Subtyping of amyloidosis by direct targeted proteomics analysis of biopsy samples

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Introduction

Amyloidosis is a disease where insoluble deposits of specific proteins occur in tissues. Different classes of amyloidosis have been reported and their diagnosis relies on the identification of the associated proteins: up to 10 proteins can be targeted using histochemical approaches. However they can be inconclusive in certain cases, leading to a lack of information about the underlying etiology.

Aim of the work

Subtyping has been recently demonstrated combining laser capture microdissection (LCM) and mass spectrometry [1]. Here, we show that ultrasonic treatment followed by targeted mass spectrometry analysis directly on either raw or fixed biopsy samples can be successfully used even without LCM to get closer to the clinical routine application for amyloidosis subtyping.

Materials & Methods

Samples treatment:

- Fixed tissue slices or raw tissues were collected from Tenon Hospital (France).
- Enzymatic digestion with porcine trypsin, after reduction and alkylation using an ultrasonic probe (MicroSon XL) on a wet ice bath [2].
- Samples were filtered on Proxeon stage tips and reconstituted in the same volume, roughly 0.15 mm^3 were injected for each LC analysis on a nano C18 Acclaim PepMap100 75µm i.d. x 15cm length column (Dionex).

Discovery LC-MS Analysis:

- LC gradient from 2% to 40%B in 170 min [buffer A: H_2O/ACN/FA 98:2:0.1 (v/v/v), buffer B: H_2O/ACN/FA 10:90:0.1 (v/v/v)].
- Proteolytic peptide mixtures were analyzed by LTQ-FTICR Ultra mass spectrometer (Thermo Scientific) in FullMS - data dependent MS^2 scan. Data were queried using Proteome Discoverer 1.4 combining Mascot 2.4 and Sequest on UniProt-SwissProt 2013_02 database, 5ppm MS, 800mDa MS/MS, up to 2 miss-cleavages, full tryptic peptides, Ox(M) and CAM(C) as partial modifications.
- Protein abundance was evaluated according to [3] taking into account the area of the three most intense peptides. Unidentified species were given the global minimum area that could be detected. Only biopsies containing the two common proteins to all amyloid deposits : Serum amyloid P component (SAMP) and Apolipoprotein E (APoE), were considered as potential amyloid candidate. Finally amyloidosis were classified according to the relative intensity of the amyloidogenic proteins [4].

Targeted LC-MS Analysis:

- LC gradient from 2% to 40%B in 60min.
- The best 10 peptides of each amyloidogenic protein are selected for targeted experiments: Parallel reaction monitoring (PRM / QExactive, Thermo Scientific) and Selected reaction monitoring (SRM / TSQ Vantage, Thermo Scientific). Data were processed using Pinpoint 3.0. Only peptides seen in three replicates were considered in this study.

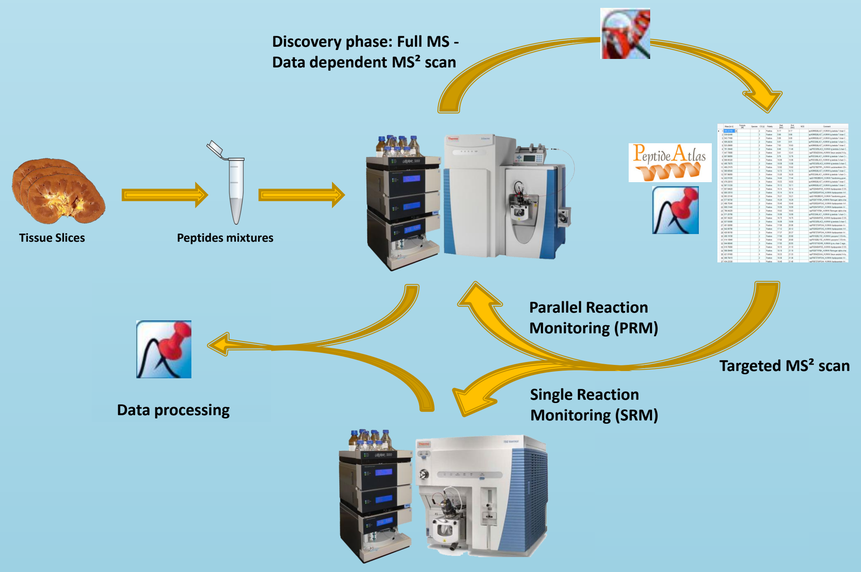

Figure 1 : Proteomics workflow

Discovery results

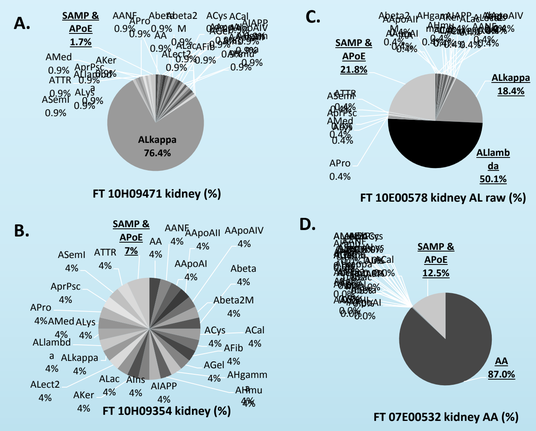

Figure 2 : Kidney biopsies of negative controls (A and B) and amyloidosis patients (C and D).

Patients with non amyloid pathologies were used as controls (figures 2A and 2B). Thus, they are not always pure negative (figure 2A) since these patients presented different pathologies. Nevertheless negative control biopsies contain neither SAMP nor ApoE proteins (amyloid fibrils biomarkers).

In figures 2C and 2D, the two fibrils markers are cleary detected. The patient shown in figure 2C has an AL amyloidosis since Ig kappa and Ig lambda are dominant. The patient shown on figure 2D has an AA amyloidosis (SAA proteins deposit).

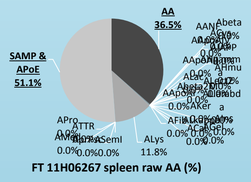

Figure 3 : Spleen biopsy of AA amyloidosis patient.

Same data processing has been applied for spleen biopsies (controls not shown). SAMP and APoE have been detected in the experiment (51.1% of all amyloidogenic proteins) and more than 36% of SAA protein. This patient has an AA amyloidosis. This sample was then used for targeted quantification.

Targeted results

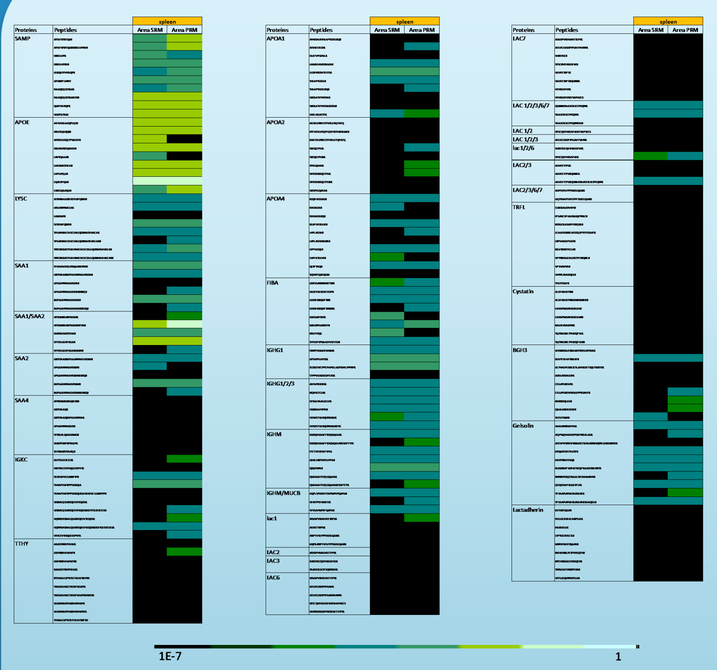

Figure 4 : HeatMap of the average peptide normalized area obtained from SRM and PRM analyses of a spleen raw tissue.

The normalized area was averaged over three replicates for each peptide. The color of each cell reflects the value of the calculated average. This HeatMap shows that higher number of peptides is identified in PRM than in SRM analyses. However, SAMP and APoE proteins, the two amyloid fibrils biomarkers, were detected in both cases. Among the amyloidogenic proteins, Serum Amyloid A 1 & 2 (SAA1 & SAA2) peptides are the most intense. The diagnosis is clear in the two analyses: this patient has an AA amyloidosis.

Conclusions & Perspectives

This first PRM and SRM quantification attempts were a success. Hence, it could be easily transferred to the French clinical departments.
Targeted analysis allows specific and sensitive subtyping of amyloidosis with either research mass spectrometers such as a QqOrbitrap or clinical application mass spectrometers such as a QqQ. Both instruments offer satisfying and similar results. In order to increase the robustness of the methods, reference proteins will be used to generate transitions for other amyloidogenic proteins.
Roughly 10 slices are required and the whole analysis lasts 2 days: 1 day for enzymatic treatment and 1 day for triplicate nanoLC-MS/MS analysis and data processing.

References

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