Single-Tear Proteomics and Precision Medicine

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The tear film is formed by two distinct layers: an outer lipid layer and an inner aqueous layer containing proteins, metabolites, electrolytes, mucins, and transmembrane glycoproteins. The composition of these layers reflects the pathophysiological state of the tissues underneath, as well as that of the whole body, which makes tears an attractive source of potential biomarkers for the evaluation of health and disease states .Thanks to the high protein concentration (approximately ranging between 4 and 10 µg/µL in basal, open-eye tears) , tears can be easily analyzed by proteomics approaches, despite the low amount of sample that can be normally collected (around 6 µL from a single withdrawal).

Keywords: lacrimal film ; liquid biopsies ; single-tear analysis ; mass-spectrometry-based proteomics

1. Introduction

Liquid biopsies, such as blood, urine, and cerebrospinal fluid, are commonly employed to diagnose and monitor systemic diseases. Among these, tears have attracted growing interest, thanks to their relatively low complexity and easy accessibility. The tear film is formed by two distinct layers: an outer lipid layer and an inner aqueous layer containing proteins, metabolites, electrolytes, mucins, and transmembrane glycoproteins [1].

Protein identification and quantification in tear samples can be achieved by fast, accurate, and high-throughput approaches based on mass spectrometry (MS) [2]. Several authors have employed label-based techniques with isobaric tags [3][4][5]. Recently, label-free, quantitative proteomics approaches are gaining interest, thanks to the improved performance of the most advanced liquid chromatography (LC)-MS platforms [6]. Thus, MS-based proteomics of tear samples is a promising approach to biomarker discovery for human diseases, which can then be combined with orthogonal techniques, such as Western blotting, for validation.

Most of the studies reported in the literature approach the investigation of tear proteomes from pooled specimens [7][8][9][10][11]. The pioneering work by Li and coworkers describes proteomics analysis of a single tear, i.e., a single withdrawal from one eye, leading to the identification of 54 proteins [7]. So far, this study has represented the only example of single-tear proteomics. Analyses conducted on pooled samples have led to improved identification efficacy [8][9][10][11]. However, the pooling procedure loses the information on individual variability.

Improved single-tear analysis is required, in order to obtain patient-specific profiles of protein expression levels and their response to controlled changes in lifestyle, treatments, and time. Modern medicine has devoted increasing attention to patient stratification, based on better-defined omics profiles [12]. Such levels of molecular description can lead to a significant contribution of quantitative proteomics to precision and personalized medicine, which has been based, instead, mainly on genetic profiling [13].

Another common strategy employed to maximize the number of identifications is the addition of a fractionation step prior to LC-MS/MS analysis, such as offline strong cation exchange (SCX) [9][10] or electrophoresis [7][8][11]. These procedures, however, increase the complexity of the experimental protocol, making its application to high-throughput studies more challenging. Moreover, additional steps increase experimental error, sample losses, and analysis costs. In this specific case, greater advantages can be provided by ultrahigh-resolution MS analysis and high-performance LC separation, which were made possible by recent technological advances [14].

Vibrational spectroscopy has proved to be a complementary technique to MS-based approaches. In particular, Fourier transform infrared (FTIR) and Raman (RS) spectroscopies are commonly documented to be the primary approach for the analysis of chemicals, tissues, isolated cells, and liquid biopsies [15]. They rely on different principles, each of them having its peculiarities: FTIR measures the absolute frequencies at which a given sample absorbs radiation, whereas RS measures the relative frequencies at which it scatters photons. For this distinction, some vibrations are FTIR active, such

as the ones related to heteronuclear polar bonds or to nonsymmetrical apolar bonds and some others are Raman active, e.g., differentiation between single, double, and triple carbon bonds.

MS-based proteomics, supported by vibrational spectroscopies, can now provide the accuracy and sensitivity necessary for single-tear profiling without upstream fractionation. With this aim, the present work describes a straightforward and high-performance approach to in-depth, single-tear quantitative proteomics.

2. Single-Tear Proteomics: A Feasible Approach to Precision Medicine

High-performance analytical procedures allow for reliable quantitative protein profiling from “single-tear” (5 µL) lacrimal fluid collected by the microcapillary method, without fractionation steps upstream from the LC-MS protocol. This work described a pipeline for data collection and analysis that can be of relevance for a wide array of biomedical applications. A systematic comparison of the alternative quantitation methods usually employed in the literature revealed a good equivalence of normalized peak intensity, peak area, and PSM over 70 runs. LDA and hierarchical clustering analyses revealed 41 descriptors for male vs. female stratification and 27 descriptors for morning vs. afternoon stratification. The effect of gender underscores the potential of tear as an informative biofluid and the importance of single-tear analysis in preserving sample identity. The effect of withdrawal time hints at the need for highly controlled sample-collection conditions, with variable protein profiles in afternoon vs. morning samples. Further studies will be needed to investigate the turnover of tear proteins and the role of sleep on tear proteome homeostasis. It would also be of interest to strengthen tear protein characterization and identification of group descriptors by multicentric studies. This work also suggests that proteomic and spectroscopic profiling could provide complementary information for biochemical and biophysical characterization of lacrimal fluid, possibly leading to the discovery of composite biomarkers.

Detailed stratification of individual patients by proteomics analysis is crucial to translate personalized medicine into practice. Tissue biopsies are informative, but their collection is not always simple or possible. For instance, practical and ethical issues restrict the collection of brain biopsies to post-mortem sampling. Localized biofluids, such as tears, contain contributions from different organs and tissues, making them interesting for both organ-specific and systemic diseases. In this frame, tear fluid has elicited a growing interest, thanks to its continuous accessibility, minimal storage requirements, high protein concentration, and responsiveness to both ocular and systemic conditions, particularly those linked to neurodegeneration (i.e., multiple sclerosis, Alzheimer’s disease, and Parkinson’s disease).

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