce strong and prolonged anti-cancer immune reaction to eradicate the tumors and thus serve as good candidates for cancer immunotherapeutics. Mass spectrometry (MS)-based methodology has been demonstrated to have great potential to identify such peptides directly.

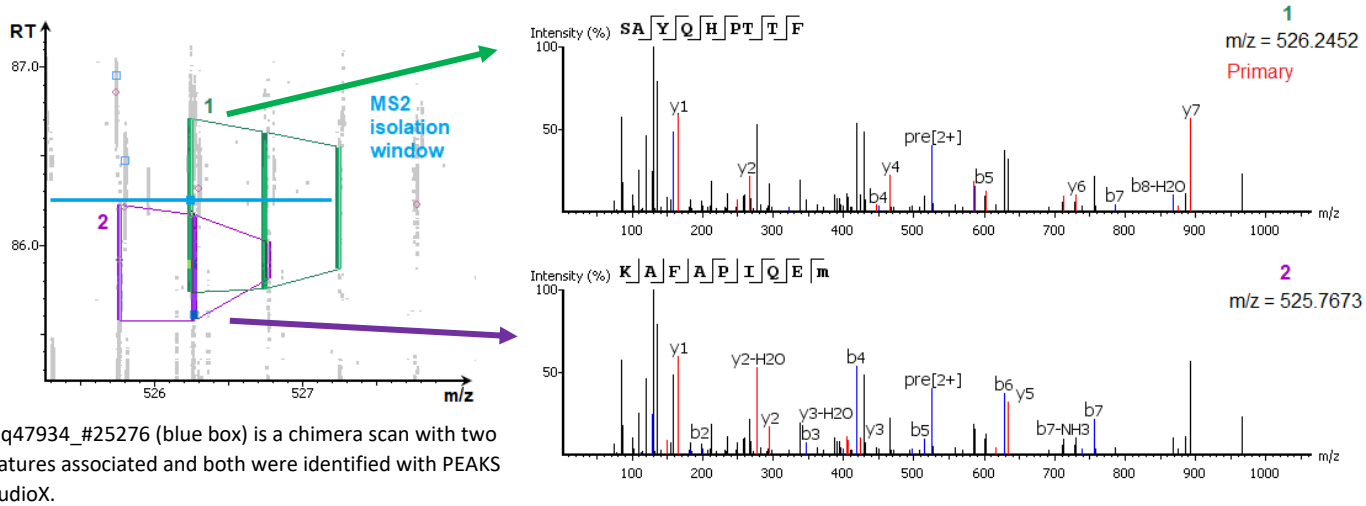
Methods

HLA peptidomes of glioblastoma multiforme (GBM), the most aggressive brain tumor with poor prognosis, was analyzed by high resolution LC-MS/MS previously [1]. Briefly, HLA-presenting peptides were extracted from GBM patient peripheral blood by using the immunoaffinity purification method. Then, bound peptides were eluted, purified and analyzed using a Q-Exactive-Plus mass spectrometer in a data-dependent manner. The dataset was re-analyzed with PEAKS Studio 8.5 and PEAKS Studio X and the results were compared. A MS2 isolation window of ± 0.9 Da was used. Thermo .RAW files were analyzed with PEAKS Studio 8.5 and PEAKS Studio X. Associating feature with chimera scan was enabled on PEAKS X. 1% FDR was applied at peptide-spectrum match (PSM) level.

Analysis parameters	Settings
Precursor mass tolerance	10 ppm
Fragment mass tolerance	0.03 Da
Enzyme	None
Variable PTMs	Oxidation (M), acetylation (N-terminal)
Database	Uniprot Human Complete Proteome database (v201808)
Peptide length filter	Between 8~14 AAs

Results

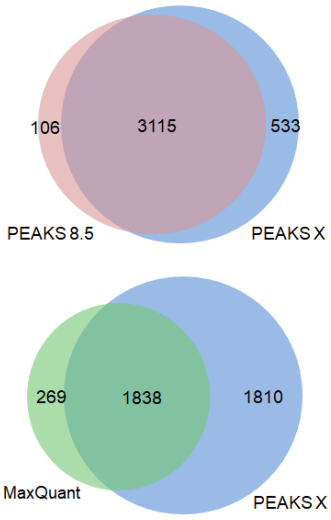
➢ MS1 feature-based peptide identification



Seq47934_#25276 (blue box) is a chimera scan with two features associated and both were identified with PEAKS StudioX.

➢ Increased peptide identifications in PEAKS Studio X results compared to PEAKS Studio 8.5 and MaxQuant

By enabling chimera scan analysis, PEAKS Studio X identifies a total of 3648 peptide sequences of soluble HLA-bound peptides in patient 11-002-V1* (Seq47934) plasma sample, 13% more than analyzed with PEAKS Studio 8.5. In addition, 97% of the peptides identified with PEAKS Studio 8.5 were also present in the PEAKS Studio X results. Furthermore, we compared our results with the publication. PEAKS Studio X identified 73% more peptide sequences and 87% of peptides identified by MaxQuant were also identified by PEAKS Studio X.



Conclusions

Feature-based identification workflow in PEAKS Studio X allows chimera scan analysis and enables higher numbers of identifications of peptides. When this method is applied to immunopeptidomics analysis, it achieves superior performance than other workflows.

References

[1] Shraibman B, et al., Identification of tumor antigens among the HLA peptidomes of Glioblastoma tumors and plasma. Mol Cell Proteomics. 2018 Aug 2.