A Proteomic Approach to the Study of Systemic Amyloidoses

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Introduction Protein deposition as amyloid is the basis of diseases that, overall, have an enormous social and medical impact. At least 24 different proteins are known to be causative agents (1). In the systemic forms, amyloid deposition is associated with dysfunction of vital organs, and molecular typing of the deposits is necessary for diagnosis and treatment (2). The traditional diagnostic approach is multidisciplinary, but sometimes fails to identify the correct type. A proteomic approach could help in diagnosis, through the direct molecular characterization of fibrillar proteins in tissues, and cast insights into the mechanisms of tissue damage. We developed a method to characterize amyloid deposits in abdominal subcutaneous fat obtained by fine needle aspiration (3) from patients with systemic amyloidosis, using 2D-PAGE followed by MALDI-TOF MS and peptide mass fingerprinting.

Methods Abdominal subcutaneous fat tissue samples were obtained, with institutional human studies approval, from individuals affected by various forms of systemic amyloidoses and from unaffected volunteers, to be used as controls. Proteins were extracted from fat tissue by homogenization directly in IEF buffer followed by ultracentrifugation to clear debris and delipidate samples (4). Samples were then subjected to 2D-PAGE analysis and Coomassie or silver staining. Protein spots were imaged and quantitated using PDQuest™ software. Spots indicating differentially expressed proteins were excised and subjected to in-gel digestion by trypsin. Peptides were analyzed by MALDI-TOF MS; MoverZ™ or MassLynx™ were used to analyze the spectra, and peptide mass fingerprinting was performed using MASCOT and/or BUPID.

Results A rapid methodology to prepare samples for high-grade 2D-PAGE analysis from small amounts (20-30 mg) of fat tissue was developed. Samples from 6 non amyloid-affected volunteers were used to generate 2D control maps for comparison with those obtained from patients with systemic amyloidoses. A first set of 70 spots from control maps has been identified by MALDI-TOF MS and peptide mass fingerprinting. Six amyloid-infiltrated samples, from patients with a known type of amyloidosis, have been analyzed overall (4 light chain amyloidosis, or AL, and 2 transthyretin amyloidosis, or ATTR). In all cases, the comparison with control maps allowed identifying significant differences, which can be summarized in 3 categories. (i) Proteins were observed in regions of the gels from patient samples consistent with the expected migration of amyloidogenic proteins (Figure 1). We performed MALDI-TOF MS and peptide mass fingerprinting to identify this group of abnormal spots in one case of AL λ amyloidosis and in one case of ATTR (caused by the amino acid substitution Val122Ile in transthyretin). In both cases, spots were recognized as formed from the predicted amyloidogenic protein or fragments, and correct amyloid typing was possible with this approach. Serum contamination did not appear to be significant. In the case of ATTR, the peptides containing the mutation were identified (5). In the AL case, the masses of the peptides from the tryptic digest of the spots matched those predicted from the theoretical digestion of the patient’s monoclonal λ light chain, whose sequence was obtained from bone marrow plasma cells (Figure 2). A heterogeneous population of N-terminal fragments of the light chain, missing portions of the constant region, was also detected in the gel using mass spectrometry. (ii) Additional spots were observed in patient samples that were absent in the controls. Some of them were characterized as proteins commonly associated with amyloid deposits, such as Serum Amyloid P and apolipoprotein E (6). (iii) Apparent changes in the intensity of some spots expressed also in control fat tissue (e.g., α-crystallin) were observed in some of the patients. Additional investigation is needed to assess the role of these differences in the context of the disease.

Conclusions The use of 2D-PAGE as a tool to highlight the differences between diseased and normal states is well known. We developed a proteomic approach using this technique in the analysis of fat tissues that provides a reliable way to directly characterize protein deposits in patients with amyloid...
disease. This approach is practical and feasible, given that fat aspirates of potential amyloid patients are routinely acquired for histological analysis. Ongoing analyses may provide identification of new aspects of the disease mechanisms, including the involvement of novel proteins and protein post-translational modifications.

**Figure 1.** 2D gel from a patient with AL\(\lambda\) amyloidosis. Boxed spots are not visible in controls. **Figure 2.** MALDI-TOF spectrum of the tryptic digest from spot 1 (\(\lambda\) light chain).

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**References**


