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Review

Using immunoproteomics to identify tumor-associated antigens (TAAs) as biomarkers in cancer immunodiagnosis

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ABSTRACT

Since intracellular proteins involved in carcinogenesis have been shown to provoke autoantibody responses, autoantibodies can be used as probes in immunoproteomics to isolate, identify, and characterize potential tumor-associated antigens (TAAs). Once a TAA is identified, several approaches will be used to comprehensively characterize and validate the identified TAA/anti-TAA systems that are potential biomarkers in certain types of cancer. Our ultimate goal is to establish rigorous criteria for designation of an autoantibody to a TAA as a cancer biomarker, examine candidate TAAs for sensitivity and specificity of anti-TAA antibody response, and further develop customized TAA arrays that can be used to enhance anti-TAA antibody detection in cancer. This review will mainly focus on the recent advances in our studies using immunoproteomic approach to identify and characterize TAAs as biomarkers in cancer.

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1. Introduction

Cancer has long been recognized as a multi-step process, which involves not only genetic changes conferring growth advantage but also factors that disrupt regulation of growth and differentiation [1–3]. It is possible that some of these factors could be identified and their functions evaluated with the aid of autoantibodies arising during tumorigenesis. Although the mechanisms leading to autoantibody production in cancer patients are not completely understood, emerging evidence indicates that most tumor-associated antigens (TAAs) are cellular proteins whose aberrant regulation of function could be linked to malignancy [4]. Several approaches are currently available for the identification of TAAs in cancer. One of the approaches is the utilization of serum antibodies from cancer patients to immunoscreen cDNA expression library to identify TAAs in cancer, and some of these identified TAAs may have potential diagnostic values in cancer diagnosis. Another approach involving the use of a proteome-based methodology, which is generally named as immunoproteomics, has been recently implemented in our laboratory for the identification of TAAs in cancer [5–7]. The practical utility of these approaches remains to be established with the proviso that efforts should be made to identify tumor-associated from tumor-irrelevant antigens. This review will mainly focus on the recent advances in our studies using immunoproteomic approach to identify and characterize TAAs as biomarkers in cancer.

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2. Tumor-associated antigens (TAAs) and anti-TAA autoantibodies

As described above, many studies have demonstrated that cancer sera contain antibodies that react with a unique group of autologous cellular antigens called TAAs [8,9]. The types of cellular proteins that induce these autoantibody responses are quite varied and include the tumor suppressor p53 [10,11] and p16 [12], oncogene products such as c-Myc [13], HER-2/neu [14], and CIP2A/p90 [15,16], proteins that protect mRNAs from degradation such as IMPs [17] and onconeural antigens [18], differentiation-antigens such as tyrosinase and cancer/testis antigens [19], and anti-apoptotic proteins such as survivin [20] and LEDGF [21]. The different factors leading to the increased production of such autoantibodies are not completely understood. However, available data show that many of the target antigens are cellular proteins like p53 whose aberrant regulation or overexpression could lead to tumorigenesis [10,11]. A highly informative study showed that in lung tumors containing several types of p53 gene mutations, including missense, stop codon and frameshift mutations, only the missense p53 mutations, with overexpression of a protein that altered function and increased the protein stability, correlated with autoantibody production [22]. In the case of the mRNA binding protein p62, a fetal protein absent in adult tissues, the presence of autoantibodies relates to abnormal expression of p62 in tumor cells [23]. The immune systems of certain cancer patients appear to sense these aberrant tumor-associated proteins as foreign antigens and have the capability to respond by producing autoantibodies which we generally called anti-TAA antibodies [24]. Thus, these anti-TAA antibodies might be regarded as reporters identifying aberrant de novo or dysregulated cellular mechanisms in tumorigenesis [4,8,9]. In recent years, the potential utility of TAAs and anti-TAA antibody systems as early cancer biomarker tools to monitor therapeutic outcomes or as indicators of disease prognosis has been extensively explored.

Interest in the use of anti-TAA antibodies as serological markers for cancer diagnosis derives from the recognition that these antibodies are generally absent, or present in very low titers, in normal individuals and in non-cancer conditions [8,9,25]. Their persistence and stability in the serum of cancer patients is an advantage over other potential markers, including the TAAs themselves, which are released by tumors but rapidly degrade or are cleared after circulating in the serum for a limited time [24]. Furthermore, the widespread availability of methods and reagents to detect serum autoantibodies facilitates their characterization in cancer patients and assay development. However, in contrast to autoimmune diseases, where the presence of a particular autoantibody may have diagnostic value, cancer-associated autoantibodies, when evaluated individually, have little diagnostic value primarily because of their low frequency. We have observed that this drawback can be overcome by using mini-arrays of carefully selected TAAs, and that different types of cancer may require different TAA arrays to achieve the sensitivity and specificity required to make immunodiagnosis a feasible adjunct to tumor diagnosis [26].

3. Immunoproteomic approach in the identification of TAAs

The methods which we have used in the identification of putative TAAs have involved initially examining the sera from cancer patients using extracts of tissue culture cells as source of antigens in western blotting and by indirect immunofluorescence on whole cells. With these two techniques, we identify sera which have high-titer fluorescent staining or strong signals to cell extracts on western blotting and subsequently use the serum antibodies either in isolating cDNA clones from cDNA expression libraries or in immunoproteomics to identify tumor-associated proteins. Using the approach of immunoscreening cDNA expression libraries, several novel TAAs including HCC1 [27], SG2NA [28], CENP-F [29], p62/IMP2 [30] and p90/CIP2A [15,16] have been identified. Several novel as well as previously defined tumor antigens have been also identified with autoantibodies from patients with different types of cancer [31] using a methodology called SEREX (serological analysis of recombination cDNA expression libraries) [32], which is essentially a modification of our previous approach [27–30]. Immunoscreening of cDNA libraries with serum antibodies for identifications of autoantigens is a well-established method and has been used to identify not only TAAs but also antigens in autoimmune diseases [33]. This methodology was the basis of the methods described in SEREX with the difference that cDNA expression libraries constructed from autologous patient tumor were used as substrate in immunoscreening. Subsequent reports using the SEREX technique have shown that the TAAs identified are not different from standard methods using cDNA expression libraries from cell lines derived from different sources, so that there did not appear to be any advantage to using cDNA libraries from autologous patients.

In the past decade, the proteomic approach has been extensively implemented for identifying tumor-associated proteins in cancer patients [34,35]. Compared to the approach of immunoscreening cDNA expression libraries, which we have previously used, the immunoproteomic technology allows individual screening of a large number of sera, as well as determination of a large number of cancer-related antigens. The immunoproteomic approach can also distinguish isoforms and the detection of autoantibodies directed against post-translational modifications (PTMs) of specific targets. It is well known that mRNA levels do not necessarily correlate with corresponding protein abundance [36]. Additional complexity of protein is conferred by PTMs including phosphorylation, acetylation, and glycosylation, as well as protein cleavage [37]. These modifications may not reflect any change at the mRNA level but play important roles in protein stability, activity and functions. Intracellular proteins may also participate in the transformation of a healthy cell into a neoplastic cell. Therefore, protein levels may be more accessible and relevant to therapeutic targets than mRNA levels.

A brief description of the immunoproteomic approach we have used to identify and characterize TAAs is shown in Fig. 1. Briefly, the sera from cancer patients were initially examined using extracts of tissue culture cells as the source of antigens in western blot and by indirect immunofluorescence (IIF) on whole cells. With these two techniques, we identified sera that have high-titer fluorescent staining or strong signals to cell extracts on western blot and subsequently used the antibodies in these sera as probes in a proteomic approach to isolate potential TAAs. Cell extract of cultured cancer cells was applied onto the first dimension isoelectrofocusing gel (1D-IEF), and subsequently loaded onto the second-dimension gel (2D-SDS-PAGE). The protein was transferred to the nitrocellulose membrane or visualized by Coomassie blue staining (or silver staining). After immunoblotting with cancer sera and normal human sera (as controls), a number of protein spots of interest were excised from the 2D gels, digested by trypsin, and subsequently analyzed by mass spectrometry (MS). In subsequent studies, we will use several approaches such as enzyme-linked immunosorbent assay (ELISA) and western blot and immunohistochemistry (IHC) with tissue arrays to comprehensively characterize and validate the identified tumor-associated antigen-antibody systems that are potentially useful in cancer immunodiagnosis, and then evaluate the sensitivity and specificity of different antigen-antibody systems as markers in certain type of cancer for further developing "TAA array" systems for cancer diagnosis, for prediction, and for following the response of patients to treatment.

A potential pitfall in using an immunoproteomic approach is that most likely several potential TAAs will not be detected, because of various limitations of two-dimensional gel electrophoresis (2-DE) followed by in-gel trypsin digestion [38,39]. First, highly hydrophobic, very large, or highly post-translationally modified (i.e., glycosylated, phosphorylated) proteins are difficult to be resolved in the 2-DE. Secondly, in-gel digestion of proteins resolved by 2-DE results in very low recovery of peptides. In solving the problem, we suggest to use