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Review

Autoimmune profiling with protein microarrays in clinical applications ☆

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ABSTRACT

In recent years, knowledge about immune-related disorders has substantially increased, especially in the field of central nervous system (CNS) disorders. Recent innovations in protein-related microarray technology have enabled the analysis of interactions between numerous samples and up to 20,000 targets. Antibodies directed against ion channels, receptors and other synaptic proteins have been identified, and their causative roles in different disorders have been identified. Knowledge about immunological disorders is likely to expand further as more antibody targets are discovered. Therefore, protein microarrays may become an established tool for routine diagnostic procedures in the future.

The identification of relevant target proteins requires the development of new strategies to handle and process vast quantities of data so that these data can be evaluated and correlated with relevant clinical issues, such as disease progression, clinical manifestations and prognostic factors. This review will mainly focus on new protein array technologies, which allow the processing of a large number of samples, and their various applications with a deeper insight into their potential use as diagnostic tools in neurodegenerative diseases and other diseases. This article is part of a Special Issue entitled: Biomarkers: A Proteomic Challenge.

1. Introduction

Historically, immunology research has focused on the role of host defense and the immune response against foreign entities. However, in recent years, there has been an increasing awareness of the role of immunology in surveillance against aberrations in the body; for example, in neoplastic disorders. Antibodies directed against endogenous proteins serve as valuable markers for neoplastic diseases, e.g., prostate cancer. The innate and adaptive components of the immune system act together to maintain the functional integrity of the human body. When it deviates from its natural function, the immune system may become misdirected against the constituents of the body, thus changing from friend to foe. The underlying pathogenic mechanism of neuromuscular diseases, for example, may involve antibodies directed toward synaptic proteins in the neuromuscular junctions. A variety of antibodies against different target proteins in the neuromuscular synapse have been identified [1], and the pathogenic roles of these antibodies have been elucidated using various in vitro and in vivo approaches. In addition to aiding diagnosis, the identification of these antibodies enables physicians to treat neuromuscular diseases, which were previously often lethal.

The functions of self-antigens and their recognition by immune cells are critical for the development of these autoimmune diseases, but the mechanisms involved are not completely understood. Antibodies that recognize proteins of an individual's own cells are called autoantibodies.

Autoantibodies have been identified as key players in several immune-mediated diseases. Tissue damage and repair are of special importance in the initiation of these diseases. The identification of biomarkers for autoimmune-mediated diseases would facilitate the accurate diagnosis of different diseases with identical clinical symptoms. Additionally, such biomarkers would assist physicians in applying more specific therapies and in monitoring the patient's response to them. Furthermore, autoantibodies or antigens as marker molecules could be used to control disease progression [2].

In addition to their pathogenic involvement, some autoantibodies function as highly specific markers, even if they are not involved in the disease pathology itself. This is especially true for intracellular proteins, which are normally protected from the immune system. Neurodegenerative diseases in particular involve a shift from beneficial to detrimental autoimmunity [3]. Antibodies recognize internal and

Abbreviations: ELISA, enzyme-linked immunosorbent assay; PURE, protein synthesis using recombinant elements; NMDA, N-methyl-D-aspartate; LOD, level of detection; QD, quantum dot; CV, coefficient of variation

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external proteins primarily to eliminate these proteins. The presence of antibodies directed toward intracellular antigens, regardless of whether they modulate the disease, could serve as a window into the underlying pathological process to aid diagnosis.

The presence of these antibodies in different body fluids, such as blood, cerebrospinal fluid, saliva or urine, could facilitate early disease diagnosis because the antibody concentrations can be determined using minimally invasive methods, e.g., enzyme-linked immunosorbent assay (ELISA), LUMINEX® or protein microarrays (see Fig. 1). Thus, antibodies could be considered a good starting point in the search for candidates for early diagnosis due to their high sensitivity and specificity as well as to their possible ability to monitor disease progression in autoimmune-mediated diseases [2]. This review provides a short overview of the currently used methods for biomarker identification, with a special focus on autoantibodies in neurodegenerative diseases and their identification via protein microarrays.

2. High-throughput microarrays

To analyze numerous samples or analytes in parallel, different high-throughput tools have been developed during the last 20 years. Especially for autoimmune diseases, which often produce similar symptoms, high-throughput tests are necessary for achieving an earlier and more specific diagnosis and a suitable treatment plan [4]. One of these techniques involves DNA microarrays, which were first described in 1995 by Schena and colleagues [5]. Those authors amplified different DNA fragments from *Arabidopsis thaliana*, placed each amplified DNA fragment into a single well of a 96-well microtiter plate and printed the fragments onto glass microscope slides in duplicate. They predicted that 20,000 different DNA fragments could be printed onto one slide using the robotic spotting technique available in 1995. Today, the Genome-Wide Human SNP Array 6.0 created by Affymetrix contains 1.8 million DNA sequences on a single glass slide [6]. By isolating mRNA from a sample of interest, synthesizing cDNAs by reverse transcription with fluorescent labeling and performing a hybridization step, the abundance of each mRNA in the sample can be quantified by determining the light intensity of each spot using a fluorescence scanner [7]. Because changes in cellular protein composition may be associated with different diseases, e.g., Alzheimer's disease, Parkinson's disease or Huntington's disease [8–10], the up- or down-regulation of proteins in the affected tissue is of special interest. Because the cellular mRNA levels do not necessarily correlate with the abundance of the corresponding proteins, scientists have attempted to transfer DNA microarray technology to a protein-based method [11]. Whereas DNA microarrays can be performed using denatured DNA fragments, protein–protein interactions are often detectable only if the immobilized proteins exhibit their naturally occurring 3D structures [12]. The preservation of this structure requires a more careful handling of the samples and of the arrays themselves. Even disregarding future improvements, protein microarrays are one of the leading high-throughput tools for biomarker detection and drug development; because they contain up to 20,000 proteins, they allow the nearly automatic processing of numerous samples in a short time [13]. These microarrays can be used to analyze antigen–antibody interactions, which are of great interest in several autoimmune and progressive diseases, including Alzheimer's and Parkinson's diseases. First, one must decide whether to spot antigens (for example, proteins, peptides or other molecules) or to immobilize a large number of different antibodies. In a second step, the array is incubated with the above-mentioned samples containing the interacting biomolecules (antigens or antibodies). Bound molecules are detected by species-specific labeled secondary antibodies. To discover more about the binding circumstances, protein–protein and protein–peptide interactions can be examined, which are a major focus in studies of disease progression and signal pathway identification. In principle, protein microarrays can be incubated with a sample solution of interest containing potential interacting proteins/peptides. Interacting proteins that have already been identified can be detected using labeled antibodies. If new interaction partners are found, it is necessary to apply additional analytic tools, e.g. mass spectrometry [14]. Furthermore, peptide arrays can also be used to identify the epitopes of a protein, which is highly beneficial for drug discovery and therapy development [15].

A promising alternative to traditional antibodies is aptamers, which are equivalent to synthetic antibodies [16]. These short, single-stranded oligonucleotides can bind to proteins, peptides or small molecules with high specificity. Aptamers are utilized in microarray analyses because they possess several advantages compared to antibodies, such as their higher chemical stability and easier synthesis and modification processes. The first utilization of aptamers in combination with microarrays was reported by Brody and coworkers in 1999 [17]. Even if their microarray protocol with photoaptamers could be used for protein detection, a protein–protein interaction analysis would be impossible due to the need for harsh washing conditions. Typical aptamers would circumvent this problem, but so far, the leakage of aptamers specific to the proteome of an organism prevents the wide application of aptamer microarrays to protein–protein interaction studies or pathogenetic analyses [18]. However, some research groups have used aptamers in biomarker discovery. For example, the slow off-rate modified aptamers designed by Gold et al. contain functional groups and can be used in proteomics for disease diagnosis and biomarker identification. Those authors reported detecting and identifying 813 proteins with low detection limits using this technique [19].

3. Types of functional protein assays

In general, microarrays allow the study of various interactions, such as protein–protein, enzyme–substrate, receptor–ligand and antibody–antigen interactions (see Fig. 2). Two main types of arrays can be differentiated, depending on the immobilized molecule. In the first type of array, thousands of potential targets are immobilized on a surface and then incubated with a sample containing anywhere from one to thousands of molecules. This technology is applied, for example, to identify

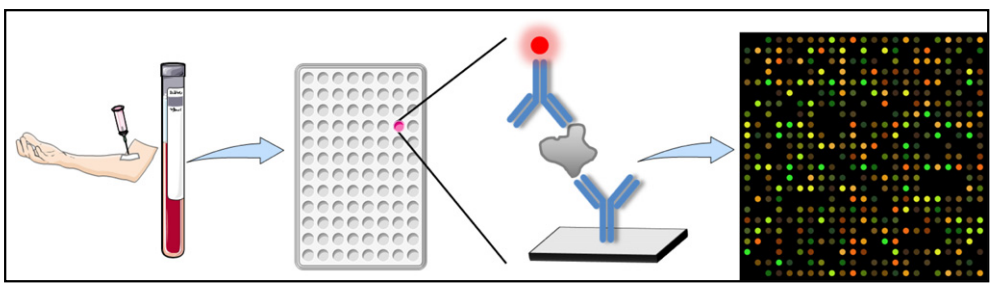

Fig. 1. Schematic presentation of a microarray analysis. A sample of blood (or another body fluid) is collected from the patient. The sample is prepared and incubated with the microarray. Previously, a corresponding binding partner for the molecule of interest was immobilized on the surface of the microarray. Here, an interacting antibody and antigen are depicted. Detection is performed by labeling a secondary antibody, which results in intensive signals if the molecule of interest is present.