

Tandem Mass Spectrometry Analysis of Protein Deposits in Human Subcutaneous Fat Tissues of a Patient with Immunoglobulin Light Chain Amyloidosis

Yanyan Lu; Roger Th  berge; Tatiana Prokaeva; Nancy Leymarie; Brian H. Spencer; Pamela T. Soo Hoo; Lawreen H. Connors; Catherine E. Costello
Boston University School of Medicine, Boston, MA

Abstract

For the systemic amyloidosis of immunoglobulin (Ig) light chains (LCs), we have found that deposited LCs are extensively processed, especially at the C-terminus, leading to fragment patterns that differ from patient to patient. We are presently using both primary mass spectrometry (MS) and tandem (MS/MS) methods to compare LC fragments in amyloid deposits from fat biopsies.

The fat biopsy described herein showed deposition of Ig λ LC proteins (3+ score, Congo red stain). No cDNA information of the LC gene is available. Proteins extracted from the biopsy sample were subjected to 2D gel analysis. Tryptic peptides derived from gel spots were analyzed with 1) a Reflex IVTM MALDI-TOF MS (Bruker), 2) an ultrafleXtremeTM MALDI-TOF/TOF MS (Bruker) and 3) an LTQ-OrbitrapTM MS (ThermoFisher) with an Acuity nanoUPLC (Waters) and TriVersa NanoMateTM robot (Advion). Data was analyzed with MASCOTTM; peak assignments were verified manually.

The amino acid sequence and post-translational modifications of the Ig LC were determined by de novo sequencing with TOF/TOF MS and with HCD fragmentation followed by detection in the Orbitrap. Mass fingerprinting searches on the MALDI-TOF MS data returned a hit for λ LC constant region. Other proteins found in the spots included clusterin, serum amyloid P-component, APOA4 and APOE.

As the MW decreased, the abundances of the peaks for constant region peptides, e.g., C 4-22, diminished, while the serially truncated products from this peptide appeared. LC/MS/MS data confirmed the peak assignments. Sequences for some variable region peptides were assigned and aligned with germline gene IGLV1-51. MALDI-TOF/TOF MS revealed the oxidation of Trp (W) to kynurenine and N-formylkynurenine.

This research is supported by NIH grants P41 RR010888/ GM104603, S10 RR015942, S10 RR020946, S10 RR025082 and NIH-NHLBI contract N01 HV00239. and the BUMC Amyloid Treatment and Research Program. We thank Bruker Daltonics for access to the Bruker ultrafleXtremeTM.