Characterization of RAS proteoforms by top-down mass spectrometry at 21T

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Introduction

The *KRAS* oncogene is frequently mutated in human cancer, with hotspots located at Gly12, Gly13, and Gln61. The membrane association and downstream signaling activity of the two gene products, KRAS4A and KRAS4B, are regulated by post-translational modification (PTM). Prior attempts to connect KRAS PTMs with oncogenic mutations by standard proteomic techniques were hampered by the significant sequence identity (~90%) between the four RAS isoforms (KRAS4A, KRAS4B, HRAS, NRAS). Therefore, we performed immunoprecipitation (IP) and subsequent analysis by top-down mass spectrometry (TD) to precisely identify and characterize intact and modified KRAS protein forms, or proteoforms, from cancer cell lines or patient tumor samples on a QE-HF mass spectrometer (MS) [1]. We subsequently hypothesized that the QE-HF limit of detection might be impacting our ability to detect lower-abundance KRAS proteoforms. To test this, we translated our novel IP-TD assay to the 21 Tesla (T) FT-ICR MS at NHMFL, as we believed that the multiple fills and alternative MS2 fragmentation methods available on this platform would dramatically improve our ability to detect and characterize endogenous KRAS proteoforms from multiple cancer contexts.

Experimental

Recombinant KRAS4B WT, KRAS4B G13D, and KRAS4A protein standards (NCI RAS Initiative) were resolved by liquid chromatography (LC) and analyzed by TD on the 21T FT-ICR MS by L.C. Anderson in March 2018. The objective was to identify the LC and MS parameters that would provide the best accurate mass determination and MS2-based sequence characterization of each target KRAS isoform. These parameters were then employed during a series of IP-TD analyses of KRAS-overexpressing MEF cell lines (NCI RAS Initiative) and replicate cancer cell line samples to those analyzed previously (NCI RAS Initiative) [1] performed on the 21T FT-ICR MS by L.C. Anderson and C.J. DeHart in May 2018. Manual data analysis and proteoform validation were performed using the Xtract algorithm present in Xcalibur (Thermo) and ProSight Lite.

Results and Discussion

The dramatic improvements in signal-to-noise ratio and MS2 fragmentation provided by the 21T FT-ICR MS led to the identification of at least 24 novel endogenous KRAS4B proteoforms by IP-TD (examples shown in **Figure 1**) and indicated multiple apparent stages of KRAS4B proteoform processing. Moreover, these experiments provided the first ever visualization of endogenous KRAS4A by IP-TD, leading to the identification of 6 novel proteoforms. These exciting results will be included in two upcoming publications, once all novel proteoform species have been fully characterized.

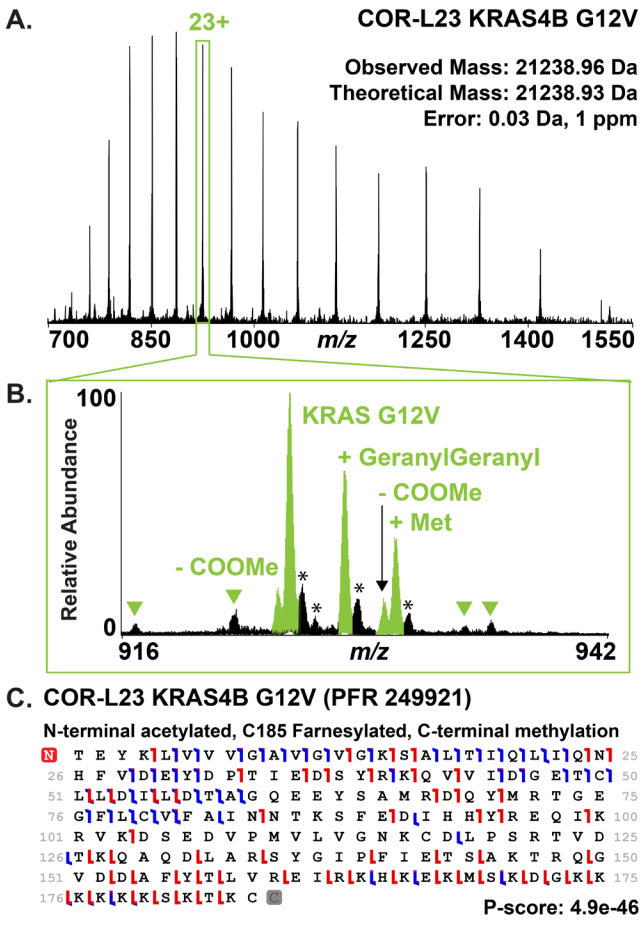

Fig.1 Enhanced detection of KRAS4B proteoforms at 21 Tesla. A. Broadband MS1 spectrum of endogenous KRAS4B G12V. **B.** Examples of novel KRAS4B proteoforms detected by IP-TD at 21T. **C.** Sequence coverage of the canonical KRAS4B G12V proteoform obtained by data-dependent CID (blue) and ETD (red) MS2 fragmentation.

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References

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