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Structural studies of serum clusterin in ATTRwt amyloidosis

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Background

Wild-type transthyretin (ATTRwt) amyloidosis is an age-related disease that causes heart failure in older adults. This disease frequently features fibril deposits in cardiac tissue that originate from dissociation of the tetrameric protein, transthyretin (TTR). Unlike hereditary TTR amyloidosis where amino acid replacements destabilize native TTR, in ATTRwt amyloidosis the amyloid protein lacks sequence alterations. Thus, it seems plausible that other factors may be responsible for destabilization of the protein and unregulated accumulation of wild-type TTR fibrils. We believe that clusterin (CLU), a plasma circulating glycoprotein known to exhibit chaperone-like activity, may play a role in the pathobiology of ATTRwt amyloidosis. In this study, we present mass spectrometry (MS) data from comparative proteomic and glycoproteomic analyses of CLU purified from healthy control and ATTRwt patient sera.

Material and methods

Serum samples from patients with biopsy-proven ATTRwt amyloidosis featuring cardiac involvement were obtained from the Boston University Amyloidosis Center IRB-approved amyloid specimens repository; control sera were purchased from Bioreclamation (Westbury, NY). Control and ATTRwt sera were from Caucasian males over 60 years of age. Equivalent aliquots of serum from 10 separate self-reported healthy controls and 10 individual patients were pooled as distinct samples. CLU was purified by immunoaffinity chromatography from the pooled sera (control, CLUc; ATTRwt patient, CLUp) and each sample spiked with a known amount of bovine alpha 1-acid glycoprotein (bAGP) as an internal control for quantification purposes in the MS analyses. Glycopeptides were recovered after CLU denaturation, reduction, alkylation and cleavage with trypsin or chymotrypsin. Further enzymatic treatment of the glycopeptides with PNGase F removed N-linked oligosaccharides to obtain de-glycosylated peptides. Samples were subjected to LC-MS/MS and the output analyzed using PEAKS studio 8.5 and GlycReSoft softwares. For PEAKS analysis, a customized database containing 77 non-synonymous single nucleotide CLU

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variants concatenated with BAGP and the human Uniprot proteome was generated. The CLU variants included those reported in 16 types of cancers (categorized as benign or probably damaging) or associated with an increased risk for Alzheimer's disease [1–4]. Cosine similarity was used to assess the degree of similarity between glycan abundances.

Results

Identification of each individual de-glycosylated peptide confirmed that CLU was the source for the vast majority of the tryptic and chymotryptic digest products in both CLUc and CLUp preparations. Matching of de-glycosylated peptides derived from CLUc to the customized database identified 20 chymotrypsin-derived and 47 trypsin-derived peptides after enzymatic digestion; these results accounted for 35% and 56% sequence coverage in the control sample, respectively. A slightly higher coverage was obtained for CLUp (38% and 63% after database-matching of chymotryptic and tryptic peptides, respectively). Taken together, an overall sequence coverage of 67% for CLUc and 69% for CLUp was achieved; importantly, these MS data demonstrate the absence of amino acid sequence variation (pathological or otherwise) in a major portion of both, CLUc and CLUp.

Glycopeptide analyses yielded a description of oligosaccharide occupancies and abundances for 3 out of the 6 known glycosylation sites in CLU. At α 81N (α subunit), CLUp displayed lower glycosylation diversity and abundances (cosine similarity= 0.502; Fig. 1A) while the oligosaccharide profile found at β 352N (β subunit) was similar for both CLUc and CLUp (cosine similarity= 0.929; Fig. 1B). Moreover, at β 332N, the glycan HexNAc:4; Hex:5; Fuc:0; Neu5Ac:2 was highly abundant (relative abundance= 8×10^8) and identified only in CLUc (not shown).

Discussion and conclusions

MS analysis of CLU derived from ATTRwt serum suggests that there is no pathologic (or non-pathologic) amino acid sequence variation present in the protein; thus, the putative chaperone role attributed to CLU may not be compromised in ATTRwt disease due to primary structure alterations. However, structural differences between CLUc and CLUp at the level of oligosaccharide occupancies and abundances, as reported in this study, may account for a reduced binding capacity of CLU towards non-native TTR, and thereby contribute to amyloid fibril formation and accumulation of wild-type TTR amyloid deposits in tissue. Manual analysis of the MS data will be necessary to identify the remaining 3 glycosylation sites and their glycan occupancies and abundances.

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Figure 1.
Glycans at α 81N and β 352N in CLU derived from control and ATTRwt sera