



AUTOANTIBODY PROFILING AS DIAGNOSTIC BIOMARKERS BY PROTEOMICS APPROACHES

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ABSTRACT: Tumor-associated autoantibodies (tumor-associated antigens) are a group of serum biomarkers which have a long half-life and are easily accessible in blood. Small quantity of antigen in the early stage of tumorigenesis can trigger a larger immune response, so makes it useful as an early diagnosis marker. Testing for autoantibodies may be helpful for the diagnosis, differential diagnosis, prognostication, or monitoring of autoimmune diseases. The proteomic technologies can be used for discovering of many autoantigens and it can generate a panel of tumor-associated antigens that exhibit better diagnostic value than a single tumor-associated antigen marker. This review will focus on proteomics technologies that enable characterization of autoantibody responses and also discuss about autoantibodies as biomarkers for diagnosing diseases.

Keywords: Autoantibody Profiling, Diagnostic Biomarkers, Proteomics Approaches.

INTRODUCTION

The immune system defends the body from external agents. The cancer cells are another important target of the immune system because proteins expression patterns change in the process of tumorigenesis from those of normal cells, which may be recognized by the defense system as external agents and elicit humoral immune responses (1-8) The important aspect of immunoproteomics is the development pattern of tumor-associated autoantigens and their specific autoantibodies in the process of tumorigenesis.(1)

Recently autoantibody profiling as prognostic biomarkers and anti-cancer vaccine immunotherapy have been studied(1, 2). Therefore, further understanding of the early processes of tumorigenesis and related autoantibodies might show important implications for tumor biology and, from these results, additional biomarkers that could potentially assist in improved diagnosis or treatment will be identified(1, 2, 9-11). In recent years, there have been more studies in autoantibody profiling cancer patient sera for the detection of tumor associated antigens with using of new proteomic technologies(1, 11).

PROTEOMICS TECHNOLOGIES IN DETECTION OF AUTOANTIBODY PROFILING

Proteomics' is the large-scale study of expression, function and interactions of proteins(12, 13). These days, proteomics approaches, Especially, 2-dimensional liquid chromatography/tandem MS, or a combination of 2-dimensional gel electrophoresis (2-DE) and matrix laser desorption/ionization time-of-flight MS (MALDI-TOF-MS), followed by database-searching (sequence tag or peptide mass fingerprinting), are used by most studies for the discovery of tumor-associated autoantibodies. The combined assay of different autoantibodies is suggested as a promising approach for the diagnosis of cancer as well.(1, 14-16)

Autoantigen microarray technology enables the comprehensive analysis of autoantibodies with microliter volumes of serum in a high-throughput manner.(17, 18) Initial studies demonstrated that autoantigen microarrays are both sensitive and specific for detecting autoantigens.(19)

MS-based antigen profiling approaches will become a useful clinical tool for the diagnosis and monitoring of therapeutic intervention in autoimmune diseases, infection and cancer.(16)

Serological Proteome Analysis (SERPA)

Immunoproteomics is a concept used to identify disease-associated antigens that elicit immune responses by combining protein separation (two dimensional (2D) electrophoresis (2-DE), gel-free separation), immunological detection (Western blotting) and MS mass spectrometry (MS).(3, 14, 16)

2-DE is a classical proteomic technique which separates proteins in a complex mixture based on charge and also molecular weight (Mr). Separation in the first dimension is based on the isoelectric point (pI) and in the second dimension is upon their molecular weight.(20, 21)

The proteomics-based approach termed 'SERPA' is useful technique for the identification of TAAs.(22)

Proteins from tumor tissues or cell lines were separated by 2D electrophoresis. Patient serum, which may include the antibodies specific to a disease, is applied and subsequently, antigenic proteins are detected by using enzyme-labelled secondary antibodies. To identify immunogenic proteins, the corresponding spots are excised from gel and are in-gel digested. The digest is analyzed by MS or tandem MS.(16)

The limitations of 2D electrophoresis are the drawbacks of SERPA, including ; bias to abundant proteins, limitations in resolving certain classes of proteins (the potential loss of small (<15 kDa), very large (>200 kDa), very acidic (pI < 3), very basic (pI > 10) and very hydrophobic proteins) and also difficulty in producing reproducible 2D gels(23). In addition, due to the denaturing conditions and the way that western blots are prepared, only sequential epitopes can be detected.(1, 21)

Multiple Affinity Protein Profiling (MAPPING)

Affinity purification is used to enrich targeted proteins of interest from a complex sample. Recently, for generating immunoaffinity columns which capture autoantigens from cancer tissue lysates, autoantibodies from the sera of cancer patients or healthy controls have been used (21, 24, 25).

2D immunoaffinity chromatography followed by the identification of TAAs by tandem MS (nano-LC MSMS) are used in MAPPING technique(25). This first dimensional separation effectively removes autoantigens that do not elicit a cancer specific immune response but are present in the healthy population. Unbound proteins are then applied to an immunoaffinity column created with autoantibodies from cancer patients (second dimension). Captured TAAs are subsequently eluted and identified by online tandem MS.(21, 24)

MAPPING maintains tumor antigen in solution and allows for the potential identification of conformational epitopes. However, immunoprecipitation using affinity columns often restricted the discovery of TAAs to antibody interactions with low dissociation rate constant.(1)

Reverse-Capture Microarrays

According to this technique IgGs from patient and control sera are purified and labeled with different fluorescent dyes. Each antigen is immobilized on a different spot in their native configurations and Cancer cell lysates or tumor lysates are incubated with commercial antibody arrays. (26) This allows the instant identification of cancer-specific autoantibodies using native antigens expressed in tumor cells, which allows for the detection of TAAs presenting post-translational modifications.

However, the only known antigens with commercially available antibodies can be analyzed using reverse-capture microarray, which is appropriate for the validation rather than the discovery of biomarkers.(1)

Protein Microarrays

Protein microarrays are widely used technology for screening protein-protein interactions, such as antibody-antigen binding, in a high-throughput and automated setting. (19, 27-33). The two types of Protein microarray used to explore the immunoproteome are forward-phase and reverse-phase microarray. the nature of the capture/bait molecule is different between them. Reverse-phase microarrays employ the antigenic nature of proteins to capture antibodies where as Forward-phase microarrays utilise immobilised antibodies to capture TAAs.(21, 34-38)

The array platforms can be two dimensional (e.g. glass slides, nitrocellulose membranes and microtiter plates) or three dimensional (e.g. beads and nano-particles)(3). This approach depends on exposing serum samples from patients to an ordered array of putative antigens, capturing those antibodies that bind the antigens on the arrays. (14, 16, 23). Because of its miniature platform, the amount of samples and reagents needed are greatly reduced.

Protein array technology enables the identification of antigens in native configuration and especially useful for the discovery of post-translationally modified antigens. Because the microarray technology provides multiplexed analyses of thousands of proteins, this method permits high-throughput identification of TAA signatures for the development of cancer diagnostics (1, 3).

However, two major disadvantages of the array are that they are biased, given that antigens are selected based on the likelihood that they play a role in the disease and that because only molecules represented on the arrays can be identified, the analytical comprehensiveness of this technique is fundamentally limited.(16)

BIOMARKER AND CLINICAL ROLES OF THE TAA

The vast majority of biomarkers are proteins which are produced by the cancer. Although these biomarkers can be identified in the sera of patients which is a simple and inexpensive manner, the inability to detect them at early stages of cancer development is the major limitation(21, 39). The immune system acts as an extremely sensitive reporter for identification of new altered proteins. (1, 40)

TAA's are tumour specific proteins and peptides which are subject to dysregulation, Mutation or post translational modification (PTM) during cancer development and have been reported as potential causes of an (auto-) antibody response.(1, 3, 21) The antibody molecules are stable in the blood and small quantity of antigen can trigger a larger immune response that is reflected in relative antibody concentrations and are also detectable at early stages of disease. They are naturally resistant to proteolysis and metabolism experienced by other molecules, attributing to their long half-life of approximately 21 days. (21, 39, 41-43) So the autoantibodies are useful as biomarkers and can be applied to cost-efficient and diagnostically and clinically relevant assays(2, 44). TAA's might become interesting therapeutic targets(45-47). Identification of antigen proteins capable of triggering a significant humoral immune system response is important for purposes such as diagnostic applications and vaccine design.(48)

Autoantibodies could be used for early prediction of the disease onset because they can exist years before the diagnosis of an autoimmune disease(49, 50). personalized profiles of TAA's and autoantibodies should be used to identify therapeutic targets to develop vaccines for targeted immunotherapy against cancer.(1)

CONCLUSION

Discovery of new serum biomarkers expressing an increased sensitivity and specificity for cancer are important for diagnosis improvement. Identification of autoantigens and the detection of autoantibody are useful in biomarker discovery. During these past few years proteomic approaches such as SERPA and one dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis or enzyme-linked immunosorbent assay (ELISA) studies on tumor-associated antigens have allowed identification of great numbers of antigens and their autoantibodies in different types of cancer. However, they are also time consuming, and lack sensibility and specificity. New proteomic techniques may hold the key to overcoming these limitations based on protein or antibody arrays which allow high throughput analysis of multiple targets in a single experiment. These proteomic technologies are useful to screen large numbers of antigens at once with small numbers of sera and can be used for the generation of a panel of TAA's that exhibit better diagnostic value than a single TAA marker. In conclusion, these approaches would improve sensitivity and specificity of detection for autoantibody profiling and promise great advances in the field of biomarkers for cancer. Comprehensive profiling of (auto) antigens provide insight into the novel diagnosis and treatment strategies for autoimmune diseases, cancer, infection, and transplantation therapy. Nevertheless, extensive validation of array results will be essential for regulatory approval and for entry into clinical application on large populations.

REFERENCES

- [1] Heo C-K, Bahk YY, Cho E-W. Tumor-associated autoantibodies as diagnostic and prognostic biomarkers. *BMB reports*. 2012;45(12):677-85.
- [2] Murphy MA, O'Leary JJ, Cahill DJ. Assessment of the humoral immune response to cancer. *Journal of proteomics*. 2012;75(15):4573-9.
- [3] Tan HT, Low J, Lim SG, Chung M. Serum autoantibodies as biomarkers for early cancer detection. *FEBS journal*. 2009;276(23):6880-904.

- [4] Sahin U, Türeci O, Schmitt H, Cochlovius B, Johannes T, Schmits R, et al. Human neoplasms elicit multiple specific immune responses in the autologous host. *Proceedings of the National Academy of Sciences*. 1995;92(25):11810-3.
- [5] Brichory FM, Misek DE, Yim A-M, Krause MC, Giordano TJ, Beer DG, et al. An immune response manifested by the common occurrence of annexins I and II autoantibodies and high circulating levels of IL-6 in lung cancer. *Proceedings of the National Academy of Sciences*. 2001;98(17):9824-9.
- [6] Minenkova O, Pucci A, Pavoni E, De Tomassi A, Fortugno P, Gargano N, et al. Identification of tumor-associated antigens by screening phage-displayed human cDNA libraries with sera from tumor patients. *International journal of cancer*. 2003;106(4):534-44.
- [7] Zhong L, Peng X, Hidalgo GE, Doherty DE, Stromberg AJ, Hirschowitz EA. Anti-bodies to HSP70 and HSP90 in serum in non-small cell lung cancer patients. *Cancer detection and prevention*. 2003;27(4):285-90.
- [8] Fischer E, Kobold S, Kleber S, Kubuschok B, Braziulis E, Knuth A, et al. Cryptic epitopes induce high-titer humoral immune response in patients with cancer. *The Journal of Immunology*. 2010;185(5):3095-102.
- [9] Zhong L, Ge K, Zu J-c, Zhao L-h, Shen W-k, Wang J-f, et al. Autoantibodies as potential biomarkers for breast cancer. *Breast Cancer Res*. 2008;10(3):R40.
- [10] Wang X, Yu J, Sreekumar A, Varambally S, Shen R, Giacherio D, et al. Autoantibody signatures in prostate cancer. *New England Journal of Medicine*. 2005;353(12):1224-35.
- [11] Ran Y, Hu H, Zhou Z, Yu L, Sun L, Pan J, et al. Profiling tumor-associated autoantibodies for the detection of colon cancer. *Clinical Cancer Research*. 2008;14(9):2696-700.
- [12] Geysen HM, Meloen RH, Barteling SJ. Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. *Proceedings of the National Academy of Sciences*. 1984;81(13):3998-4002.
- [13] Hueber W, Utz PJ, Steinman L, Robinson WH. Autoantibody profiling for the study and treatment of autoimmune disease. *Arthritis research*. 2002;4(5):290-5.
- [14] Anderson KS, LaBaer J. The sentinel within: exploiting the immune system for cancer biomarkers. *Journal of proteome research*. 2005;4(4):1123-33.
- [15] Won CH, Kang YJ, Lee DH, Kim KH, Park NH, Kwon SO, et al. Comparative secretome analysis of human follicular dermal papilla cells and fibroblasts using shotgun proteomics. *Biochemistry and Molecular Biology Reports*. 2012;45(4):253-8.
- [16] Ohyama K, Kuroda N. Challenge of Mass Spectrometry toward the Elucidation of Life Phenomena. *Biol Pharm Bull*. 2012;35(9):1409-12.
- [17] Balboni I, Limb C, Tenenbaum JD, Utz PJ. Evaluation of microarray surfaces and arraying parameters for autoantibody profiling. *Proteomics*. 2008;8(17):3443-9.
- [18] Balboni I, Chan SM, Kattah M, Tenenbaum JD, Butte AJ, Utz PJ. Multiplexed protein array platforms for analysis of autoimmune diseases. *Annu Rev Immunol*. 2006;24:391-418.
- [19] Robinson WH, DiGennaro C, Hueber W, Haab BB, Kamachi M, Dean EJ, et al. Autoantigen microarrays for multiplex characterization of autoantibody responses. *Nature medicine*. 2002;8(3):295-301.
- [20] Friedman DB, Hoving S, Westermeier R. Isoelectric focusing and two-dimensional gel electrophoresis. *Methods in enzymology*. 2009;463:515-40.
- [21] Martin K, Ricciardelli C, Hoffmann P, Oehler MK. Exploring the immunoproteome for ovarian cancer biomarker discovery. *International journal of molecular sciences*. 2011;12(1):410-28.
- [22] Klade CS, Voss T, Krystek E, Ahorn H, Zatloukal K, Pummer K, et al. Identification of tumor antigens in renal cell carcinoma by serological proteome analysis. *Proteomics*. 2001;1(7):890-8.
- [23] Canelle L, Bousquet J, Pionneau C, Deneux L, Imam-Sghiouar N, Caron M, et al. An efficient proteomics-based approach for the screening of autoantibodies. *Journal of immunological methods*. 2005;299(1):77-89.
- [24] Caron M, Choquet-Kastylevsky G, Joubert-Caron R. Cancer immunomics using autoantibody signatures for biomarker discovery. *Molecular & Cellular Proteomics*. 2007;6(7):1115-22.
- [25] Hardouin J, Lasserre JP, Canelle L, Duchateau M, Vlieghe C, Choquet-Kastylevsky G, et al. Usefulness of autoantigen depletion to detect autoantibody signatures by multiple affinity protein profiling. *Journal of separation science*. 2007;30(3):352-8.

- [26] Ehrlich JR, Qin S, Liu BC. The reverse capture autoantibody microarray: a native antigen-based platform for autoantibody profiling. *Nature protocols*. 2006;1(1):452-60.
- [27] Maki T. Challenge of Mass Spectrometry toward the Elucidation of Life Phenomena. *Biol Pharm Bull*. 2012;35(9):1413-6.
- [28] Bouwman K, Qiu J, Zhou H, Schotanus M, Mangold LA, Vogt R, et al. Microarrays of tumor cell derived proteins uncover a distinct pattern of prostate cancer serum immunoreactivity. *Proteomics*. 2003;3(11):2200-7.
- [29] Kijanka G, Murphy D. Protein arrays as tools for serum autoantibody marker discovery in cancer. *Journal of proteomics*. 2009;72(6):936-44.
- [30] Kricka LJ, Master S, Joos T, Fortina P. Current perspectives in protein array technology. *Annals of clinical biochemistry*. 2006;43(6):457-67.
- [31] Davies DH, Liang X, Hernandez JE, Randall A, Hirst S, Mu Y, et al. Profiling the humoral immune response to infection by using proteome microarrays: high-throughput vaccine and diagnostic antigen discovery. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;102(3):547-52.
- [32] Vigil A, Chen C, Jain A, Nakajima-Sasaki R, Jasinskas A, Pablo J, et al. Profiling the humoral immune response of acute and chronic Q fever by protein microarray. *Molecular & Cellular Proteomics*. 2011;10(10).
- [33] Yu X, Schneiderhan-Marra N, Hsu H-Y, Bachmann J, Joos TO. Protein microarrays: effective tools for the study of inflammatory diseases. *Reverse Chemical Genetics*: Springer; 2009. p. 199-214.
- [34] Gnjatic S, Ritter E, Büchler MW, Giese NA, Brors B, Frei C, et al. Seromic profiling of ovarian and pancreatic cancer. *Proceedings of the National Academy of Sciences*. 2010;107(11):5088-93.
- [35] Ramachandran N, Hainsworth E, Bhullar B, Eisenstein S, Rosen B, Lau AY, et al. Self-assembling protein microarrays. *Science*. 2004;305(5680):86-90.
- [36] Madoz-Gúrpide J, Wang H, Misek DE, Brichory F, Hanash SM. Protein based microarrays: a tool for probing the proteome of cancer cells and tissues. *Proteomics*. 2001;1(10):1279-87.
- [37] Nam MJ, Madoz-Gurpide J, Wang H, Lescure P, Schmalbach CE, Zhao R, et al. Molecular profiling of the immune response in colon cancer using protein microarrays: Occurrence of autoantibodies to ubiquitin C-terminal hydrolase L3. *Proteomics*. 2003;3(11):2108-15.
- [38] Qiu J, Madoz-Gurpide J, Misek DE, Kuick R, Brenner DE, Michailidis G, et al. Development of natural protein microarrays for diagnosing cancer based on an antibody response to tumor antigens. *Journal of proteome research*. 2004;3(2):261-7.
- [39] Anderson NL, Anderson NG. The human plasma proteome history, character, and diagnostic prospects. *Molecular & Cellular Proteomics*. 2002;1(11):845-67.
- [40] Leslie D, Lipsky P, Notkins AL. Autoantibodies as predictors of disease. *Journal of Clinical Investigation*. 2001;108(10):1417-22.
- [41] Tragglia E, Puzone R, Lanzavecchia A. Antigen dependent and independent mechanisms that sustain serum antibody levels. *Vaccine*. 2003;21:S35-S7.
- [42] Slifka MK, Ahmed R. Long-lived plasma cells: a mechanism for maintaining persistent antibody production. *Current opinion in immunology*. 1998;10(3):252-8.
- [43] Morell A, Terry WD, Waldmann TA. Metabolic properties of IgG subclasses in man. *Journal of Clinical Investigation*. 1970;49(4):673.
- [44] Hanash S. Harnessing immunity for cancer marker discovery. *Nature biotechnology*. 2003;21(1):37-8.
- [45] Knutson K, Disis M. Tumor antigen-specific T helper cells in cancer immunity and immunotherapy. *Cancer Immunology, Immunotherapy*. 2005;54(8):721-8.
- [46] Casal JI, Barderas R. Identification of Cancer Autoantigens in Serum. *Molecular diagnosis & therapy*. 2010;14(3):149-54.
- [47] Tan EM, Zhang J. Autoantibodies to tumor-associated antigens: reporters from the immune system. *Immunological reviews*. 2008;222(1):328-40.
- [48] Magnan CN, Zeller M, Kayala MA, Vigil A, Randall A, Felgner PL, et al. High-throughput prediction of protein antigenicity using protein microarray data. *Bioinformatics*. 2010;26(23):2936-43.
- [49] Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *New England Journal of Medicine*. 2003;349(16):1526-33.

- [50] Hu S, Vissink A, Arellano M, Roozendaal C, Zhou H, Kallenberg CG, et al. Identification of autoantibody biomarkers for primary Sjögren's syndrome using protein microarrays. *Proteomics*. 2011;11(8):1499-507.