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Research paper

An efficient proteomics-based approach for the screening of autoantibodies

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Abstract

This study presents an improved method for the complete transfer of proteins separated by two-dimensional gel electrophoresis to a membrane, specifically designed for the screening and identification of antigens recognized by autoantibodies in patients with breast cancer (BCP) and healthy volunteers. This paper reports the evaluation of this technique using proteins from MCF7 as a source of antigens following 2-DE separation. The appropriate quantity of protein to be loaded on gels (150 μg) has been determined, the aim being a complete and reproducible recovery of all separated proteins onto the polyvinylidene fluoride membrane (2D-blot) after a semi-dry electrotransfer. Several different transfer methods were tested in parallel, resulting in the selection and optimisation of one using a discontinuous buffer system, based on the isotachophoresis theory. To facilitate the comparative analysis of the different sets of 2D-blots probed with individual sera from BCP and healthy volunteers, the 2D-blots were stained with colloidal gold following the immunodetection step. The gels and 2D-blots were scanned and analysed by imaging software. The matching permitted exact localisation of particular relevant protein spots hybridised by antibodies on the 2D-blots. These spots were subsequently located on preparative gels for identification by mass spectrometry. A set of 40 2D-blots was probed with 20 sera from patients with breast cancer and 20 sera from healthy volunteers. In the protein profiles submitted to immunodetection, 15 proteins were repeatedly immunodetected by both BCP and sera from healthy people. Those proteins were identified by mass spectrometry. Conversely, some protein isoforms were preferentially immunodetected by BCP sera and may reflect the presence of this cancer. The improved isotachophoretic method

Abbreviations: 2-DE, two-dimensional gel electrophoresis; 2D-blotting, Western blotting following 2-DE; PVDF, polyvinylidene fluoride; 2D-blot, PVDF membrane after 2D-blotting; MS, mass spectrometry; ISB, isotachophoresis-based method for blotting; BCP, breast cancer patients; SERPA, serological proteome analysis; MS, mass spectrometry; MS/MS, mass spectrometry in tandem mode.

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described in this study is suitable for comparing the overall profile of autoimmunity between different populations and for subsequent identification of relevant antigens.

Keywords: Western blotting; Proteomics; SERPA; Autoantibodies

1. Introduction

Proteomics uses a combination of sophisticated methods including two-dimensional gel electrophoresis (2-DE), image analysis, and mass spectrometry (MS), and provides major opportunities for screening and identifying autoantigens (Brichory et al., 2001; Klade et al., 2001; Le Naour, 2001; Xiang et al., 2004). Western blotting, in combination with 2-DE gels (2D-blotting) (Anderson et al., 1982; Ishida et al., 1997; Geissler et al., 1999; Petersen, 2003), permits the transfer and immobilisation of proteins to a semi rigid support, allowing subsequent immunodetection of relevant antigens among the several thousand individual proteins separated using 2-DE. The term "serological proteome analysis" (SERPA) has been proposed for this approach (Klade et al., 2001). Comparative probing of blots with sera from patients and healthy subjects may allow the characterization of proteins that have the potential to be used as a marker of a particular pathology (Le Naour et al., 2001; Forne et al., 2003; Stulik et al., 2003; Wang et al., 2003; Xiang et al., 2004). To compare unequivocally the overall profile of autoimmunity in patients and healthy volunteers, it is necessary to overcome the variability of protein profiles probed by the sera on the 2D-blot. Although articles describing data obtained by 2D-blotting are numerous, it appears that there has been no report published raising awareness of technical variations (Shen et al., 1990) and the impact this has on the discovery of meaningful diagnostic markers (Samuelsson et al., 2003).

In the present study, the optimisation of a 2D-blotting method for screening autoantibodies is described. The effectiveness and reproducibility of this approach has been evaluated. The detection of proteins that were immunorecognized with differing frequencies by sera from patients with breast cancer (BCP) and healthy people is reported. Fifteen proteins were consistently immunodetected using both BCP and sera from healthy subjects. These proteins were identified by mass spectrometry. Conversely, some protein isoforms were preferentially immunodetected using BCP and may relate to cancer status.

2. Materials and methods

All chemicals used, if not otherwise stated, were purchased from Sigma (St. Louis, USA).

2.1. Sera

Sera were obtained during a routine blood test at the time of breast cancer diagnosis prior to any treatment. Sera from healthy volunteer women served as controls. Aliquots of sera were immediately frozen at −80 °C until used and were never refrozen.

2.2. Protein extraction and 2-DE

The source of antigens was the oestrogen receptor-positive MCF7 cell line. The proteins were extracted from cells under a laminar flux, wearing gloves and mask, to prevent any contamination. After several careful washes in PBS, 2×10^8 cells were extracted in 1 ml of a solution containing 7 M urea, 2 M thiourea, 5% (v/v) glycerol, 0.33% (w/v) CHAPS, 0.35% (v/v) Triton X100, 0.35% (w/v) sulfobetaine 3–10, 10% (v/v) isopropanol, 12.5% (v/v) isobutanol, 0.6% (v/v) of a mixture (2:1) of IPG buffer 4–7 and ampholytes 6–8 (Amersham, Orsay, France), 100 mM DTT (w/v) and a trace of bromophenol blue. Isoelectrofocusing was done using the IEFCell (BioRad, Hercules, CA, USA) on 17-cm-long strips (pH 5–8) as previously described (Poirier et al., 2003). 150 μg, 300 μg, and 500 μg of the protein extract were loaded on to the strips. Focusing was achieved at 80,000 Vh, 100,000 Vh, and 160,000 Vh for 150 μg, 300 μg,