

ABRF: 2008 PRG STUDY SUMMARY

As the main goal of the 2008 PRG study was to identify and to determine qualitative differences among the proteins in both samples A and B, we thought that a good strategy was to combine information provided by using two different enzymes for digestion in order to maximize protein sequence coverage.

The digested protein extracts were analyzed by mass spectrometry using nanoLC-ESI- MS/MS with an ion trap system. With this technique, the isoform 1 of the Advanced glycosylation end product-specific receptor precursor was unambiguously identified in both samples A and B. However, no differences were found between both samples.

In order to test whether the isoform 2 or other possible differences (truncations, etc) were also present in any of the samples, we decided to perform a 2D gel electrophoresis methodology. With this approach, we visually observed qualitative differences between sample A and B. The corresponding spots were excised and automatically in-gel digested with trypsin and Glu-C. The resulting proteolytic peptides were analyzed by mass spectrometry using a MALDI-TOF/TOF system. Briefly, our results demonstrated that two different truncated forms (31 and 14 kDa, respectively, sharing the N-terminal region of the protein) of the Advanced glycosylation end product-specific receptor precursor are present in sample B but not in sample A. This conclusion was reached through the identification of peptides spanning progressively shorter regions of the protein.