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Contributions of immunoaffinity chromatography to deep proteome profiling of human biofluids

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ABSTRACT

Human biofluids, especially blood plasma or serum, hold great potential as the sources of candidate biomarkers for various diseases; however, the enormous dynamic range of protein concentrations in biofluids represents a significant analytical challenge for detecting promising low-abundance proteins. Over the last decade, various immunoaffinity chromatographic methods have been developed and routinely applied for separating low-abundance proteins from the high- and moderate-abundance proteins, thus enabling much more effective detection of low-abundance proteins. Herein, we review the advances of immunoaffinity separation methods and their contributions to the proteomic applications in human biofluids. The limitations and future perspectives of immunoaffinity separation methods are also discussed.

1. Introduction

Human biofluids are biological fluids that are excreted or secreted from inside the bodies of living people, including but not limited to blood, urine, cerebrospinal fluid (CSF), saliva, tear, and synovial fluid (See Fig. 1). Human biofluids, especially blood plasma/serum and urine, are considered the most promising sources for the discovery of novel biomarkers for disease diagnosis and prognosis based on the notion that these biofluids contain disease-associated proteins secreted or leaked from pathological tissues across the body [1–3]. Comparing to other types of specimens such as tissues, biofluids are often easily obtainable through noninvasive procedures, making it particularly attractive for large-scale clinical and/or longitudinal studies. For these reasons, there has been tremendous interest in profiling the biofluid proteomes for the development of biomarkers for various diseases over the last decade [4–10].

Blood plasma or serum has been the most popular choice for biomarker development and verification/validation. One of the unique challenges in proteome profiling of plasma/serum lies in its extremely large dynamic range of protein concentrations (up to 10–12 orders of magnitude [4]). The 12 most abundant proteins (e.g., albumin, transferrin, immunoglobulins, etc) account for ~95% of total protein mass in plasma or serum, which leaves thousands of other moderate- and low-abundance proteins (MAPs and LAPs) in only 5% of total protein mass (See Fig. 1). The "masking" effect caused by these high-abundance proteins (HAPs) greatly hampers the detection of LAPs such as cytokines and other clinically important proteins that often present at the sub ng/ml level. Besides blood, urine is also an ideal source for biomarker discovery and has been utilized in more and more proteomics studies [9,11,12]. Urine is formed in the kidney by ultrafiltration from the blood, which can be easily accessed and collected in large amounts via non-invasive approaches [13]. Comparing to blood, the composition of urine is less dominated by HAPs, which provides a relatively easier access to LAPs (See Fig. 1). Other biofluids, such as cerebrospinal fluid, saliva, tear, and synovial fluid, have also been analyzed for potential biomarkers; however in a limited number of studies comparing to blood and urine [14–16].

Immunoaffinity chromatography (IAC) approaches have become the most commonly utilized strategies for digging deeper into the biofluid proteomes by both global and targeted proteomics [17–19]. IAC represents a specific type of affinity chromatography where the stationary phase is composed of immobilized antibodies or other affinity reagents on solid support matrix. The underlying

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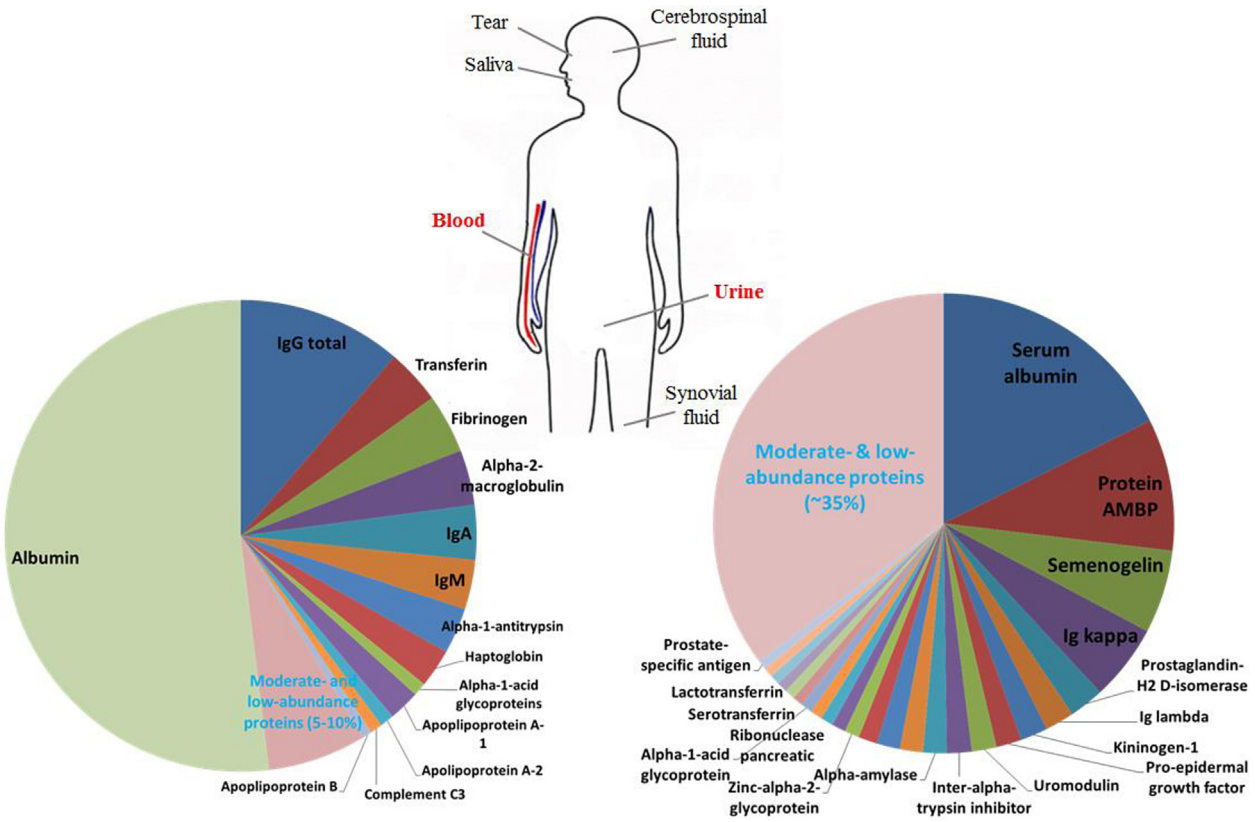

Fig. 1. Biofluids in human. Main sources and origins of human biofluids are depicted; the compositions of protein mass for both plasma/serum (data from in-house protein identification result and protein abundances were based on spectral count) and urine (data from Li et al. [162]) are shown in pie charts on the left and right hand side, respectively.

principle of IAC is based on the selective non-covalent interaction between antibodies (or affinity reagents) and their specific binding targets or antigens. The purpose of IAC separations is to enrich LAPs of interest by either removing HAPs from the complex samples through immunoaffinity depletion (immunodepletion) or directing capturing low-abundance targets of interest through immunoaffinity enrichment (immunoenrichment). Significant advances in IAC methods for both the depletion and enrichment have been made and these methods have been broadly utilized in proteomics applications for many types of biofluids related to various human diseases [18,20].

2. Overview of immunoaffinity chromatography

The most common schemes for applying IAC either by immunodepletion or immunoenrichment are illustrated in Fig. 2. In these schemes, chromatographic matrices (column) or other resins with immobilized antibodies are used to specifically capture target proteins/peptides, and the resulting flow-through fraction (immunodepletion) or bound fraction (immunoenrichment) is collected for further analyses [21,22]. The immunodepletion strategy is designed to remove the HAPs and enrich LAPs on a global scale [23]. In immunodepletion, complex samples as plasma/serum are first loaded onto a depletion column, and only specific HAPs are selectively captured by the antibodies immobilized on column, while other LAPs flow through directly and are collected (See Fig. 2A). To maximize the detection of LAPs, simultaneous removal of multiple HAPs is desired. Therefore, multiple antibodies are often mixed and immobilized onto column in order to remove multiple HAPs. Furthermore, immunodepletion columns targeting different proteins/peptides can also be used in tandem to enable the removal of a relatively large number of HAPs [21]. On the other hand, the immunoenrichment (or immunoaffinity purification) strategy is more targeted toward specific analytes (e.g., low-abundance proteins, peptides, or PTMs) to enhance the detection of these specific target low abundance analytes of interest, which is ideally suited for coupling with targeted proteomics measurements [24,25]. In immunoenrichment, only target low-abundance analytes are recognized and bound on column, while all other proteins/peptides are washed away, and the targeted proteins/peptides are further eluted from column for analysis (See Fig. 2B).

The utility of IAC is highly dependent on the quality of affinity reagents, typically antibodies [19]. A “good” antibody for IAC should meet two requirements: (a) a high intrinsic affinity toward target protein, and (b) reversible interactions between antibody and antigen that can be easily de-stabilized. Two main types of antibodies are commonly used in IAC, namely polyclonal and monoclonal antibodies [17,19]. Polyclonal antibodies are produced as a heterogeneous population of antibodies from multiple clones of B-cells, which can recognize and bind a variety of epitopes on a single antigen with diverse affinity [26]. Sera of immunized animals are the main sources of polyclonal antibodies. Due to their easy accessibility and relative low cost, polyclonal antibodies are widely used for developing IAC methods. However, there are a number of limitations in using polyclonal antibodies. Firstly, sera are usually available in limited supply and could vary from animal to animal. Therefore, it is difficult to obtain consistent quality of antibodies from multiple batches or lots. To obtain high quality antigen-specific antibodies, polyclonal antibodies must be purified against the target antigen via affinity chromatography [19]. However, it is often impractical to acquire a large amount of purified antigen proteins for this purpose. Alternatively, anti-peptide antibodies have been developed to selectively capture the proteolytic peptides of target proteins for their quantification in human