Published in final edited form as:
Cancer Biomark. 2010 ; 6(0): 257–270. doi:10.3233/CBM-2009-0138.

Usage of cancer associated autoantibodies in the detection of disease

Steven P. Dudas[a], **Madhumita Chatterjee**[a], and **Michael A. Tainsky**[a,b,*]

[a]Program in Molecular Biology and Genetics, Karmanos Cancer Institute and Department of Pathology, Wayne State University School of Medicine, Warren, Detroit, MI, USA

[b]Program in Molecular Biology and Genetics, Barbara Ann Karmanos Cancer Institute, MI, USA

Abstract

Efforts toward deciphering the complexity of the tumor specific proteome by profiling immune responses generated against tumor associated antigens (TAAs) holds great promise for predicting the presence of cancer long before the development of clinical symptoms. The immune system is capable of sensing aberrant expression of certain cellular components involved in tumorigenesis and the resultant autoantibody response provides insights to the targets that are responsible for eliciting immunogenicity to these cellular components. Analysis of the cancer-specific humoral immune response has led to panels of biomarkers that are specific and sensitive biomarkers of disease. Using multianalyte-based in vitro analytical discovery platforms which can be easily adapted into clinical diagnostic screening tests, body fluids such as serum, plasma saliva, or urine can be interrogated to detect autoantibodies against natural or recombinant antigens, which may possess etiologic significance to cancer. Non-invasive screening tests exhibiting high specificity and sensitivity to detect early stage cancer in the heterogeneous population of cancer patients potentially have the greatest impact in decreasing mortality rates. Overall, this review summarizes different experimental approaches in the development of diagnostic screening tests for the early detection of cancer and their implementation in the development of clinical multianalyte biomarker assays.

Keywords

Diagnostic biomarker; Humoral immune response; immunogenicity; protein microarray; antigen microarrays; tumor associated antigens; autoantibody

1. Introduction

In the evolving field of diagnostic assays for cancer detection, extensive research has identified a variety of mechanisms by which cancer cells provide molecular markers for their own detection. Researchers are identifying and studying different classes of analytes in the body fluids of cancer patients with the objective of developing clinically applicable assays useful in the detection, diagnosis, and treatment of the disease. We and others are exploiting the cancer patient's own immune response by evaluating cancer-associated autoantibodies generated against autologous cellular components produced by an individual's tumor cells as measurable analytes in blood. These autologous cellular components generally referred to tumor-associated antigens (TAA) have been recognized

*Corresponding author: Michael A. Tainsky, Program in Molecular Biology and Genetics, Barbara Ann Karmanos Cancer Institute, 110 E. Warren, Prentis 3115, Detroit, MI 48201-3917, USA. Tel.: +1 313 578 4340; Fax: +1 313 832 7294, tainskym@karmanos.org.

and evaluated as markers of disease state for decades (see Table 1). Indeed most of the FDA approved blood based assays for the evaluation of disease state in cancer patients is in the determination of the serum levels to these TAAs [57]. To distinguish cancer state from non-cancer, we and others are undertaking the development of serological tests that determine the presence of autoantibodies to TAAs rather than assessing the level of any particular TAA in the blood. A review of the current state of this area of research in the development of cancer biomarkers will be covered as well as a presentation of the potential advantages of this approach for future of cancer diagnostics.

A central aspect of any assay development is the search for informative biomarkers specific to the disease state. For this discussion, the definition of a biomarker is any molecule that indicates an alteration of the physiological state of an individual relevant to a disease state, drug treatment, toxins, or other environmental stimuli. Implied in this definition is that a biomarker is not static, but varies over time in relationship to the physiological state that it is reporting. In this field, cancer associated autoantibodies are being considered as excellent candidates for cancer biomarkers as they represent biosensors that the immune system provides, indicative of its response to a developing tumor. The generation of these autoantibodies in response to autologous cellular antigens would not be static, but would present as measurable physiological changes that one could relate to the disease state in question. Immunoglobulins are extremely stable in serum samples and are known to persist for extended times after the removal of the its antigenic factor [13]. This is a distinct advantage for their use over other potential markers in body fluids which are usually comprised of either proteins or polynucleotides. These classes of molecules once released by tumors are known to be quickly degraded and/or removed from circulation after a related short duration of time. The lack of long-term stability of non-immunoglobulin associated tumor markers in samples is a significant technical barrier to their implementation as biomarkers because of a limit half-life in serum and therefore their limited window of detectability. Indeed the stability and usefulness of immunoglobins have already been recognized in the development of laboratory tests for other diseases. There are numerous commercially available tests designed to indentify human antibodies in serum for various viral proteins and autoantibodies for autoimmune disease.

The main objective of autoantibody biomarker discovery is to indentify molecular markers capable of discriminating the “healthy” state from the “disease” state, preferentially in the asymptomatic, preclinical phase of the cancer, and therefore may improve the patient's outcome. Early detection of any cancer is generally accepted as the best approach to reduce mortality rates from cancer [28]. Biomarker screening tests must enhance the diagnostic value of physical examination, medical tests, or other procedures. As in cancer screening procedures such as mammography or screening colonoscopy, asymptomatic patients undergo such tests to find any initial signs of cancers. An important feature of the study designs of biomarker research projects is to envision the purpose of the test at the initial discovery phase, with a strategy to identify analytes that can be readily translated into a clinical assay. Different strategies have been employed to develop biomarkers often based on a novel technology with intrinsic technologies that direct and focus the studies to particular types of analytes and with particular uses [69,113]. At this level, significant research effort is being directed at the development of assays for the early detection of cancer. We will refer to markers identified for the early detection as diagnostic markers. The objective for such diagnostic markers would be to discriminate with adequate classification performance (sensitivity and specificity) the disease state (malignant or benign) from the healthy individual within an at risk screening population.

The hypothesis for autoantibody biomarker research is that the immune system can be exploited as a biosensor of disease-related changes in the proteome, and by evaluating this

system's reactivity to human TAAs, diagnostic assays can be created to detect small curable tumors or that prognostic assays can be developed to predict tumor behavior. The history of biomarker research has made it clear that tests dependent on single cancer markers are inadequate to identify tumor bearing patients in an at risk screening population [10,12,14, 15,31,45,50,51,58,71,95,108,109]. Rather, biomarker researchers are convinced that panels of analytes will be required to produce clinical tests with sufficient sensitivity and specificity for the early detection of cancer [82]. This lesson was learned from the lack of acceptance of single biomarkers into clinical practice to distinguish subjects with cancer from those without cancer. The objective of the autoantibody approach is to develop whole proteome-based technologies to accurately identify those protein biomarkers recognized by the cancer-specific immune system. The methods being applied in this field vary from candidate tumor-specific protein analytes to totally undirected TAA biomarker identification.

1.1. Immunologic response to cancer: Basics of the immune response

The two arms of the immune system, the humoral and cell-mediated immunity, are the integral parts of the body's defense mechanism that protects us from microbial infections. Both of these parts are known to be actively involved in the body's own response to cancer. The autologous cellular components that are aberrantly expressed in cancer and present reactive epitopes to immune system consist almost entirely of intracellular self-antigens [19]. The proteins presenting these epitopes that are evoking the production of cancer-specific autoantibodies predominately function in cell cycle regulation, DNA repair and replication, and RNA processing. The immune system has great potential in sensing these aberrant expressed TAAs. The most important contributor to this enormous bio-sensory power of immune system is the immune effector, the T-cell. These cells encounter the tumor generated self-antigens presented by antigen presenting cells (APC), and in combination with MHC, become activated to perform their immunological regulatory role. The immune response to cancer has been extensively studied not only with the objective of understanding the disease, but also with the possible hopes of developing immunotherapies to combat the illness. This has lead to a wealth of knowledge which is being utilized towards the detection of the disease.

It is now known that a number of different changes can occur in the structure or expression pattern of certain cellular components during tumorigenesis triggering the immune system to recognize self antigens as non-self/foreign. First, endogenous self-antigens have been shown to exhibit immunogenicity when they are overexpressed during tumorigenesis [26]. Although T-cells encounter most of the self antigens due to promiscuous gene expression by medullary thymic epithelial cells [47] during their maturation in thymus and get tolerized, growing evidence still supports the presence of self-reactive T-cells in the T-cell repertoire. Studies have shown that only properly processed self-antigen determinants are able to tolerize T-cells. However, self-antigens may have ‘subdominant’ or ‘cryptic’ determinants that are poorly processed from native molecule and inefficiently presented to T cells [20]. When these self-antigens are overexpressed in cancer, the cryptic determinants are eventually presented to T-cells in a co-stimulatory environment thus eliciting immunological responses [65]. Second, genetic mutations leading to the formation of structurally altered proteins bearing MHC class II-restricted immunogenic determinants are highly predominant in cancer cells. For example, autoantibodies against p53 bearing missense mutations in lung cancer has been documented [104]. Immunological responses against frame-shift mutations have been also detected in colorectal cancer patients [53, 75, 81]. Reports from different studies showed that fusion proteins produced by chromosomal translocations in cancer may bear immunogenic HLA class II-restricted epitopes that can activate CD4+ T-cells in cancer patients. For example, BCR-ABL fusion protein produced by t(9;22) is expressed in most cases of chronic myelogenous leukemia (CML). Presentation of BCR-ABL derived peptides

by CML-derived dentritic cells (DC) have been shown to activate fusion peptide specific CD4+ T lymphocytes in an HLA class-II restricted manner [107]. Third, alternative splicing of pre-messenger RNA is a key molecular mechanism resulting in the formation of different mRNA variants that encode different polypeptides endowed with different biological functions and thus could have a profound impact on tumor development and progression [85,97]. Both mutations in cis-acting splicing elements and changes in expression pattern of effector molecules involved in splicing regulatory network, severely affect splicing profile of many cancer-related genes. Bourdon et al. showed that different isoforms of p53 that originate through alternative splicing are expressed in a tissue-dependent manner and that their expression pattern is altered in human breast tumors [9]. Line et al. identified an alternatively spliced isoform known as transforming acidic coiled–coli protein1 (TACC1) in gastric cancer using SEREX technology [52]. Fourth, translation of mRNA from alternative open reading frame (AORF) can lead to the generation of proteins bearing immunogenic determinants that can trigger humoral immune responses in cancer patients. For example, antigenic peptides encoded from AORF of NY-ESO-1, a cancer testis (CT) antigen, have been shown to elicit immunological responses in cancer patients [19,98]. Another CT antigen, CAMEL, has been shown to be translated from AORF of the LAGE gene that is highly homologous to NY-ESO-1 gene [19, 98]. Studies have shown the existence of CAMEL specific CD4+ T-cell clones in melanoma patients [84]. Several mechanisms have been put forward that result in the translation from AORFs: i) Kozak et al. reported the leaky-scanning model that describes the malfunction of poor KOZAK sequence in recruiting the ribosomes to a downstream AUG instead of the correct 5′ AUG translation start site [44]; ii) Weiss et al. suggested a ribosomal frame-shifting mechanism in which the ribosome is allowed to change the reading frame because of the presence of a “slippery site” in the middle of translation [102]; and iii) termination read-through is a mechanism that allows ribosome to continue translation even after encountering stop signal [8]. Fifth, post-translational modifications of the expressed antigens such as changes in glycosylation [38], phosphorylation [27] can affect antigen processing, binding of processed antigen with MHC molecule, and interaction of antigen-MHC complex with the T-cell receptor. Aberrant glycosylation has been observed in many cancers presenting these modified glycosyl epitopes as TAAs that can override tolerance and induce humoral immunity in cancer patients. Tarp et al. reported the presence of immunodominant epitopes on GalNAc∞1-O-Ser/Thr and Neu Ac∞2-6GalNAc∞1-O-Ser/Thr glycosyl moieties of MUC1, which can induce humoral immunity in MUC-transgenic mice [87]. Deregulated phosphorylation of many oncogenes is a major event in malignant transformation. Protein phosphorylation generally occurs on tyrosine, serine or threonine residues by different cellular protein kinases and the phosphopeptide antigen derived from these phosphorylated oncogenes have been shown to be recognized by class I MHC-restricted T-cells [2].

Unfortunately, in many instances, the generation of tumor autoantibodies to many of the overexpressed proteins or other cellular abnormalities can be inhibited by several mechanisms. As example, one of the first serum cancer biomarkers described, CEA [33,34], typically does not elicit an autoimmune response. The elevated levels of this protein are associated with the prognosis of multiple cancers yet rarely are naturally occurring human antibody to the CEA protein found [90]. One mechanism that naturally inhibits generation of self-antigen recognitions is due to the promiscuous gene expression in thymus. Several TAAs such as melanoma antigen [MAGE] or differentiation antigens have been shown to be expressed in medullary thymic epithelial cells (mTECs) beside their normal expression that is restricted to male germ cells in the testis. Although melanoma antigens are being used clinically for immunotherapy, they probably impose some degree of tolerance due to their expression in mTECs thus lowering the effectiveness of immune responses in cancer patients [36]. There are also several other mechanisms by which tumors can evade immune responses-i) down-regulation of the antigen-processing machinery such as MHC class I

molecules has been observed in different cancers like breast, prostate and lung cancer [61]; ii) down-regulation of the transporter associated with antigen presentation (TAP) genes as well as components of the immunoproteosome such as LMP-2 and LMP-7 have likewise been documented in a number of tumor types [42,76]; iii) tumors and/or their surrounding stroma may produce immunosuppressive factors like TGF-β, IL-10 and vascular endothelial growth factor (VEGF) that can induce production of immature myeloid cells and regulatory T-cells (Tregs) that inhibits dendritic cell maturation and activation of T-cells in a tumor-specific immune response [46]; iv) lack of expression of co-stimulatory molecules on the tumor cells can lead to anergy of tumor reactive T-cells [17]; v) tumor cells express FasL that bind to Fas receptors on T-cells leading to T-cell apoptosis thereby providing immunologic resistance with consequent tumor growth [37].

1.2. Autoimmune disease and lessons learned

Autoimmune diseases are characterized by the activation of body's immune responses directed against its own tissues due to breakdown of immune tolerance to specific self-antigens causing prolonged inflammation and subsequent tissue destruction. This is associated with the production of increased concentration of antibodies to ribonucleoproteins, double-stranded DNA, phospholipids and other cellular constituents as observed in patients diagnosed with systemic lupus erythematosus (SLE) which provoke pro-inflammatory responses [6]. The autoantibodies most frequently detected in RA patients are rheumatoid factor (RF) and antibodies directed toward citrullinated peptides (antiCCP) [80,91]. The humoral immune response to TAAs elicited in cancer patients has many parallels to the autoimmune disease response [55]. Most of cancer-associated autoantibodies are directed toward similar classes of tumor induced self-antigens. Reports from different studies indicate that repertoire of tumor autoantibodies overlaps to a significant extent with the typical patients with autoimmune diseases. For example, antinuclear antibodies associated with RA, SLE are more prevalent in patients with cancer than in healthy individuals [30]. Antibodies to single- and double-stranded DNA, the Ro antigen, the La antigen, and the small ribo-nuclear protein particle have been observed both in cancer patients and in patients with autoimmune diseases [32, 59,110]. Thus, patients with malignancies may develop autoimmune, SLE or rheumatic manifestations. Therefore, a panel of good candidate TAAs for cancer immunotherapy should be chosen in such as way that the activation of the immune responses against those antigens promotes a favorable clinical outcome without the development of autoimmunity.

1.3. Autoantibodies as reporters of early carcinogenesis

The role of tumor autoantibodies as reporters of early carcinogenesis has been well documented. It has been reported that anti-p53 antibodies may develop months to years before the clinical diagnosis of cancer [1]. Anti-p53 antibodies have been detected in the sera of workers exposed to vinyl chloride who developed angiosarcoma of the liver and in the sera of heavy smokers who developed lung cancer [56]. In uranium workers who are also at high risk for lung cancer, anti-p53 antibodies were detected long before clinical manifestations of a tumor [21]. The collateral benefits of the surrogate role of these autoantibodies as reporters in identifying TAAs that are involved in carcinogenesis may improve survival rates in cancer patients as a result of earlier diagnosis.

1.4. Prognostic utility of tumor autoantibodies

Reliable predictive parameters of the disease course are essential particularly in therapeutic decision making to treat cancer at early stage. Cai et al. reported that monitoring of the change of serum p53 antibodies before and after treatment of patients diagnosed with esophageal carcinoma (EC) with radiotherapy can be useful for evaluating the response to the treatment and prognosis of EC patients. Their study showed that the positive rate of p53-

antibodies in patients with EC was related to histological grade, stage of the disease, lymph node metastasis but not to age, sex and site of tumor formation. There was a significant difference in the level of serum p53 antibodies before and after radiotherapy treatment. The positive rate of p53 antibodies in EC patients who responded to radiotherapy was much lower than the patients who did not respond to radiotherapy [11]. Unfortunately in their study, a correlation between serum p53 antibodies in EC patients and the presence of mutations in p53 gene was not performed. The study by Shimada et al. showed that sero-positive esophageal squamous cell carcinoma patients, whose serumanti-p53 titer did not decrease after surgery, showed worse prognosis than patients who showed sero-conversion [83]. Pallasch et al. [67] reported the detection of autoantibodies against 3 different glioma SEREX antigens, namely, GLEA1, GLEA2 and PHD-finger protein3 (PHF3). Their study showed that patients who had autoantibodies against GLEA2 and PHF3 have significant survival compared to patients who did not have GLEA2 and PHF3 antibodies [67]. Thus, a correlative study between the level of tumor autoantibodies and the overall survival outcome of cancer patients (reflected in the change in tumor status or tumor burden related to the therapy) could be extremely informative for evaluating therapeutic interventions.

1.5. Methods for the identification of tumor-specific autoantibodies

As mentioned earlier, a validated set of diagnostic markers will be needed to meet the performance criteria of adequate specificity and sensitivity to discriminate between the “healthy” state from a beginning “disease” state to form the basis for a clinically valuable screening test. A variety of research approaches are currently being used that have the potential to meet these objectives. High throughput approaches have the ability to identify large numbers of tumor associated autoantibodies and are able to relatively easily and inexpensively evaluate large numbers of patient sera. At the initial discovery stage, this ability to multiplex autoantibody detection is critical for the development of complex panels of autoantigens for reliable assays. As with any developing research area, the present work has evolved from previous efforts. Initially most of the methods dealing with self-antigen/autoantibody systems were not developed for this particular objective. Indeed, most of the literature dealing with this field represents the extensive effort that has identified hundreds of TAAs and many of which have not been evaluated for diagnostic efficacy in large, controlled cohorts or in combination with other markers.

In a recent review covering spontaneous humoral immune responses to tumor associated self-antigens, the number of confirmed responses to TAAs is rather limited [75]. A search through the existing literature and reviewing over 3,600 articles reporting evidence of humoral response in cancer patients identified only 107 different tumor antigens. This review did exclude most TAAs found via SEREX or SERPA studies and only citing those that were confirmed upon subsequence independent immunoassays. The detection frequency of tumor-associated autoantibodies seen in patients' blood taken at the time of diagnosis was typically low. This resulted from the technical limitation of SEREX antigens i.e. single TAA/autoantibody pairs as diagnostic markers. The frequency range for even the most extensively studied TAA, p53, has been reported in as little as 7% in some cancers (though it is also been reported to be up to 60% in esophageal cancer [75]. The general estimate even for the best tumor antigens is that they may evoke humoral response in only 5–10% of patients.

Selection of candidate TAA/autoantibody markers through these technologies has allowed for the evaluation of extensive portions of the proteome or epitome with multiple candidate analytes being examined as single assays for cancer diagnosis or prognosis. Nevertheless, the process for the discovery and evaluation of diagnostic markers has not been entirely reliable or reproducible in subsequent validation studies [72]. The three methodologies initially presented here represent high throughput discovery platforms capable of evaluating

large numbers of potential TAAs in combination with large sample numbers. We do not include a review of the numerous other approaches and strategies that have been successful in identifying cancer autoantibodies. Though in a number of these studies the TAA/autoantibodies pairs found were successfully employed in classification assays, secondarily to the discovery phase. The distinction of the high throughput approaches from other methodologies is that within the experimental design these technologies have the ability to discover new candidate markers and simultaneously conduct classification analyses.

1.5.1. Natural protein microarrays: 2-D liquid chromatography antigen microarrays—Initially, natural protein microarray immunoassays require multidimensional protein fractionation of cell line extracts. Printed protein arrays containing around a thousand individual fractions of proteins are then interrogated with sera from cohorts of cancer patients and controls to identify protein antigens reactive with immunoglobulins. Of the methods reviewed, this approach exploits the ability to present the native autologous cellular antigens as a target antigen for autoantibody studies. Arrays printed with cellular proteins retain native conformations and post-translational modifications. Also, this approach captures altered or aberrant proteins which may be due to disease related mutations such as translocations or frameshifts that are represented among the possible epitope repertoire as well as post-translational modifications.

By way of example for this technique, two recent studies that applied this approach, detected autoantibodies in either pre-diagnostic lung cancer sera [71] or sera from pancreatic cancer patients [68]. This approach has also been used in other studies [10, 60, 64]. In both the lung and pancreatic cancer studies, candidate target TAAs were fractionated from human cancer cell lines lysates (human lung adenocarcinoma cell lines A549; Panc-1 pancreatic adenocarcinoma cell line) by two-dimensional liquid chromatography. Proteins were separated in the first dimension either by anion exchange or according to their isoelectric point via high-performance liquid chromatography followed by a second separation by reverse-phase chromatography. Protein fractions were printed in replicates onto nitrocellulose slides to identify candidate antigens. In the lung study, fractions were printed without further treatment, whereas in the pancreatic study, the fractions were further treated with a cyanogen-bromide digestion before printing to expose additional epitopes. Microarrays of natural proteins were generated and interrogated with multiple serum samples. In lung, a series of 85 lung cancer sera and 85 matched controls were applied to individual microarray immunoassays. For the pancreatic studies, a series of 10 normal controls, 10 chronic pancreatitis and 10 pancreatic cancer cases were used. Detection of the autoantibodies was performed using an indirect immunofluorescence with Alexa 647-labeledanti-humanimmunoglobulinG (IgG). In both cases, certain spotted fractions that exhibited high reactivity to immunoglobulins in cancer sera were further analyzed by mass spectrometry (MS) for protein identification.

1.5.2. Phage display antigen microarrays (Epitomics)—Phage display antigen microarrays have recently been developed as a high throughput modification of the basic SEREX approach but allowing for the screening of thousands of antigens as monoclonal cDNA phage display clones simultaneously using only a minimal amount of sera on a microarray with fluorescent detection using scanning technology and advanced bioinformatics to analyze the results. The central strategy is a non-directed approach to isolate TAAs by screening cDNA expression libraries using serum IgGs from cancer patients as probes, and sequencing immuoreactive antigen clones. The SEREX technique was initially described by Sahin et al. in 1995 [78]. Epitomics employs cDNAs libraries prepared from mRNA from cancer cell lines or fresh tumor specimens using T7 phage display cloning technology in which each library contains approximately 10^7 original random-primed protein expression clones. Using a random-primed cDNA library permits

representation of N- and C-terminal epitopes in the library. In the SEREX protocol, primary discovery screenings are performed with plaque lifts on nitrocellulose membranes using single autologous patient for tissue based libraries or small heterogeneous sera groups for cell line derived cDNA libraries. The high throughput epitomics approach altered this strategy by conducting a liquid-phase subtractive biopanning of the phage library with heterogeneous immunoglobulins from normal sera to remove non-specific clones and cancer serum derived immunoglobulins to enrich for the specific clones. Next $1–5 \times 10^3$ phage are picked after serological enrichment of antigen clones. Individual antigen clones are then printed on microarrays allowing the analyses with minimum sample volume < 10 μl, multiple sera, and thousands of candidate antigens. As with SEREX, this epitomic approach affords the ability to rapidly determine TAA identity through DNA sequencing of the selected antigen clones that specifically binds to patients' IgGs. This approach has been employed for the identification of biomarkers in ovarian [16], head and neck [51], breast [31], prostate [99] and lung cancers [111, 112].

1.5.3. Protein microarrays—Protein microarrays are a recently developed proteomic approach that uses a high-throughput technology which allow for the screening of thousands of known proteins in TAA/autoantibody detection systems. These arrays employ known candidates as the spotted proteins often selected by literature searches and then generated by in vitro translation systems. Again, proteins are arrayed on slides and interrogated with serum immunoglobulins to determine immunoreactivity of any candidate antigens. This system was initially described in the identification of autoantigens of autoimmune diseases [77]. The disadvantage of protein microarrays is that the identity of each of the printed proteins used as potential TAAs is known and thus missing as yet undiscovered mutated, translocated, or AORF proteins. The identity of any sero-reactive antigen to autoantibodies is immediately known without further analysis. Also, as a microarray platform, thousands of protein spots can be interrogated simultaneously with a minimal volume of serum. This technology has been applied in the detection of TAA/autoantibodies reactivity in ovarian cancer [40] and breast cancer [3].

1.6. Development of clinically applicable detection assays

Appropriate cancer biomarker-based screening tests should be minimally invasive and reproducible. In addition, screening technology must be sufficiently sensitive to detect cancer at an early or even precancerous stage but specific enough to classify individuals without cancer as being free of disease. Comparison with other conventional technologies, such as ELISA, has indicated that microbead-based immunoassays (Luminex) are reliable, accurate, cost-effective, highly sensitive and have rapid turn around time for results. While there are advantages to these diagnostic platforms, there are still challenges or pitfalls that must be addressed before their acceptance as a technology platform in the routine clinical diagnostic laboratory one being that individual sera can have nonspecific background readings and that there are wide variations in the coupling efficiency of the antigen proteins to the beads.

1.6.1. Multiplex ELISA approach—The ELISA is the most commonly used antibody detection method. This technology has been applied by Imafuku et al. for the detection tumor autoantibodies in cancer patients [41]. Multiplex ELISA can be performed in 96, 384 or 1536-well microtiter plates for high-throughput screening. The avid protein-binding surface of individual wells of the microtiter plates are coated with bait molecules for the capture and detection of circulating analytes of interest in patient sera. After immunobinding of autoantibodies, a substrate-conjugated anti-human IgG is used to generate signal measured with colorimetric, chemiluminescent or fluorescent assays. The advantages of this

multiplex ELISA technology are the easy automaton and the feasibility of measuring the autoantibody titers in patient's serum.

A high throughput version of multiplex ELISA approach is the Reverse Capture Antibody Microarray. This platform is based on the dual-antibody sandwich immunoassay of enzyme-linked immunosorbent assay in which tumor or healthy tissue-derived native antigens are allowed to bind with 500 monoclonal antibodies that are spotted on the array surface. The antigen-antibody arrays are next incubated with fluorescently-labeled IgG from cancer and control samples. Another set of microarrays incubation is carried on simultaneously by swapping the dyes (used to label cancer and control IgGs) to include the dye-effects. The microarrays are next washed, analyzed in a fluorescence microarray scanner and statistical analyses are performed on scanned images. The reverse capture antibody array platform uses native proteins as bait and the autoantibody responses against post-translational modifications of native proteins are readily detectable on this array platform making it superior to other protein microarrays that use recombinant proteins. Tang et al. has applied this technology for the discovery of biomarkers that are involved in pathophysiologic pathways in mucinous ovarian cancer [86].

1.6.2. Bead assays (Luminex-xMAP)—In Luminex technology molecular reactions take place on the surface of microsphere bead sets that are dyed with differing concentrations of two fluorophores to generate distinct bead sets. Each bead set is coated with capture antibody specific for one analyte [43]. Using this method, 100 different microsphere beads each with different analytes can be created and potentially analyzed in parallel. During an assay, capture antibodies covalently bound to the surface of the beads immobilize the analytes of interest. After a washing step to remove unbound materials, detector antibodies are reacted with the beads, followed by addition of an R-phycoerythrin (RPE) conjugate that labels the immune complexes on the beads. The spectral properties of the beads are then monitored with the Luminex® xMAP® instrument where they pass through two lasers that excite the internal dyes identifying each microsphere bead, and the reporter dye (RPE) captured during the assay. Statistical analyses are next performed on the readings as each bead set contains a different antigen. The bead-based suspension array technology allows simultaneous analysis of serum antibodies or proteins with specificities for up to 100 different proteins. Luminex assays have been applied to demonstrate high levels of cytokines in cancer patients with the advantage of low serum consumption [35]. Using this technology Visintin et al. reported that a combination of 6 biomarkers namely, leptin, prolactin, osteopontin, insulin-like growth factor II, macrophage inhibitory factor, and CA-125 was able to detect OV-CA with a sensitivity of 95.3% and a specificity of 99.4% respectively [62]. Luminex technology has its own limitation because human sera contain antibodies that can directly bind to the beads, thereby increasing non-specific background [101].

1.7. Challenges of assay development

Serum levels of non-tumor associated antibodies also exist due to immunological responses to other diseases. Many of the antigen/antibodies reactions seen during the discovery phase will have little utility in the development of serological assays for cancer detection. Appropriate study designs must be implemented for these high-throughput approaches to truly distinguish TAA/autoantibody responses that are informative in profiling of class outcome (healthy verse disease) from the non-informative TAA/autoantibody responses as well as detectable non-informative disease related antigen/antibody responses. Non-informative immune signals will confound the subsequent analysis and critical selection of discovery cohorts is necessary. This requires the generation of study populations that are well documented and representative of a screening population. The samples of such a cohort

should be collected at clinics in standardized protocols. Also, the sample population should represent a targeted population consistent to that of the eventual diagnostic test. Reduction of confounding variables due to inappropriate study cohort characteristics is essential to the validity of experimental results. In addition, these novel technologies must be paired with appropriate computational methods capable of analyzing high-dimensional data generated from large scale biomarker discovery projects. These computational methods are necessary to reduce large analyte panels to smaller ones more suitable for clinical diagnostics.

2. Conclusion

Preliminary results in the development of serological diagnostic assays have demonstrated that the basic experimental tools to accomplish this goal exist. In the future, autoantibody patterns against tumor-specific proteins may achieve high specificity and sensitivity to diagnose disease in screening populations. Each of the technologies presented in this review has inherent limitations in its ability to present inclusive spectra of all possible relevant epitopes for a specific gene. Limitations are due to either the initial selection of candidate target antigens using a non-representative or few cancer sera, the antigens represent only a segment of the entire protein, and/or that the antigen is generated in a non-mammalian/non-malignant system. Individually, these limitations constrain the available spectrum of epitopes available to each approach. A diagnostic panel of antigen biomarkers useful in a large spectrum of any particular type of cancer patient may require taking the best of antigens from each of several technologies. This would not only increase possible representation of different conformations of a protein of interest, but increase the representation of relevant tumor specific genes. Regardless of the initial source of target TAAs, the eventual selection of the required minimum number of TAAs to construct definitive panel of biomarkers will come from balancing the biological studies of these heterogeneous diseases with statistical models and industrial requirements for possible clinical applicable platforms.

We and others have shown that autoantibody signatures in cancer patients' sera do allow discrimination of various cancers from healthy patients and those with related benign diseases even in these early studies. The quest for accurate panels of biomarkers requires the need for robust assays that are both reliable and reproducible that can be taken to the next phase of large scale validation studies. These studies are employing novel technologies for autoantigen biomarker discovery have focused on the goal of the development of diagnostic tests that can be implemented in a true clinical setting. Understanding the nature of the autoimmune response in cancer has helped to establish the tools that will permit detecting tumor specific autoantibodies for cancer diagnosis. These approaches are versatile to not only identify panels of screening diagnostic markers, but may be extended to determine prognostic markers for disease staging, monitoring the efficacy of therapeutic response as well as the identification of potential targets for personalized immunotherapy.

Acknowledgments

This work was supported by funds from the Gail Purtan Ovarian Cancer Research Fund, The Rais Foundation, The Barbara and Fred Erb Endowed Chair in Cancer Genetics, and by grants from National Institutes of Health (NIH), 5R21CA132723-2, R21/R33-CA100740 and U01-CA117748, The Komen Foundation, BCTR0504211, FAS0703852 and The Michigan Life Science Corridor Fund, 085P300470.

References

1. Abendstein B, Marth C, Muller-Holzner E, Wid-schwendter M, Daxenbichler G, Zeimet AG. Clinical significance of serum and asciticp53 autoantibodies in epithelial ovarian carcinoma. Cancer. 2000; 88:1432–1437. [PubMed: 10717627]

2. Andersen MH, Bonfill JE, Neisig A, Arsequell G, Sondergaard I, Valencia G, Neefjes J, Zeuthen J, Elliott T, Haurum JS. Phosphorylated peptides can be transported by TAP molecules, presented by class I MHC molecules, and recognized by phosphopeptide-specific CTL. J Immunol. 1999; 163:3812–3818. [PubMed: 10490979]
3. Anderson KS, Ramachandran N, Wong J, Raphael JV, Hainsworth E, Demirkan G, Cramer D, Aronzon D, Hodi FS, Harris L, Logvinenko T, LaBaer J. Application of protein microarrays for multiplexed detection of antibodies to tumor antigens in breast cancer. J Proteome Res. 2008; 7:1490–1499. [PubMed: 18311903]
4. Anderson KS, Wong J, Vitonis A, Crum CP, Sluss PM, LaBaer J, Cramer D. p53 autoantibodies as potential detection and prognostic biomarkersinserous ovarian cancer. Cancer Epidemiol Biomarkers Prev. 2010; 19:859–868. [PubMed: 20200435]
5. Angelopoulou K, Rosen B, Stratis M, Yu H, Solomou M, Diamandis EP. Circulating antibodies against p53 protein in patients with ovarian carcinoma. Correlation with clinicopathologic features and survival. Cancer. 1996; 78:2146–2152. [PubMed: 8918407]
6. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, Harley JB. Development of autoantibodies before the clinical onset of systemic lupus erythematosus1. N Engl JMed. 2003; 349:1526–1533. [PubMed: 14561795]
7. Blaes F, Klotz M, Huwer H, Straub U, Kalweit G, Schimrigk K, Schafers HJ. Antineural and antinuclear autoantibodies are of prognostic relevance in non-small cell lung cancer. Ann Thorac Surg. 2000; 69:254–258. [PubMed: 10654525]
8. Bonetti B, Fu L, Moon J, Bedwell DM. The efficiency of translation termination is determined by a synergistic interplay between upstream and downstream sequences in Saccharomyces cerevisiae. J Mol Biol. 1995; 251:334–345. [PubMed: 7650736]
9. Bourdon JC, Fernandes K, Murray-Zmijewski F, Liu G, Diot A, Xirodimas DP, Saville MK, Lane DP. p53 isoforms can regulate p53 transcriptional activity. Genes Dev. 2005; 19:2122–2137. [PubMed: 16131611]
10. Bouwman K, Qiu J, Zhou H, Schotanus M, Mangold LA, Vogt R, Erlandson E, Trenkle J, Partin AW, Misek D, Omenn GS, Haab BB, Hanash S. Microarrays of tumor cell derived proteins uncover a distinct pattern of prostate cancer serum immunoreactivity. Proteomics. 2003; 3:2200–2207. [PubMed: 14595819]
11. Cai HY, Wang XH, Tian Y, Gao LY, Zhang LJ, Zhang ZY. Changes of serum p53 antibodies and clinical significance of radiotherapy for esophageal squamous cell carcinoma. World J Gastroenterol. 2008; 14:4082–4086. [PubMed: 18609695]
12. Casiano CA, Mediavilla-Varela M, Tan EM. Tumor-associated antigen arrays for the serological diagnosis of cancer. Mol Cell Proteomics. 2006; 5:1745–1759. [PubMed: 16733262]
13. Chatterjee M, Draghici S, Tainsky MA. Immunotheranostics: breaking tolerance in immunotherapy using tumor autoantigens identified on protein microarrays. Curr Opin Drug Discov Devel. 2006; 9:380–385.
14. Chatterjee M, Ionan A, Draghici S, Tainsky MA. Epitomics: global profiling of immune response to disease using protein microarrays. OMICS. 2006; 10:499–506. [PubMed: 17233560]
15. Chatterjee M, Mohapatra S, Ionan A, Bawa G, Ali-Fehmi R, Wang X, Nowak J, Ye B, Nahhas FA, Lu K, Witkin SS, Fishman D, Munkarah A, Morris R, Levin NK, Shirley NN, Tromp G, Abrams J, Draghici S, Tainsky MA. Diagnostic markers of ovarian cancer by high-throughput antigen cloning and detection on arrays. Cancer Res. 2006; 66:1181–1190. [PubMed: 16424057]
16. Chatterjee M, Mohapatra S, Ionan A, Bawa G, Ali-Fehmi R, Wang X, Nowak J, Ye B, Nahhas FA, Lu K, Witkin SS, Fishman D, Munkarah A, Morris R, Levin NK, Shirley NN, Tromp G, Abrams J, Draghici S, Tainsky MA. Diagnostic markers of ovarian cancer by high-throughput antigen cloning and detection on arrays. Cancer Res. 2006; 66:1181–1190. [PubMed: 16424057]
17. Chen L, Linsley PS, Hellstrom KE. Costimulation of T cells for tumor immunity. Immunol Today. 1993; 14:483–486. [PubMed: 7506034]
18. Chen YM, Hu CP, Wong FH, Chen PH, Tsai YT, Lee SD, Liu JD, Chang CM. Circulating immune complexes in the sera and ascites of hepatocellular carcinoma or chronic hepatitis patients. Zhonghua Min Guo Wei Sheng Wu Ji Mian Yi Xue Za Zhi. 1986; 19:34–45. [PubMed: 3028719]

19. Chen YT, Scanlan MJ, Sahin U, Tureci O, Gure AO, Tsang S, Williamson B, Stockert E, Pfreundschuh M, Old LJ. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. Proc Natl Acad Sci U S A. 1997; 94:1914–1918. [PubMed: 9050879]
20. Cibotti R, Kanellopoulos JM, Cabaniols JP, Halle-Panenko O, Kosmatopoulos K, Sercarz E, Kourilsky P. Tolerance to a self-protein involves its immunodominant but does not involve its subdominant determinants. Proc Natl Acad Sci U S A. 1992; 89:416–420. [PubMed: 1370355]
21. Conrad K, Mehlhorn J. Diagnostic and prognostic relevance of autoantibodies in uranium miners. Int Arch Allergy Immunol. 2000; 123:77–91. [PubMed: 11014974]
22. Crawford LV, Pim DC, Bulbrook RD. Detection of antibodies against the cellular protein p53 in sera from patients with breast cancer. Int J Cancer. 1982; 30:403–408. [PubMed: 6292117]
23. Dalmau J, Furneaux HM, Gralla RJ, Kris MG, Posner JB. Detection of the anti-Hu antibody in the serum of patients with small cell lung cancer – a quantitative western blot analysis. Ann Neurol. 1990; 27:544–552. [PubMed: 2163235]
24. Day ED, Lassiter S, Woodhall B, Mahaley JL, Mahaley MS Jr. The localization of radioantibodies in human brain tumors. I. Preliminary exploration. Cancer Res. 1965; 25:773–778. [PubMed: 5891008]
25. Disis ML, Calenoff E, McLaughlin G, Murphy AE, Chen W, Groner B, Jeschke M, Lydon N, McGlynn E, Livingston RB. Existent T-cell and antibody immunity to HER-2/neu protein in patients with breast cancer. Cancer Res. 1994; 54:16–20. [PubMed: 7505195]
26. Disis ML, Knutson KL, Schiffman K, Rinn K, McNeel DG. Pre-existent immunity to the HER-2/neu oncogenic protein in patients withHER-2/neu overexpressing breast and ovarian cancer. Breast Cancer Res Treat. 2000; 62:245–252. [PubMed: 11072789]
27. Dittmer F, Pohlmann R, von FK. The phosphorylation pattern of oligosaccharides in secreted procathepsin D is glycosylation site-specific and independent of the expression of mannose 6-phosphate receptors. J Biol Chem. 1997; 272:852–858. [PubMed: 8995373]
28. Etzioni R, Urban N, Ramsey S, McIntosh M, Schwartz S, Reid B, Radich J, Anderson G, Hartwell L. The case for early detection. Nat Rev Cancer. 2003; 3:243–252. [PubMed: 12671663]
29. Faiderbe S, Chagnaud JL, Wafflart J, Geffard M. Autoantibodies directed against membrane phospholipid in serum in patients with malignant tumors. C R Acad Sci III. 1989; 310:49–52. [PubMed: 2516762]
30. Fernandez MF, Karvonen RL, Ensley J, Kraut M, Granda JL, Alansari H, Tang N, Tomkiel JE. Spectra of antinuclear antibodies in patients with squamous cell carcinoma of the lung and of the head and neck. Cancer Detect Prev. 2005; 29:59–65. [PubMed: 15734219]
31. Fernandez-Madrid F, Tang N, Alansari H, Granda JL, Tait L, Amirikia KC, Moroianu M, Wang X, Karvonen RL. Autoantibodies to Annexin XI-A and Other Autoantigens in the Diagnosis of Breast Cancer. Cancer Res. 2004; 64:5089–5096. [PubMed: 15289310]
32. Franceschini F, Cavazzana I. Anti-Ro/SSA and La/SSB antibodies. Autoimmunity. 2005; 38:55–63. [PubMed: 15804706]
33. Gold P, Freedman SO. Demonstration of Tumor-Specific Antigens in Human Colonic Carcinomata by Immunological Tolerance and Absorption Techniques. J Exp Med. 1965; 121:439–462. [PubMed: 14270243]
34. Gold P, Freedman SO. Specific carcinoembryonic antigens of the human digestive system. J Exp Med. 1965; 122:467–481. [PubMed: 4953873]
35. Gorelik E, Landsittel DP, Marrangoni AM, Modugno F, Velikokhatnaya L, Winans MT, Bigbee WL, Herberman RB, Lokshin AE. Multiplexed immunobead-based cytokine profiling for early detection of ovarian cancer. Cancer Epidemiol Biomarkers Prev. 2005; 14:981–987. [PubMed: 15824174]
36. Gotter J, Brors B, Hergenhahn M, Kyewski B. Medullary epithelial cells of the human thymus express a highly diverse selection of tissue-specific genes colocalized in chromosomal clusters. J Exp Med. 2004; 199:155–166. [PubMed: 14734521]
37. Hahne M, Rimoldi D, Schroter M, Romero P, Schreier M, French LE, Schneider P, Bornand T, Fontana A, Lienard D, Cerottini J, Tschopp J. Melanoma cell expression of Fas(Apo-1/CD95)

ligand: implications for tumor immune escape. Science. 1996; 274:1363–1366. [PubMed: 8910274]

38. Hakomori S. Glycosylation defining cancer malignancy: new wine in an old bottle. Proc Natl Acad Sci U S A. 2002; 99:10231–10233. [PubMed: 12149519]
39. Hardouin J, Lasserre JP, Sylvius L, Joubert-Caron R, Caron M. Cancer immunomics: from serological proteome analysis to multiple affinity protein profiling. Ann N Y Acad Sci. 2007; 1107:223–230. [PubMed: 17804550]
40. Hudson ME, Pozdnyakova I, Haines K, Mor G, Snyder M. Identification of differentially expressed proteins in ovarian cancer using high-density protein microarrays. Proc Natl Acad Sci U S A. 2007; 104:17494–17499. [PubMed: 17954908]
41. Imafuku Y, Omenn GS, Hanash S. Proteomics approaches to identify tumor antigen directed autoantibodies as cancer biomarkers. Dis Markers. 2004; 20:149–153. [PubMed: 15502247]
42. Imanishi T, Kamigaki T, Nakamura T, Hayashi S, Yasuda T, Kawasaki K, Takase S, Ajiki T, Kuroda Y. Correlation between expression of major histocompatibility complex class I and that of antigen presenting machineries in carcinoma cell lines of the pancreas, biliary tract and colon. Kobe J Med Sci. 2006; 52:85–95. [PubMed: 16855371]
43. Jones LP, Zheng HQ, Karron RA, Peret TC, Tsou C, Anderson LJ. Multiplex assay for detection of strain-specific antibodies against the two variable regions of the G protein of respiratory syncytial virus. Clin Diagn Lab Immunol. 2002; 9:633–638. [PubMed: 11986272]
44. Kozak M. Determinants of translational fidelity and efficiency in vertebrate mRNAs. Biochimie. 1994; 76:815–821. [PubMed: 7880897]
45. Koziol JA, Zhang JY, Casiano CA, Peng XX, Shi FD, Feng AC, Chan EK, Tan EM. Recursive partitioning as an approach to selection of immune markers for tumor diagnosis. Clin Cancer Res. 2003; 9:5120–5126. [PubMed: 14613989]
46. Kusmartsev S, Gabrilovich DI. Role of immature myeloid cells in mechanisms of immune evasion in cancer. Cancer Immunol Immunother. 2006; 55:237–245. [PubMed: 16047143]
47. Kyewski B, Derbinski J. Self-representation in the thymus: an extended view. Nat Rev Immunol. 2004; 4:688–698. [PubMed: 15343368]
48. Laurent-Puig P, Lubin R, Semhoun-Ducloux S, Pelletier G, Fourre C, Ducreux M, Briantais MJ, Buffet C, Soussi T. Antibodies against p53 protein in serum of patients with benign or malignant pancreatic and biliary diseases. Gut. 1995; 36:455–458. [PubMed: 7698709]
49. Le NF, Misek DE, Krause MC, Deneux L, Giordano TJ, Scholl S, Hanash SM. Proteomics-based identification of RS/DJ-1 as a novel circulating tumor antigen in breast cancer. Clin Cancer Res. 2001; 7:3328–3335. [PubMed: 11705844]
50. Leidinger P, Keller A, Ludwig N, Rheinheimer S, Hamacher J, Huwer H, Stehle I, Lenhof HP, Meese E. Toward an early diagnosis of lung cancer: an autoantibody signature for squamous cell lung carcinoma. Int J Cancer. 2008; 123:1631–1636. [PubMed: 18649359]
51. Lin HS, Talwar HS, Tarca AL, Ionan A, Chatterjee M, Ye B, Wojciechowski J, Mohapatra S, Basson MD, Yoo GH, Peshek B, Lonardo F, Pan CJ, Folbe AJ, Draghici S, Abrams J, Tainsky MA. Autoantibody approach for serum-based detection of head and neck cancer. Cancer Epidemiol Biomarkers Prev. 2007; 16:2396–2405. [PubMed: 18006929]
52. Line A, Slucka Z, Stengrevics A, Li G, Rees RC. Altered splicing pattern of TACC1 mRNA in gastric cancer. Cancer Genet Cytogenet. 2002; 139:78–83. [PubMed: 12547166]
53. Line A, Slucka Z, Stengrevics A, Silina K, Li G, Rees RC. Characterisation of tumour-associated antigens in colon cancer. Cancer Immunol Immunother. 2002; 51:574–582. [PubMed: 12384809]
54. Lokshin AE, Winans M, Landsittel D, Marrangoni AM, Velikokhatnaya L, Modugno F, Nolen BM, Gorelik E. Circulating IL-8 and anti-IL-8 autoantibody in patients with ovarian cancer. Gynecol Oncol. 2006; 102:244–251. [PubMed: 16434085]
55. Lu H, Goodell V, Disis ML. Humoral immunity directed against tumor-associated antigens as potential biomarkers for the early diagnosis of cancer. J Proteome Res. 2008; 7:1388–1394. [PubMed: 18311901]
56. Lubin R, Zalcman G, Bouchet L, Tredanel J, Legros Y, Cazals D, Hirsch A, Soussi T. Serum p53 antibodies as early markers of lung cancer. Nat Med. 1995; 1:701–702. [PubMed: 7585154]

57. Ludwig JA, Weinstein JN. Biomarkers in cancer staging, prognosis and treatment selection. Nat Rev Cancer. 2005; 5:845–856. [PubMed: 16239904]
58. Ludwig N, Keller A, Comtesse N, Rheinheimer S, Pallasch C, Fischer U, Fassbender K, Steudel WI, Lenhof HP, Meese E. Pattern of Serum Autoantibodies Allows Accurate Distinction between a Tumor and Pathologies of the Same Organ. Clin Cancer Res. 2008; 14:4767–4774. [PubMed: 18676746]
59. Lv S, Zhang J, Wu J, Zheng X, Chu Y, Xiong S. Origin and anti-tumor effects of anti-dsDNA autoantibodies in cancer patients and tumor-bearing mice. Immunol Lett. 2005; 99:217–227. [PubMed: 15869804]
60. Madoz-Gurpide J, Kuick R, Wang H, Misek DE, Hanash SM. Integral protein microarrays for the identification of lung cancer antigens in sera that induce a humoral immune response. Mol Cell Proteomics. 2008; 7:268–281. [PubMed: 17916591]
61. Marincola FM, Jaffee EM, Hicklin DJ, Ferrone S. Escape of human solid tumors from T-cell recognition: molecular mechanisms and functional significance. Adv Immunol. 2000; 74:181–273. [PubMed: 10605607]
62. Mor G, Visintin I, Lai Y, Zhao H, Schwartz P, Rutherford T, Yue L, Bray-Ward P, Ward DC. Serum protein markers for early detection of ovarian cancer. Proc Natl Acad Sci U S A. 2005; 102:7677–7682. [PubMed: 15890779]
63. Morton DL, Malmgren RA, Holmes EC, Ketcham AS. Demonstration of antibodies against human malignant melanoma by immunofluorescence. Surgery. 1968; 64:233–240. [PubMed: 4173159]
64. Nam MJ, Madoz-Gurpide J, Wang H, Lescure P, Schmalbach CE, Zhao R, Misek DE, Kuick R, Brenner DE, Hanash SM. Molecular profiling of the immune response in colon cancer using protein microarrays: occurrence of autoantibodies to ubiquitin C-terminal hydrolase L3. Proteomics. 2003; 3:2108–2115. [PubMed: 14595809]
65. Nanda NK, Sercarz EE. Induction of anti-self-immunity to cure cancer. Cell. 1995; 82:13–17. [PubMed: 7606778]
66. Nayak SK, Knotts FB, Drogemuller CR, Pilch YH. Detection of Antibodies Bound to Tumor Cell Surface Antigens with Radlolodlnated Staphylococcus aureus Protein A (SPA). Cancer Immunol Immunother. 1979; 5:243–252.
67. Pallasch CP, Struss AK, Munnia A, Konig J, Steudel WI, Fischer U, Meese E. Autoantibodies against GLEA2 and PHF3 in glioblastoma: tumor-associated autoantibodies correlated with prolonged survival. Int J Cancer. 2005; 117:456–459. [PubMed: 15906353]
68. Patwa TH, Wang Y, Simeone DM, Lubman DM. Enhanced detection of autoantibodies on protein microarrays using a modified protein digestion technique. J Proteome Res. 2008; 7:2553–2561. [PubMed: 18452326]
69. Pepe MS, Feng Z, Janes H, Bossuyt PM, Potter JD. Pivotal evaluation of the accuracy of a biomarker used for classification orprediction: standards for study design. J Natl Cancer Inst. 2008; 100:1432–1438. [PubMed: 18840817]
70. Qin S, Qiu W, Ehrlich JR, Ferdinand AS, Richie JP, O'leary MP, Lee ML, Liu BC. Development of a “reverse capture” autoantibody microarray for studies of antigen-autoantibody profiling. Proteomics. 2006; 6:3199–3209. [PubMed: 16596707]
71. Qiu J, Choi G, Li L, Wang H, Pitteri SJ, Pereira-Faca SR, Krasnoselsky AL, Randolph TW, Omenn GS, Edelstein C, Barnett MJ, Thornquist MD, Goodman GE, Brenner DE, Feng Z, Hanash SM. Occurrence of Autoantibodies to Annexin I, 14-3-3 Theta and LAMR1 in Pre-diagnostic Lung Cancer Sera. J Clin Oncol. 2008; 26:5060–5066. [PubMed: 18794547]
72. Ransohoff DF. How to improve reliability and efficiency of research about molecular markers: roles of phases, guidelines, and study design. J Clin Epidemiol. 2007; 60:1205–1219. [PubMed: 17998073]
73. Ratel D, Nasser V, Dupre I, Benabid AL, Berger F. Antibodies to endostatin in a multifocal glioblastoma patient. Lancet. 2000; 356:1656–1657. [PubMed: 11089829]
74. Reipert BM, Tanneberger S, Pannetta A, Bedosti M, Poell M, Zimmermann K, Stellamor MT. Increase in autoantibodies against Fas (CD95) during carcinogenesis in the human colon: a hope for the immunoprevention of cancer? Cancer Immunol Immunother. 2005; 54:1038–1042. [PubMed: 15864586]

75. Reuschenbach M, von Knebel DM, Wentzensen N. A systematic review of humoral immune responses against tumor antigens. Cancer Immunol Immunother. 2009; 58:1535–1544. [PubMed: 19562338]
76. Ritz U, Momburg F, Pilch H, Huber C, Maeurer MJ, Seliger B. Deficient expression of components of the MHC class Iantigen processing machinery in human cervical carcinoma. Int J Oncol. 2001; 19:1211–1220. [PubMed: 11713591]
77. Robinson WH, Steinman L, Utz PJ. Protein arrays for autoantibody profiling and fine-specificity mapping. Proteomics. 2003; 3:2077–2084. [PubMed: 14595805]
78. Sahin U, Tureci O, Schmitt H, Cochlovius B, Johannes T, Schmits R, Stenner F, Luo G, Schobert I, Pfreundschuh M. Human neoplasms elicit multiple specific immune responses in the autologous host. Proc Natl Acad Sci U S A. 1995; 92:11810–11813. [PubMed: 8524854]
79. Scanlan MJ, Chen YT, Williamson B, Gure AO, Stockert E, Gordan JD, Tureci O, Sahin U, Pfreundschuh M, Old LJ. Characterization of human colon cancer antigens recognized by autologous antibodies. Int J Cancer. 1998; 76:652–658. [PubMed: 9610721]
80. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, van Venrooij WJ. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum. 2000; 43:155–163. [PubMed: 10643712]
81. Schwitalle Y, Kloor M, Eiermann S, Linnebacher M, Kienle P, Knaebel HP, Tariverdian M, Benner A, von Knebel DM. Immune response against frameshift-induced neopeptides in HNPCC patients and healthy HNPCC mutation carriers. Gastroenterology. 2008; 134:988–997. [PubMed: 18395080]
82. Shaw PA, Pepe MS, Alonzo TA, Etzioni R. Methods for Assessing Improvement in Specificity when a Biomarker is Combined with a Standard Screening Test. Stat Biopharm Res. 2009; 1:18–25. [PubMed: 20054437]
83. Shimada H, Shiratori T, Takeda A, Matsushita K, Okazumi S, Akutsu Y, Matsubara H, Nomura F, Ochiai T. Perioperative changes of serum p53 antibody titer is a predictor for survival in patients with esophageal squamous cell carcinoma. World J Surg. 2009; 33:272–277. [PubMed: 19052812]
84. Slager EH, Borghi M, van der Minne CE, Aarnoudse CA, Havenga MJ, Schrier PI, Osanto S, Griffioen M. CD4+ Th2 cell recognition of HLA-DR-restricted epitopes derived from CAMEL: a tumor antigen translated in an alternative open reading frame. J Immunol. 2003; 170:1490–1497. [PubMed: 12538712]
85. Srebrow A, Kornblihtt AR. The connection between splicing and cancer. J Cell Sci. 2006; 119:2635–2641. [PubMed: 16787944]
86. Tang L, Yang J, Ng SK, Rodriguez N, Choi PW, Vitonis A, Wang K, McLachlan GJ, Caiazzo RJ Jr, Liu BC, Welch WR, Cramer DW, Berkowitz RS, Ng SW. Autoantibody profiling to identify biomarkers of key pathogenic pathways in mucinous ovarian cancer. Eur J Cancer. 2010; 46:170–179. [PubMed: 19926475]
87. Tarp MA, Sorensen AL, Mandel U, Paulsen H, Burchell J, Taylor-Papadimitriou J, Clausen H. Identification of a novel cancer-specific immunodominant glycopeptide epitope in the MUC1 tandem repeat. Glycobiology. 2007; 17:197–209. [PubMed: 17050588]
88. Taylor G, Odili JL. Tumour specific T-like antigen of human breast carcinoma. Br J Cancer. 1970; 24:447–453. [PubMed: 4097648]
89. Tureci O, Mack U, Luxemburger U, Heinen H, Krummenauer F, Sester M, Sester U, Sybrecht GW, Sahin U. Humoral immune responses of lung cancer patients against tumor antigen NY-ESO-1. Cancer Lett. 2006; 236:64–71. [PubMed: 15992994]
90. Ura Y, Ochi Y, Hamazu M, Ishida M, Nakajima K, Watanabe T. Studies on circulating antibody against carcinoembryonic antigen (CEA) and CEA-like antigen in cancer patients. Cancer Lett. 1985; 25:283–295. [PubMed: 2578868]
91. Vittecoq O, Pouplin S, Krzanowska K, Jouen-Beades F, Menard JF, Gayet A, Daragon A, Tron F, Le L. X Rheumatoid factor is the strongest predictor of radiological progression of rheumatoid arthritis in a three-year prospective study in community-recruited patients. Rheumatology (Oxford). 2003; 42:939–946. [PubMed: 12730503]
92. Volkmann M, Sinn HP, Gaugel D, Frey M, Hajjar Y, Ludwig J, Hansel S, Bastert G, Wallwiener D, Fiehn W, Zentgraf H, Huober J. Anti-p53 in breast cancer: concordance of different assay

procedures and association with p53 antigen expression. Oncology. 2002; 63:297–305. [PubMed: 12381910]

93. von Mensdorff-Pouilly S, Gourevitch MM, Kenemans P, Verstraeten AA, van Kamp GJ, Kok A, van UK, Snijdewint FG, Paul MA, Meijer S, Hilgers J. An enzyme-linked immunosorbent assay for the measurement of circulating antibodies to polymorphic epithelial mucin (MUC1). Tumour Biol. 1998; 19:186–195. [PubMed: 9591045]
94. von KS, Burtin P. On the specificity of autoantibodies presentincoloncancer patients. Immunology. 1966; 10:507–515. [PubMed: 4958052]
95. Wadle A, Kubuschok B, Imig J, Wuellner B, Wittig C, Zwick C, Mischo A, Waetzig K, Romeike BF, Linde-mann W, Schilling M, Pfreundschuh M, Renner C. Serological immune response to cancer testis antigens in patients with pancreatic cancer. Int J Cancer. 2006; 119:117–125. [PubMed: 16432832]
96. Wandall HH, Blixt O, Tarp MA, Pedersen JW, Bennett EP, Mandel U, Ragupathi G, Livingston PO, Hollingsworth MA, Taylor-Papadimitriou J, Burchell J, Clausen H. CancerCancer biomarkers defined by autoantibody signatures to aberrant O-glycopeptide epitopes. Cancer Res. 2010; 70:1306–1313. [PubMed: 20124478]
97. Wang ET, Sandberg R, Luo S, Khrebtukova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP, Burge CB. Alternative isoform regulation in human tissue transcriptomes. Nature. 2008; 456:470–476. [PubMed: 18978772]
98. Wang RF, Johnston SL, Zeng G, Topalian SL, Schwartzentruber DJ, Rosenberg SA. A breast and melanoma-shared tumor antigen: T cell responses to anti-genic peptides translated from different open reading frames. J Immunol. 1998; 161:3598–3606. [PubMed: 9759882]
99. Wang X, Yu J, Sreekumar A, Varambally S, Shen R, Giacherio D, Mehra R, Montie JE, Pienta KJ, Sanda MG, Kantoff PW, Rubin MA, Wei JT, Ghosh D, Chinnaiyan AM. Autoantibody signatures in prostate cancer. N Engl J Med. 2005; 353:1224–1235. [PubMed: 16177248]
100. Wasserman J, Glas U, Blomgren H. Autoantibodies in patients with carcinoma of the breast. Correlation with prognosis. Clin Exp Immunol. 1975; 19:417–422. [PubMed: 1081928]
101. Waterboer T, Sehr P, Pawlita M. Suppression of nonspecific binding in serological Luminex assays. J Immunol Methods. 2006; 309:200–204. [PubMed: 16406059]
102. Weiss RB, Dunn DM, Atkins JF, Gesteland RF. Slippery runs, shifty stops, backward steps, and forward hops: −2, −1, +1, +2, +5, and +6 ribosomal frameshifting. Cold Spring Harb Symp Quant Biol. 1987; 52:687–693. [PubMed: 3135981]
103. Wild CP, Ridanpaa M, Anttila S, Lubin R, Soussi T, Husgafvel-Pursiainen K, Vainio H. p53 antibodies in the sera of lung cancer patients: comparison with p53 mutation in the tumour tissue. Int J Cancer. 1995; 64:176–181. [PubMed: 7622306]
104. Winter SF, Minna JD, Johnson BE, Takahashi T, Gazdar AF, Carbone DP. Development of antibodies against p53 in lung cancer patients appears to be dependent on the type of p53 mutation. Cancer Res. 1992; 52:4168–4174. [PubMed: 1322237]
105. Wong J, Sibani S, Lokko NN, LaBaer J, Anderson KS. Rapid detection of antibodies in sera using multiplexed self-assembling bead arrays. J Immunol Methods. 2009; 350:171–182. [PubMed: 19732778]
106. Yamamoto A, Shimizu E, Ogura T, Sone S. Detection of autoantibodies against L-myc oncogene products in sera from lung cancer patients. Int J Cancer. 1996; 69:283–289. [PubMed: 8797869]
107. Yasukawa M, Ohminami H, Kojima K, Hato T, Hasegawa A, Takahashi T, Hirai H, Fujita S. HLA class II-restricted antigen presentation of endogenous bcr-abl fusion protein by chronic myelogenous leukemia-derived dendritic cellstoCD4(+)Tlymphocytes. Blood. 2001; 98:1498–1505. [PubMed: 11520800]
108. Zhang JY. Tumor-associated antigen arrays to enhance antibody detection for cancer diagnosis. Cancer Detect Prev. 2004; 28:114–118. [PubMed: 15068835]
109. Zhang JY, Casiano CA, Peng XX, Koziol JA, Chan EK, Tan EM. Enhancement of antibody detection in cancer using panel of recombinant tumor-associated antigens. Cancer Epidemiol Biomarkers Prev. 2003; 12:136–143. [PubMed: 12582023]

110. Zhang L, Liu J, Zhang H, Wu S, Huang L, He D, Xiao X. Discovery and identification of anti-U1-A snRNP antibody in lung cancer. Sci China C Life Sci. 2005; 48:641–647. [PubMed: 16483144]

111. Zhong L, Coe SP, Stromberg AJ, Khattar NH, Jett JR, Hirschowitz EA. Profiling tumor-associated antibodies for early detection of non-small cell lung cancer. J Thorac Oncol. 2006; 1:513–519. [PubMed: 17409910]

112. Zhong L, Hidalgo GE, Stromberg AJ, Khattar NH, Jett JR, Hirschowitz EA. Using protein microarray as a diagnostic assay for non-small cell lung cancer. Am J Respir Crit Care Med. 2005; 172:1308–1314. [PubMed: 16109979]

113. Zolg JW, Langen H. How industry is approaching the search for new diagnostic markers and biomarkers. Mol Cell Proteomics. 2004; 3:345–354. [PubMed: 14749446]

Table 1

Timeline of diagnostic technologies used for the detection of tumor autoantibodies in cancer

Year of invention	Technology for tumor autoantibody detection	Application in cancer diagnostics	References
1965	Radioiodination of serum antibody	This technology was applied to determine the *in vivo* localization of radioantibodies in human brain tumors using animal models.	[24]
1966	Passive haemagglutination	Tumor autoantibodies were detected in patients with colonic cancer or other diseases.	[94]
1968	Immunofluorescence	Presence of tumor autoantibodies against malignant human melanoma was demonstrated in this study.	[63]
1970	Compliment fixation method and Passive agglutination technique	Autoantibodies against T like antigen were detected in breast carcinoma.	[88]
1975	Indirect Immunofluoresence	Tumor autoantibodies were detected in patients with breast carcinoma.	[100]
1979	Radioiodination of Staphylococcus protein A (SPA)	This assay was employed for the detection of antibodies in melanoma and colon carcinoma patients.	[66]
1982	Immunoprecipitation and sodium dodecyl poly-acrylamide gel (SDS-PAGE)	Autoantibodies against cellular p53 were detected in the sera obtained from patients with breast cancer.	[22]
1985	Immunoelectrophoresis and radioimmunoelec-trophoresis In conjunction with I-125 labeled CEA	Autoantibodies against CEA were detected in the serum of colonic cancer patients.	[90]
1986	Polyethylene glycol (PEG) and C1q solid-phase microassay (C1q-SPMA)	Circulating immune complexes were detected in sera or ascites of patients with hepatocellular carcinoma.	[18]
1989	Adapted immunoenzymatic assay (ELISA method)	This technolgoy was applied for the detection of autoan-tibodies against membrane phospholipids such as, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, phosphatidylethanolamine, gangliosides, sphingomyelin, sph-ingosin, and cardiolipin in the serum of patients with malignant tumors.	[29]
1990	Avidin-biotin immunoperoxidase method and highly sensitive quantitative western blot analysis	Anti-Hu antibodies were detected in the serum of patients diagnosed with small cell lung cancer.	[23]
1994	Recombinant baculovirus containing tumor Ag and western blot	Autoantibodies to Her2/neu were detected in breast cancer patients.	[25]
1995	Enzyme linked immunosorbent assay (ELISA)	This technology was used for the detection of serum p53 antibodies in patients with benign or malignant pancreatic and biliary diseases. Another group reported the detection of p53 antibodies in the sera of lung cancer patients in the same year.	[48,103]
1995	SEREX technology[3]	Circulating autoantibodies against melanoma antigens, renal carcinoma antigens, brain tumor antigens, antigens expressed in Hodgin diseases were detected in serum of cancer patients.	[78]
1996	This methodology was basedonthe preparation of bacterially synthesized glutathione S-transferase (GST)-tumor Ag fusion proteins and western blot analysis	Autoantibodies directed against L-myc oncogene products were detected in the sera of patients with lung cancer.	[106]
1996	Time-resolved immunofluorometric procedure	Circulating p53 antibdies were detected in patients with ovarian carcinoma.	[5]
1997	SEREX technology	Autoantibodies against cancer testis antigen NY-ESO-1 were detected in osephageal squamous cell carcinoma patients.	[19]

Year of invention	Technology for tumor autoantibody detection	Application in cancer diagnostics	References
1998	SEREX technology	Forty eight human colon cancer antigens (NY-CO-1-NYCO-48) were identified by SEREX analysis in patients with colon cancer.	[79]
1998	ELISA (PEM.C1g) employed a 60 mer MUC1 peptide conjugated to bovine serum albumin and peroxidase-labeled antihuman immunoglobulin G or M antibodies	Circulating antibodies to polymorphic epithelial mucin (MUC1) were detected in breast and ovarian carcinoma patients.	[93]
2000	Indirect immunofluorescence test (IFT)	Antineural and antinuclear autoantibodies were detected in patients with non-small cell lung cancer.	[7]
2000	SDS-PAGE and western blot analysis	This technology was applied for the detection of antibodies against endostatin in patients with multifocal glioblastoma.	[73]
2001	Two dimensional (2D) PAGE, western blotting, and Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF)	Occurrence of autoantibodies against novel tumor antigen RS/DJ-1were detected in breast cancer patients.	[49]
2002	ELISA, immunoblot and indirect fluorescence.	p53 antibodies were detected in breast cancer.	[92]
2003	Two dimensional liquid chromatography and protein microarrays	The study showed that microarrays of fractionated proteins could be a powerful tool for tumor antigen discovery and cancer diagnosis.	[10]
2005	Expression of recombinant tumor antigen, SDS-PAGE, western blotting and ELISA	Increased level of Fas (CD95) autoantibodies was detected during colon carcinogenesis.	[74]
2006	Expression of recombinant His-tagged tumor Ag, and ELISA	Circulating autoantibodies of cancer testis antigen NY-ESO-1 were detected in lung cancer patients.	[89]
2006	SEREX and Luminex technology	Autoantibodies against IL-8 were elevatedinpatients with ovarian cancer.	[54]
2006	Epitomics: Combination of phage display cloning of tumor Ag, differential biopanning and protein microarray	Autoantibodies directed against 65 tumor antigens were detected in patients with ovarian cancer.	[16]
2006	Reverse capture autoantibody microarray	This technology was applied for antigen-autoantibdy profiling with sera from prostate cancer and benign prostate hyperplasia.	[70]
2007	Serological proteome profiling (SERPA)	Autoantibodies signatures produced in response to the breast or colorectal cancer was reported.	[39]
2008	Nucleic acid programmable protein microarray (NAPPA) and ELISA	This technology reported that of 1705 nonreduntant expressed antigens, dominant antibodies were detected in patients diagnosed with melanoma, breast and ovarian cancer.	[3]
2008	Luminex bead array technology	This technology allowed rapid detection of tumor autoantibody repertoire in the serum of cancer patients using *in vitro* expressed epitope-tagged tumor Ag (either GST or FLAG tagged) that were captured later onto anti-epitope coupled Luminex SeroMap beads.	[105]
2010	Chemoenzymatic synthesis of O-glycopeptides and O-Glycopeptide array print	Autoantibody signatures to aberrant O-glycopeptide epitopes in the serum of cancer patients were reported.	[96]
2010	RAPID ELISA	Autoantibodies to p53 were detected in 42% of patients with advanced serous ovarian cancer.	[4]

[3]SEREX database can be found at their web site (http://ludwig-sun5.unil.ch/CancerImmunomeDB/).