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Opinion

DRUG ANALYSIS USING LUMINESCENCE METHODS

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INTRODUCTION

One of the most common post-translational modifications (PTMs) is glycosylation, which regulates a variety of biological processes like protein stability and cell signaling. As a result, glycoproteins are also a consistent component of the human tear film proteome. They form the first defense barrier of the ocular immune system and keep the ocular surface functioning properly. Glycoproteins are a valuable resource for the discovery of biomarkers or drug targets because irregularities in the glycoproteomic composition of the tear film may contribute to the onset of chronic eye diseases. As a result, the goal of this study was to use high-resolution mass spectrometry (MS) to identify the specific N-glycosylation sites of tear glycoprotein glycopeptides and create an affinity method based on lectins for their enrichment and concentration. We first collected native glycoproteins from human tear sample pools and used 1D gel electrophoresis and specific protein stainings to assess the enrichment efficacy of various lectin column systems for method development and evaluation.

DISCUSSION

Glycopeptide enrichment from human tear sample digests was carried out using the most effective multi-lectin column system. After that, the specific N-glycosylation sites of the glycopeptides were identified and located using MS. Our study's main finding was that healthy tear sample pools contained 26 N glycosylation sites for 11 N-glycoproteins. We found, among other things, that the tear film proteins lactotransferrin, Ig heavy chain constant 1, prolactin-inducible protein, and extracellular lacritin are significant and highly reliable N glycoproteins that are already linked to the development of various chronic eye diseases like dry eye syndrome. In conclusion, future research on human tear film and ocular surface-related inflammatory diseases will benefit greatly from the current study's N-glycoprotein catalog [1].

The ocular surface, cornea, and conjunctiva are all covered by the complex mixture of proteins, electrolytes, metabolites, lipids, and other components that make up the human tear film. It has a thickness of 3 millimeters, a volume of 7–10 milliliters, and important functions like delivering oxygen and nutrients to the cornea or protecting the ocular surface from the immune system are carried out by it. The health of the ocular surface is reflected in the tear film's proper formation;

however, any abnormality in its composition or function may lead to inflammation and the development of chronic eye diseases like dry eye syndrome (DES). Because of these facts, tears are an excellent source for the discovery of biomarkers because they are simple to obtain and can be collected using non-invasive sample collection techniques. As a result, Schirmer's strip technology or capillary tubes are used to collect basal tears, both of which have their advantages and disadvantages in routine clinical practice. However, as a potential biomarker pool, tears are suitable for the discovery of biomarkers in other neurodegenerative or systemic diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis, and even cancer. However, pathologies affecting the ocular surface or other structures related to the eye are not the only potential biomarker pool [2,3].

One of the most common post-translational modifications (PTMs), protein glycosylation is essential to numerous biological processes like protein function, stability and degradation, cell signaling, immune responses, and cell cycle regulation. In addition, this particular PTM plays an important role in preserving the homeostasis of the ocular surface and creating a natural barrier that protects the eye from various microorganisms and pathogens. As a result, pathogenic glycomic changes have already been found in the basal tears of diabetic retinopathy and keratoconjunctivitis patients. This shows how powerful protein glycosylations are as a sensitive indicator for the discovery of biomarkers and early diagnosis. Depending on the side chain of the amino acid (aa) at the relevant PTM site, protein glycosylations can generally be broken down into O-linked and N-linked glycosylations. As a result, N-glycosylations naturally occur at asparagine (N) residues that exhibit the standard protein sequence motif N–X–serine (S), threonine (T), and cysteine (C), where X denotes any aa other than proline (P). O-glycosylations, on the other hand, do not require a predetermined canonical protein sequence and can occupy any S or T residue. Additionally, peptide N-glycosidase F can enzymatically release N-glycosylations from the protein backbone, facilitating the independent analysis of N-glycan motifs and the associated protein N-glycosylation site of N to D (aspartic acid), favoring mass spectrometry detection of the original N-glycosylation site with a mass shift of +0.984 Da [4].

O-glycoprotein deglycosylation, on the other hand, cannot be accomplished through enzymatic means and can only be accomplished through chemical reactions like -elimination. Peeling reactions take place during this chemical deglycosylation process, which can lead to undesirable changes in the protein sequence and natural O-glycan motifs. As a result, the goal of this study was to identify and describe N-glycosylation sites in human tear film, which will form the foundation for future ocular biomarker discovery. In addition, previous investigations into the glycomic composition of basal tears revealed a predominant presence of 50–150 N-glycans in comparison to 8 O-glycan motifs, indicating that this glycosylation type plays a regulatory role in the human tear film. Other proteomic markers, such as the polymeric immunoglobulin receptor (PIGR) or the immunoglobulin heavy constant -1 (IGHA1), were also found to be reliable glycoproteins. These glycoproteins are important parts of the ocular defense system and make up the natural barrier. However, the primary goal of this study was to create an effective affinity technology based on lectins that could first enrich native glycoproteins from human tear sample pools. In this study, 11 tear N-glycoproteins with 26 distinct N-glycosylation sites were reliably identified using MS after the affinity method was developed and improved to its full potential using established affinity technology [5,6].

CONCLUSION

These findings are particularly interesting because any imbalances in the natural tear film proteome's composition and N-glycosylation levels could cause tear film instabilities that are irreversible and contribute to the development of chronic ocular surface diseases like DES. Because of this, it is of the utmost significance to comprehend the precise function that N-glycosylations play in preserving the homeostasis and functionality of the ocular surface, as well as the possibility of applying this knowledge to specific medical treatment in the future. In addition, important structural and functional information about the attached N-glycan motifs can be obtained through MS-based intact glycopeptide characterization, which may also be a promising strategy for ocular biomarker discovery in the future.

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