149 SIMULTANEOUS IDENTIFICATION OF KRAS MUTATION-CARRYING MULTIPLE NEOEPITOPES BY DIFFERENTIAL ION MOBILITY-ASSISTED TARGETED-MASS SPECTROMETRY

[1]Yuriko Minegishi*, [2]Noriko Iwamoto, [2]Takashi Shimada, [1,3]Kazuma Kiyotani, [1,4]Satoshi Nagayama, [5]Eric Tran, [6]Yoshinobu Koguchi, [6]William L Redmond, [6]Brian Piening, [6]Bernard A Fox, [1]Koji Ueda. *[1]Japanese Foundation for Cancer Research, Ariake, Tokyo, Japan; [2]Shimadzu Corporation, Kyoto, Japan; [3]National Institutes of Biomedical Innovation, Health and Nutrition, Ibaraki, Osaka, Japan; [4]Uji-Tokushukai Hospital, Uji, Kyoto, Japan; [5]Providence Cancer Institute, Portland, OR, USA; [6]Earle A. Chiles Research Institute, Providence Cancer Institute, Portland, OR, USA*

Background Extracellularly presented HLA class I antigen (immunopeptide) is one of the central mediators for cancer immunity. Immunopeptidomics, an analysis that directly identifies immunopeptides by mass spectrometry (MS), is the sole technique to investigate actually presented antigens. With the rapid progress in cancer immunotherapies targeting shared neoantigens, including non-canonical alternative neoantigens (cancer's dark matter), screening system will become increasingly important to verify whether neoantigens of interest are actually presented in patient materials. We have previously established the differential ion mobility (DIM)-assisted immunopeptidogenomics that enabled direct identification of KRAS-G12V-carrying neoepitope from 40 mg of clinical tissue.[1 2] Here, we applied this DIM to establish targeted-immunopeptidomics that identifies multiple neoantigens of interest simultaneously by one analysis.

Methods The status of KRAS mutations of cell lines was obtained from the RAS Initiative and COSMIC website. Based on that information, possible KRAS neoantigen sequences were predicted by NetMHCpan4.1 and synthesized as peptides. Immunopeptide samples were routinely prepared and first analyzed by DIM-assisted immunopeptidomics. For targeted-immunopeptidomics, parallel reaction monitoring (PRM) was applied. To identify multiple neoantigens from one analysis, optimal CV and retention time (RT) obtained by actual analyses of synthetic peptides in advance were utilized for PRM. As an analytical instrumentation for LC/DIM/MS system, Vanquish Neo UHPLC, FAIMS-Pro interface for DIM and the mass spectrometer ORBITRAP FUSION LUMOS (all by ThermoFisher Scientific) respectively were connected and used. Raw data for PRM was acquired using detector Orbitrap at 60K resolution. Proteome discoverer 3.0 was used to identify peptides.

Results DIM-assisted PRM has enabled the efficient identification of neoantigens of interest. In the example of Colo-668 cells, we previously identified only one KRAS-G12V-carrying neoepitope, but by using DIM-PRM, all two predicted neoepitopes were successfully identified from the sample. When using the Colo-668 cells as a vessel that embodies the potential to naturally present KRAS neoepitope, by stable expression of KRAS-G12D, additional presentation of two G12D-carrying neoepitopes were identified by DIM-PRM. So far by DIM-PRM, a total of 6 sequences of naturally-presented KRAS-neoepitopes from 3 cell lines were successfully identified.

Conclusions Targeted-immunopeptidomics by DIM-PRM holds promise for detecting actually presented antigens of interest. This could contribute to screen the best antigen(s) to target for cancer immunotherapy. Accumulation of data about naturally presented neoantigens by DIM-PRM, is also expected to contribute to the larger goal of completing an HLA-type-specific neoantigen catalog that supports the prompt selection of combined cancer immunotherapy.

Acknowledgements Scientific and Funding Support by Shimadzu Corporation.

Grant Support by Japanese Society for Promotion of Science (JSPS: 23K04971).

Grant Support by Japan Agency for Medical Research and Development (AMED: 20ae0101074s0302).

REFERENCES

1. Minegishi Y, Kiyotani K, Nemoto K, Inoue Y, Haga Y, Fujii R, Saichi N, Nagayama S, Ueda K. Differential ion mobility mass spectrometry in immunopeptidomics identifies neoantigens carrying colorectal cancer driver mutations. *Commun Biol*. 2022 Aug 18;**5**(1):831.
2. Haga Y, Minegishi Y, Ueda K. Frontiers in mass spectrometry-based clinical proteomics for cancer diagnosis and treatment. *Cancer Sci*. 2023 May;**114**(5):1783–1791

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0149