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Cancer Epidemiology, Biomarkers & Prevention

Review

Serologic Autoantibodies as Diagnostic Cancer Biomarkers—A Review

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Abstract

Current diagnostic techniques used for the early detection of cancers are successful but subject to detection bias. A recent focus lies in the development of more accurate diagnostic tools. An increase in serologic autoantibody levels has been shown to precede the development of cancer disease symptoms. Therefore, autoantibody levels in patient blood serum have been proposed as diagnostic biomarkers for early-stage diagnosis of cancers. Their clinical application has, however, been hindered by low sensitivity, specificity, and low predictive value scores. These scores have been shown to improve when panels of multiple diagnostic autoantibody biomarkers are used. A five-marker biomarker panel has been shown to increase the sensitivity of prostate cancer diagnosis to 95% as compared with 12.2% for prostate-specific antigen alone. New potential biomarker panels were also discovered for lung, colon, and stomach cancer diagnosis with sensitivity of 76%, 65.4%, and 50.8%, respectively. Studies in breast and liver cancer, however, seem to favor single markers, namely α-2-HS-glycoprotein and des-γ-carboxyprothrombin with sensitivities of 79% and 89% for the early detection of the cancers. The aim of this review is to discuss the relevance of autoantibodies in cancer diagnosis and to outline the current methodologies used in the detection of autoantibodies. The review concludes with a discussion of the autoantibodies currently used in the diagnosis of cancers of the prostate, breast, lung, colon, stomach, and liver. A discussion of the potential future use of autoantibodies as diagnostic cancer biomarkers is also included in this review. *Cancer Epidemiol Biomarkers Prev; 22(12); 2161–81. ©2013 AACR.*

Introduction

Worldwide, cancer is the second leading cause of death (1, 2). Despite tremendous efforts to develop strategies against cancer-related mortality, the battle with high cancer mortality rates continues (3, 4). To counteract these mortality rates, research has focused on the development of diagnostic tools that enable the diagnosis of a cancer earlier before it progresses to an often incurable metastatic stage (5). Autoantibody levels in patient blood serum have been proposed as diagnostic biomarkers for early-stage diagnosis of cancers, as an increase in serum levels of certain autoantibodies has been shown to precede the development of disease symptoms (6, 7) and correlate with cancer incidence (8) for cancers of the breast (9), lung and small cell lung (10, 11), colon (12), ovary (13), prostate (14), and head and neck cancer (15, 16).

Theories of the process of autoantibody production in cancer are complex and not yet fully understood. The immune response toward tumor-associated antigens (TAA) presented in early stages of carcinogenesis is thought to occur in response to cancer immunosurveillance, the process by which the immune system recognizes and destroys invading pathogens as well as host cells that have become cancerous (17–19). It has also been suggested that genetic, hormonal, and environmental influences may play a part in triggering autoimmunity.

Immunologic processes causing autoantibody production are believed to be generated by the immune system in response to mutations, degradation, overexpression of proteins, and/or the release of proteins from damaged tissue (20–23). Autoantibody production is also believed to be caused by mis-presentation or misfolding of proteins, which may be recognized by the immune system leading to autoantibody production and therefore, TAAs or proteins that have undergone alternate posttranslational modifications (PTM) may be recognized as nonautologous (17, 19, 24), that is, their phosphorylation, glycosylation, oxidation, or proteolytic cleavage could generate a neo-epitope or enhance self-epitope presentation and affinity to the MHC or T-cell receptor, inducing an immune response (25). A neo-epitope is an epitope that is located within an unexposed region of the protein, preventing any interaction between the molecule and antibodies or lymphocytes, therefore avoiding the induction of an immune response against the molecule. The neo-epitope may only cause an immune response or tolerance when its

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structure is exposed by a conformational change or stereochemical alteration of the protein structure (26).

Here, we discuss the relevance of autoantibodies in cancer diagnosis, autoantibody production in response to cancers, current methodologies used in the detection of autoantibodies, currently used autoantibodies in the diagnosis of cancers of the prostate, breast, lung, colon, stomach, and liver as well as the potential future use of autoantibodies as diagnostic cancer biomarkers. A comprehensive search of electronic databases such as PubMED, NIH, UWA library, and Edith Cowan University (ECU; Perth, WA, Australia) library and others was carried out from November 2012 to August 2013. This review included studies that were published within the last 10 years from 2003 to 2013 that reported on "currently utilized autoantibody detection methods," "serological diagnostic cancer biomarkers," and "diagnostic autoantibody cancer biomarkers."

Diagnostic Relevance of Autoantibodies as Biomarkers in Cancer

Currently, the diagnosis of the majority of cancers is restricted to the examination of the patient's primary tumor by morphologic and immunohistochemical analysis. More recently, the use of autoantibodies toward autologous TAAs has been gathering momentum as these have been detected in the asymptomatic stage of cancer and may therefore serve as diagnostic biomarkers (27–31). In fact, autoantibodies have been found to precede the manifestation of clinical signs of tumor progression by several months to years (17, 32–34). One example of the potential of serologic autoantibodies to diagnose early-stage cancer is the discovery of the extracellular protein kinase A (ECPKA) autoantibody as a universal cancer biomarker. In healthy mammalian cells, cAMP-dependent protein kinase A (PKA) is an intracellular enzyme. In most cancers, including those forming the subject of this review, this enzyme is secreted into the circulatory system. Once secreted, the protein is known as ECPKA. This antibody was found to be elevated in a wide range of cancers of various stages of malignancies in different cell types including bladder, breast, cervical, colon, esophageal, gastric, liver, lung, ovarian, prostate, pancreatic, renal, small bowel, rectal, adenocystic carcinomas, melanoma, sarcoma, thyoma, liposarcoma, and leiomyosarcoma compared with healthy controls. Blood ECPKA levels are increased and ECPKA levels decreased after surgical removal of solid tumors (35). With the assumption that this excretion results in the production of anti-ECPKA antibodies, an enzyme immunoassay measuring the immunoglobulin G (IgG) of this autoantibody was developed and the sensitivity and specificity of this biomarker for detecting the incidence of 20 different cancers was calculated to be 90% and 87%. Anti-ECPKA autoantibody was detected in 90% of the patient samples and in only 13% of the control samples, indicating that the presence of the ECPKA autoantibody in sera correlates with cancer incidence (8). Furthermore, autoantibodies are easily extracted from blood serum and are generally stable and bind with high specificity to their specific antigenic proteins (36).

To date, no single autoantibody biomarker has been used as a cancer biomarker due to the low sensitivity and specificity of single markers. Panels of multiple tumor-associated autoantibodies with high specificity and sensitivity are sought therefore for translation into simple biomarker panel tests for routine clinical diagnosis of early-stage cancer (17, 19, 37–40).

Methodology of Autoantibody Detection

To advance the discovery of novel combinations of autoantibody biomarkers, techniques that allow the simultaneous screening of multiple biomarkers are required. Examples of such methodologies include serological analysis of tumor antigens by recombinant cDNA expression cloning (SEREX), phage display, serological proteome analysis (SERPA), multiple affinity protein profiling (MAPPing), or protein microarrays. Please refer to Fig. 1 and Table 1 for a comprehensive overview and comparison of methodologies and associated processes used to detect multiple autoantibodies simultaneously.

SEREX

SEREX was first developed in 1995 (41, 42). This technique uses antibody reactivity with autologous cancer patient sera to identify immunogenic tumor proteins (17, 39). The cDNA expression library used in this methodology is constructed from tumor specimens of interest and then cloned into λ-phage expression vectors that are used to transfect *Escherichia coli*. The resulting recombinant proteins are then transferred onto a nitrocellulose membrane, which is incubated with diluted patient sera. Clones that are reactive with high-titer IgG antibodies are identified using an enzyme-conjugated secondary antibody specific for human IgG. The cDNA clone is sequenced and the autoantigen identified. The major advantage of using SEREX is the fact that it allows the identification of TAAs from *in vivo* material. Another advantage of this technology is that it allows the identification of several tumor-specific antigens in one experiment. Furthermore, both the tumor-specific antigen and its coding cDNA are present in the same plaque when immunoscreening is performed that allows the subsequent sequencing of matched cDNA immediately. The disadvantage of SEREX is the high likelihood of false-positive results. Second, the use of tumor tissue from a single patient with cancer followed by screening with autologous patient sera limits the identification of TAAs to that patient. Moreover, this complex methodology does not detect alternate tumor-associated PTMs of antigens (17). Patients may also exhibit autoimmunity to autologous proteins and therefore irrelevant non-cancer-associated proteins may be detected. Furthermore, parallel analysis with healthy donor sera as controls cannot be performed easily.

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Phage display

Alternatively, a cDNA phage display library is constructed directly from tumor tissue or a cancer cell line derived from patient tumor material (43). Phage clones that bind to cancer sera are identified through a differential biopanning approach (44). Alternatively, a more cost-effective method is to construct the cDNA phage display library by expressing the phage proteins fused to the antigens on the surface of bacteriophages. The phage display method has the advantage of allowing the simultaneous screening of a large number of antigens against the sera of cancer patients relative to serum of healthy individuals (14, 43). The phage-display method has a higher throughput value than the SEREX method, but again, antigens with alternate PTMs cannot be detected using the phage-display method (19, 45).

Protein microarray

The protein array methods are advantageous in that they require only minute amounts of patient sera (46) while enabling the simultaneous screening of large numbers of antigens in a single test (47–52). In this methodology, purified or recombinant as well as synthetic proteins are used. Alternatively, fractured proteins of tumor origin are spotted onto the microarray platform. Arrays are then incubated with patient and control sera (17, 19, 53, 54). The array platform can be either two-dimensional (2D; such as nitrocellulose membranes, microtiter plates, or glass slides) or three-dimensional (3D; such as nanoparticles or beads). Although protein microarray methods are commonly used to analyze recombinant proteins expressed from *Escherichia coli* cells, alternatively, other host expression systems, such as yeast and insect cells, have been used to produce libraries presenting proteins with the correct PTMs. The disadvantage associated with this method is the requirement for high-quality protein synthesis (55). Furthermore, studies using protein microarrays are time restricted because of the short shelf-life of protein arrays (19, 56).

Reverse-capture microarray

In this method, the antibodies reacting with specific proteins are spotted onto the microarray. Similar to the protein microarray, the reverse-capture microarray is incubated with tumor lysate and serum proteins and the microarrays with captured proteins are then further

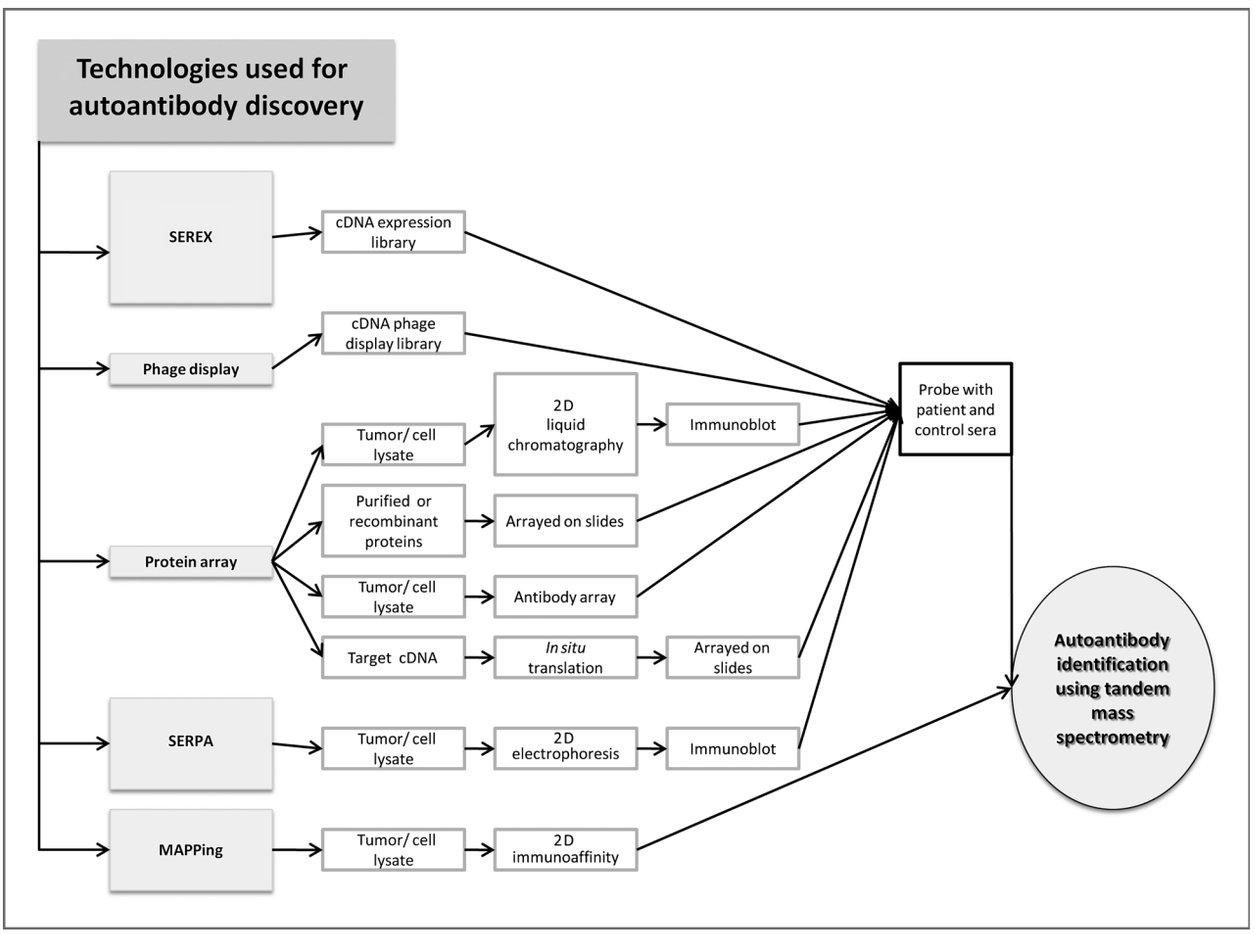

Figure 1. Technologies utilized for autoantibody discovery.

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Table 1. Summary of autoantibody detection methodologies

Methodology name	High-throughput	Cost	Time	Advantages	Disadvantages
SEREX	Yes		• May take several days • This is the most time consuming of all the methods due to the need to construct the cDNA library	• Allows detection from *in vivo* material • Use of multiantigen-specific patient serum allows the identification of several tumor-specific antigens in one experiment • Both the tumor-specific antigen and its coding cDNA are present in the same plaque when immunoscreening is performed that allows the subsequent sequencing of the matching cDNA immediately • Slightly more sensitive than SERPA	• High likelihood of false-positive results • Does not detect alternate tumor-associated PTMs of antigens • Use of tumor tissue from a single cancer followed by screening with autologous patient serum limits identification of TAAs to that of a single patient • Parallel analysis of tumor proteins with healthy donor sera as controls cannot be performed easily
Phage display	Yes—higher throughput than SEREX	More cost-effective if phage proteins are fused to antigens on bacteriophage surface	• May take several days	• Constructed directly from tumor tissue or patient tumor material–derived cell line	• Does not detect alternate tumor-associated PTMs of antigens
Protein microarray	Yes	Production of thousands of recombinant proteins is very expensive	• Time restriction due to short shelf-life of protein arrays	• Large numbers of antigens can be tested against large numbers of sera in a single test • Purified, recombinant or synthetic proteins may be used • Array platform may be 2D or 3D • Yeast or insect cells may be used as alternative expression systems to produce libraries with correct PTMs • 3D structure is often intact optimizing antigen–antibody interaction for recombinant	• High-quality protein synthesis is required • Other than high-quality antibodies or antigens, only commercially available proteins can be studied • Time restriction due to short shelf-life of protein arrays • High reproducibility is difficult to achieve • Enormous data collection requires specialized software tools

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Table 1. Summary of autoantibody detection methodologies (Cont'd)

Methodology name	High-throughput	Cost	Time	Advantages	Disadvantages
				proteins produced in mammalian systems • Requires only minute amounts of sera	• Production of thousands of recombinant proteins is very labor-intensive • Recombinant proteins produced in non-mammalian systems may not have the correct PTMs and may therefore be misfolded
SERPA	• Yes • Liquid-based separations are amenable to automation and the ELISA format can be coupled to mass spectrometric analysis that increases the throughput	More cost-effective than SEREX	• May be completed within hours	• Effective separation of a complex mixture of proteins based on their isoelectric points and molecular weights • Allows detection from *in vivo* material • Allows for the identification of tumor-specific PTMs and isoforms • Avoids the time-consuming construction of cDNA libraries • Parallel analysis of tumors proteins with healthy donor sera as controls can be performed easily • 2D immunoblots provide a global view of the antibody–tumor-associated antigen interaction	• Limited identification of low-abundance and transmembrane TAAs • Because of the use of Western blot analyses only linear epitopes can be detected • Separation of cell membrane proteins remains a challenge due to their insoluble nature in aqueous buffers • This method of autoantibody detection is very labor-intensive
MAPPing	Yes	Similar cost-efficiency to SERPA	• May be completed within hours	• Tumor antigens are maintained in solution that allows the identification of structural epitopes	• Restricted tumor antigen identification to antibody interactions with a low dissociation rate constant • Limited detection of tumor antigens in more complex protein solutions due to the use of immunoprecipitation

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incubated with sera from patients and controls. The autoantibodies are detected with fluorescent-labeled secondary antibody (57–59). The advantage of the utilization of "reverse-capture" microarray technology is the elimination of the need for recombinant proteins and allows the instant identification of cancer-specific autoantibodies. However, only known antigens and their commercially available antibodies can be analyzed and immunoreactivity with posttranslationally modified antigens cannot be differentiated unless antibodies that bind exclusively to these antigens are commercially available.

SERPA

SERPA (60) is also known as PROTEOMEX. This technique is very useful for detection of TAAs as it incorporates an effective separation of a complex mixture of proteins based on their isoelectric points and molecular weights through 2D electrophoresis and Western blotting followed by identification by mass spectrometry (19, 61, 62). Proteins from the tumor tissue of interest are transferred onto a nitrocellulose membrane and immobilized. The sera from patients with cancer and controls are separately screened using the immobilized proteins. The appropriate immunoreactive profiles are compared and the cancer-associated antigenic spots are identified by mass spectrometry. Similar to the SEREX technique, the advantage of the SERPA technique is the use of *in vivo*–derived TAAs. Furthermore, the SERPA technique allows for the identification of tumor-specific PTMs and isoforms but is limited in terms of the identification of low-abundance and transmembrane TAAs (17, 34, 51). SERPA also enables the easy parallel analysis of tumor proteins with healthy donor sera as controls and avoids the time-consuming construction of cDNA libraries, enabling this methodology to be completed within a few hours as compared with several days for SEREX and phage-display technology. However, due to the way that Western blot analyses are prepared, SERPA can only be used to detect linear epitopes (63).

MAPPing

The MAPPing methodology incorporates 2D immunoaffinity chromatography, which is followed by the identification of TAAs by tandem mass spectrometry analysis (64). In the first phase of the initial immunoaffinity chromatography, lysate from cancer cell lines or tumor tissue containing nonspecific TAAs is bound to IgG that was obtained from healthy controls in an immunoaffinity column. The flow-through fraction is then subjected to 2D immunoaffinity in a column that contains IgG from patients with cancer and columns can be used in parallel (65). The tumor antigens that are captured in the patient columns are eluted and digested for identification by nano-liquid chromatography mass spectrometry. MAPPing ensures that the tumor antigens are maintained in a solution that allows the potential identification of structural epitopes. The disadvantages associated with this method include the restriction of the tumor antigen identification to antibody interactions with a low dissociation rate constant. Furthermore, immunoprecipitation using these affinity columns limits the detection of tumor antigens in more complex protein solutions, such as cell lysate.

Currently Used Diagnostic Autoantibody Cancer Biomarkers

According to epidemiologic statistics from the Cancer Research UK (66), the most commonly diagnosed cancers worldwide include lung, breast, colorectum, stomach, prostate, and liver cancers. Here, we discuss currently used or investigated autoantibodies that may serve as diagnostic biomarkers for the cancers mentioned above. Please refer to Table 2 to see a detailed summary of the major studies described in this review, including information such as sample size, methods used, protein abbreviations, full names, encoding genes, alternative protein names, and their associated cancer type as well as the accuracy of each potential biomarker and/or biomarker panel.

Prostate cancer

The prostate-specific antigen (PSA), also known as kallikrein 3 (KLK3), is part of a family of proteases that are known as kallikreins. These proteases are encoded by a cluster of genes that are located within a 300-kb region on chromosome 19q13.4 (67). PSA is responsible for the cleavage of the proteins seminogelin I and II, which leads to the liquefaction of the semen in seminal fluid (68). PSA activity is normally confined to prostatic glandular structures only, however, disturbances of this structure such as by formation of a tumor, may result in leakages of PSA into the circulatory system (69). The PSA blood test measures the amount of PSA within a patient's circulation. Any PSA level between 0 and 4 ng/mL is considered normal, whereas PSA levels between 4 and 10 ng/mL are slightly elevated, PSA levels between 10 an 20 ng/mL are moderately elevated, and any PSA levels above 20 ng/mL are highly elevated. A positive PSA serum level above 4 ng/mL concentration has diagnostic potential in patients with prostate cancer (70).

Although PSA serum levels are the most commonly used diagnostic test for this cancer to date, its specificity is less than 50%, resulting in frequent false-positive results (71). The primary limitation of the use of PSA as a diagnostic biomarker is the inability to distinguish between benign and malignant stages of the disease (72). Increased PSA serum levels may also arise due to noncancerous conditions such as enlargement of the prostate, prostatitis, and urinary infection (69). Xie and colleagues (73) developed a new multiplex assay that they termed the "A+PSA" assay (the autoantibody+PSA assay). This assay used B-cell epitopes from previously defined prostate cancer–associated antigen (PCAA), including New York esophageal squamous cell carcinoma (NY-ESO-1), synovial sarcoma X breakpoint 2,4 (SSX-2,4), X antigen family

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Table 2. List of studies mentioned in this review including the study sample size, methods used, biomarker abbreviation, full biomarker names, cancer sites associated with biomarker for possible diagnosis, encoding genes, alternative names, and the accuracy of individual biomarkers or biomarker panels

Reference	Sample size	Methods used	Protein abbreviation	Protein full name	Cancer associated with protein for possible diagnosis	Encoding gene	Alternative names	Accuracy of marker/marker panel
Xie and colleagues (73)	131 Presurgery biopsy confirmed prostate cancer patients and 121 patients with prostatitis and/or benign prostatic hyperplasia Total cohort = 252	Novel seroMAP-based multiplex A+PSA assay versus PSA assay alone	NY-ESO-1	New York esophageal squamous cell carcinoma	Prostate, breast, lung	*NY-ESO-1*		Sensitivity = 79% Specificity = 84%
			SSX-2,4	Synovial sarcoma, X breakpoint 2,4	Prostate	*SSX2*		
			XAGE-1b	X antigen family, member 1B	Prostate	*XAGE1B*		
			LEDGF	Lens epithelium–derived growth factor p75	Prostate	*PSIP1*	PC4, SFRS1 interacting protein 1, DFS 70, p75/p52, PSIP1	
			AMACR	α-Methylacyl-CoA racemase	Prostate	*AMACR*		
			p90	Transferrin receptor protein 90	Prostate	*TFRC*		
			PSA	Prostate-specific antigen	Prostate	*KLK3*	KLK3, γ-seminoprotein	
			PSA	Prostate-specific antigen	Prostate	*KLK3*	KLK3, γ-seminoprotein	Sensitivity = 52% Specificity = 79%
Wang and colleagues (14)	119 Patients with prostate cancer and 138 healthy controls Total cohort = 257	Phage protein microarray	BRD2	Bromodomain-containing protein 2	Prostate	*BRD2*		Sensitivity = 81.6% Specificity = 88.2%
			eIF4G1	Eukaryotic translation initiation factor 4 γ 1	Prostate	*EIF4G1*		
			RPL22	Ribosomal protein L22	Prostate	*RPL22*		
			RPL13a	Ribosomal protein LBa	Prostate	*RPL13A*		
			XP_373908	Hypothetical protein XP_373908	Prostate	To be determined		
O'Rouke and colleagues (74)	41 Patients with prostate cancer and 39 patients with benign prostate hyperplasia Total cohort = 80	Reverse capture microarray	PSA	PSA	Prostate	*KLK3*	KLK3, γ-seminoprotein	Sensitivity = 12.2% Specificity = 80%
			TLN1	Talin 1	Prostate	*TLN1*		Sensitivity = 95% Specificity = 80%

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Reference	Sample size	Methods used	Protein abbreviation	Protein full name	Cancer associated with protein for possible diagnosis	Encoding gene	Alternative names	Accuracy of marker/marker panel
			TARDBP	TAR DNA-binding protein	Prostate	*TARDBP*		
			LEDGF	Lens epithelium–derived growth factor p75	Prostate	*PSIP1*	PC4, SFRS1 interacting protein 1, DFS 70, p75/p52, PSIP1	
			CALD1	Caldesmon	Prostate	*CALD1*		
			PARK7	Parkinson disease (autosomal recessive, early onset) 7 oncogene	Prostate, Breast	*PARK7*	DJ-1	
Looi and colleagues (81)	479 Patients with various types of cancer (including 41 breast cancer sera) and 82 healthy controls Total cohort = 561	ELISA, Western blotting	c-myc	Avian myelocytomatosis viral oncogene homolog	Breast, lung, colon	*Myc*	Myc	Sensitivity = 43.9% Specificity = 97.6%
			p16	Protein 16	Breast, colon	*p16*		
			p53	(Tumor) protein 53	Breast, lung, colon, stomach, liver	*TP53*		
Zhong and colleagues (82)	87 Patients with breast cancer and 87 healthy controls Total cohort = 174	ELUSA, SEREX	SERAC1	Serine active site containing 1	Breast	*SERAC1*		Sensitivity = 77% Specificity = 82.8%
			RELT	Receptor expressed in lymphoid tissues	Breast	*RELT*		
			ASB-9	Ankyrin repeat and suppressor of cytokine signaling (SOCS) box protein 9	Breast	*ASB9*		
Desmetz and colleagues (83)	49 Patients with DCIS, 58 patients with early-stage breast cancer, 20 patients with other types of cancer, 20 patients with various autoimmune diseases, and 93 healthy controls totoal cohort = 240	ELISA, SERPA	HSP60	Heat shock protein 60	Breast, colon	*HSP60*		Sensitivity = 31.8% Specificity = 95.7%

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Reference	Sample size	Methods used	Protein abbreviation	Protein full name	Cancer associated with protein for possible diagnosis	Encoding gene	Alternative names	Accuracy of marker/marker panel
Chapman and colleagues (84)	40 Patients with DCIS, 97 patients with primary breast cancer, and 94 healthy controls Total cohort = 231	ELISA	p53	(Tumor) protein 53	Breast, lung, colon, stomach, liver	*TP53*		Sensitivity = 45%–64% (DCIS-primary breast cancer) Specificity = 85%
			c-myc	Avian myelocytomatosis viral oncogene homolog	Breast, lung, colon	*Myc*	Myc	
			HER2	Human epidermal growth factor receptor 2	Breast, lung	*ERBB2*	HER2/*neu*, ErbB-2, CD340, p185	
			NY-ESO-1	New York esophageal squamous cell carcinoma	Prostate, breast, lung	*NY-ESO-1*		
			BRCA1	Breast cancer type I susceptibility protein	Breast	*BRCA1*		
			BRCA2	Breast cancer type II susceptibility protein	Breast	*BRCA2*		
			MUC1	Mucin 1, cell surface associated	Breast, lung	*MUC1*	PEM	
Hamrita and colleagues (85)	40 Patients with invasive breast cancer and 42 healhy controls Total cohort = 82	SERPA	HSP60	Heat shock protein 60	Breast, colon	*HSP60*		Sensitivity = 47.5% Specificity = 95.3%
Yi and colleagues (80)	81 Patients with presurgery breast cancer and 73 healthy controls Total cohort = 154	2DE, immunoblot, mass spectrometry	AHSG	α-2-HS-glycoprotein	Breast	*AHSG*	Fetuin-A	Sensitivity = 79% Specificity = 90.4%
Pereira-Faca and colleagues (87)	45 Patients with newly diagnosed lung cancer, 18 patients with prediagnostic lung cancer, and 62 matched healthy controls Total cohort = 125	1D-SDS-PAGE, 2D-PAGE, Western blotting, mass spectrometry	14-3-3 Θ	14-3-3 Θ	Lung	*YWHAQ*		Sensitivity = 55% Specificity = 95%

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Reference	Sample size	Methods used	Protein abbreviation	Protein full name	Cancer associated with protein for possible diagnosis	Encoding gene	Alternative names	Accuracy of marker/marker panel
			PGP 9.5	Protein gene product 9.5	Lung	*PGP*		
			ANXA1	Annexin I	Lung	*ANXA1*	Lipocortin I	
Qiu and colleagues (88)	85 Patients with prediagnostic lung cancer and 85 matched healthy controls Total cohort = 170	Protein microarray	ANXA1	Annexin I	Lung	*ANXA1*	Lipocortin I	Sensitivity = 51% Specificity = 82%
			14-3-3 θ	14-3-3 θ	Lung	*YWHAQ*		
			LAMR1	Laminin receptor 1	Lung	*RPSA*	Ribosomal protein SA	
Yang and colleagues (89)	40 Patients with newly diagnosed lung squamous carcinoma, 30 patients with various other types of cancer, and 50 healthy controls Total cohort = 120	2D-PAGE, ELISA	TPI	Triose-phosphate isomerase	Lung	*TPI*	TIM	Sensitivity = 47.5% Specificity = 90%
			MnSOD	Mitochondrial superoxide dismutase 2	Lung, Liver	*SOD2*	SOD2	
He and colleagues (90)	94 Patients with non–small cell lung cancer, 15 patients with small cell lung cancer, 10 patients with gastric cancer, 8 patients with colon cancer, 9 patients with *Mycobacterium avium* complex infection of the lung, and 60 healthy controls Total cohort = 196	2D-PAGE, Western blotting, mass spectrometry, ELISA	CEA	Carcinoembryonic antigen	Breast, colon, lung, stomach	*CEACAM* genes		Sensitivity = 69.3% Specificity = 98.3%
			α-Enolase	α-Enolase1	Lung	*ENO1*		
			CYFRA 21-1	Cytokeratin fragment 21-1	Lung, colon, stomach	*GRP*	Fragment of cytokeratin 19	
Chapman and colleagues (91)	82 Patients with non–small cell lung cancer, 22 patients with small cell lung cancer, and 50 healthy controls Total cohort = 154	ELISA	p53	(Tumor) protein 53	Breast, lung, colon, stomach, liver	*TP53*		Sensitivity = 76%] Specificity = 92%

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			c-myc	Avian myelocytomatosis viral oncogene homolog	Breast, lung, colon	*Myc*	Myc	
			HER2	Human epidermal growth factor receptor 2	Breast, lung	*ERBB2*	HER2/*neu*, ErbB-2, CD340, p185	
			MUC1	Mucin 1, cell surface associated	Breast, lung	*MUC1*	PEM	
			NY-ESO-1	New York esophageal squamous cell carcinoma	Prostate, breast, lung	*NY-ESO-1*		
			CAGE	Cancer antigen 1	Lung	*CAGE1*		
			GBU4-5	TAA GBU4-5	Lung	*GBU4-5*		
Liu and colleagues (92)	46 Patients with colon cancer and 58 healthy controls Total cohort = 104	ELISA mini-array	CEA	Carcinoembryonic antigen	Breast, colon, lung, stomach	*CEACAM* genes		Sensitivity = 82.6% Specificity = 89.7%
			Imp 1	IMP dehydrogenase 1	Colon	*IMPDH1*	Inosine-5′-monophosphate dehydrogenase 1 (IMPDH1)	
			p62	Nucleoporin p62	Colon, liver	*NUP62*		
			Koc	K homology domain containing protein overexpressed in cancer	Colon	*KOC*		
			p53	(Tumor) protein 53	Breast, lung, colon, stomach, liver	*TP53*		
			c-myc	Avian myelocytomatosis viral oncogene homolog	Breast, lung, colon	*Myc*	Myc	
Reipert and colleagues (93)	38 Patients with colorectal adenoma, 21 patients with colorectal adenocarcinoma, and 38 healthy controls Total cohort = 97	ELISA	Fas/CD95	FAS receptor	Colon	*TNFRSF6*	Apoptosis antigen 1 (APO-1 or APT), cluster of differentiation 95 (CD95), TNF receptor superfamily member 6 (TNFRSF6)	Sensitivity = 17% Specificity = 100%
		ELISA	MUC5AC	Mucin-5AC	Colon	*MUC5AC*		

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Table 2. List of studies mentioned in this review including the study sample size, methods used, biomarker abbreviation, full biomarker names, cancer sites associated with biomarker for possible diagnosis, encoding genes, alternative names, and the accuracy of individual biomarkers or biomarker panels (Cont'd)

Reference	Sample size	Methods used	Protein abbreviation	Protein full name	Cancer associated with protein for possible diagnosis	Encoding gene	Alternative names	Accuracy of marker/marker panel
Kocer and colleagues (94)	20 Patients with colorectal polyp, 30 patients with colorectal cancer, and 22 healthy controls Total cohort = 72							Sensitivity = 54% Specificity = 73%
He and colleagues (98)	25 Patients with colorectal cancer and 15 healthy controls Total cohort = 40	SERPA, 2D-PAGE, Western blotting, mass spectrometry, immunohisto-chenistry, ELISA	HSP60	Heat shock protein 60	Breast, colon	*HSP60*		Sensitivity = 52% Specificity = 93.3%
Chen and colleagues (99)	52 Patients with colon cancer, 39 patients with breast cancer, 16 patients with cervical cancer, 70 patients with esophageal cancer, 73 patients with gastric cancer, 62 patients with hepatic cancer, 104 patients with lung cancer, 14 patients with nasopharyngeal cancer, 17 patients with ovarian cancer, and 82 healthy controls Total cohort = 447	ELISA, Western blotting, immunohosto-chemistry	c-myc	Avian myelocytomatosis viral oncogene homolog	Breast, lung, colon	*Myc*	Myc	Sensitivity = 65.4% Specificity = 93.9%
			p53	(Tumor) protein 53	Breast, lung, colon, stomach, liver	*TP53*		
			Calnuc	Nucleobindin 1	Colon	*NUCB1*		
			CCNB1	G_2/mitotic-specific cyclin-B1	Colon	*CCNB1*		
			CCND1	G_1–S-specific cyclin-D1	Colon	*CCND1*		
Shimizu and colleagues (101)	40 Patients with gastric cancer who had undergone gastric resection Total cohort = 40	ELISA	p53	(Tumor) protein 53	Breast, lung, colon, stomach, liver	*TP53*		Sensitivity = 42.5%
			CEA	Carcinoembryonic antigen	Breast, colon, lung, stomach	*CEACAM* genes		
			CA 19-9	Carbohydrate antigen 19-9	Stomach	*MUC1*	Cancer antigen 19-9, sialylated Lewis (a) antigen	
		ELISA	p53	(Tumor) protein 53		*TP53*		

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Reference	Sample size	Methods used	Protein abbreviation	Protein full name	Cancer associated with protein for possible diagnosis	Encoding gene	Alternative names	Accuracy of marker/marker panel
Qiu and colleagues (100)	61 Preoperative gastric carcinoma patients, 10 patients with gastritis, 10 patients with gastric ulcers, and 10 patients with gastrospasm Total cohort = 91				Breast, lung, colon, stomach, liver			Sensitivity = 50.8% Specificity = 100%
			CEA	Carcinoembryonic antigen	Breast, colon, lung, stomach	*CEACAM* genes		
Farinati and colleagues (109)	1,158 Patients with hepatocellular carcinoma Total cohort = 1,158	ELISA	AFP	α-Fetoprotein	Liver	*AFP*	α-Fetoprotein, α-1-fetoprotein, α-fetoglobulin	Sensitivity = 54%
Marrero and colleagues (111)	48 Healthy controls, 51 patients with noncirrhotic hepatitis, 53 patients with compensated cirrhosis, and 55 patients with hepatocellular carcinoma Total cohort = 207	ELISA	AFP	α-Fetoprotein	Liver	*AFP*	α-Fetoprotein, α-1-fetoprotein, α-fetoglobulin	Sensitivity = 77% Specificity = 73%
			DCP	Des-γ-carboxy-prothrombin	Liver	*DCP*	Protein induced by vitamin K absence/ antagonist-II (PIVKA-II)	Sensitivity = 89% Specificity = 95%
			AFP	α-Fetoprotein	Liver	*AFP*	α-Fetoprotein, α-1-fetoprotein, α-fetoglobulin	Sensitivity = 88% Specificity = 95%
			DCP	Des-γ-carboxy-prothrombin	Liver	*DCP*	Protein induced by vitamin K absence/ antagonist-II (PIVKA-II)	
Takashima and colleagues (113)	15 Patients with hepatocellular carcinoma and 20 healthy controls Total cohort = 35	2DE, 2D immunoblot, SEREX	HSP70	Heat shock 70kDa protein 1	Liver	*HSP70*		Sensitivity = 46.7% Specificity = 90%
			Prx	Peroxiredoxin	Liver	*PRDX*		Sensitivity = 33.3% Specificity = 100%
			MnSOD	Mitochondrial superoxide dismutase 2	Lung, liver	*SOD2*	SOD2	Sensitivity = 40% Specificity = 90%
			GAPDH		Liver	*GAPDH*	G3PDH	Sensitivity = 33.3% specificity = 65%

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Reference	Sample size	Methods used	Protein abbreviation	Protein full name	Cancer associated with protein for possible diagnosis	Encoding gene	Alternative names	Accuracy of marker/marker panel
				Glyceraldehyde 3-phosphate dehydrogenase				
Zhou and colleagues (112)	128 Patients with hepatocellular carcinoma, 76 patients with chronic hepatitis, 22 patients with liver cirrhosis, 54 patients with nasopharynx cancer, 54 patients with gastric-intestine, and 80 healthy controls Total cohort = 421	SEREX, Western blot analysis, ELISA	HCC-22-5	Hepatocellular carcinoma–associated antigen HCC-22-5	Liver	*HCC*		Sensitivity = 40% Specificity = 100%
Li and colleagues (115)	174 Patients with hepatocellular carcinoma, 63 patients with chronic hepatitis, 66 patients with other types of cancer, and 71 healthy controls Total cohort = 374	SERPA, 2DE, Western blotting, protein microarray	DDX3X	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked	Liver	*DDX3X*		Sensitivity = 85.6% Specificity = 69.8%
			AIF	Apoptosis-inducing factor	Liver	*AIF*		Sensitivity = 55.9% Specificity = 81.4%
			EEF2K	Eukaryotic elongation factor 2 kinase	Breast, Liver	*EEF2K*	CaMKIII	Sensitivity = 78.8% Specificity = 78.5%
			PBP	Prostatic-binding protein	Liver	*PBP*		Sensitivity = 48.3% Specificity = 82.6%
			hnRNP A2	Heterogeneous nuclear ribonucleoprotein A2	Liver	*HNRNPA2*		Sensitivity = 64.4% Specificity = 70.9%
			TPI	Triose-phosphate isomerase	Lung, Liver	*TPI*	TIM	Sensitivity = 64.4% Specificity = 75%
			AFP	α-Fetoprotein	Liver	*AFP*	α-Fetoprotein, α-1-fetoprotein, α-fetoglobulin	Sensitivity = 72.9% Specificity = 75%

NOTE: Only cancer types described in this review are included.
Abbreviation: 2DE, two-dimensional electrophoresis.

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member 1B (XAGE-1b), lens epithelium–derived growth factor (LEDGF), transferrin receptor protein 9 (p90), and α-methylacyl-CoA racemase (AMACR). The platform allowed the simultaneous screening of these six autoantibodies alongside PSA, and PSA screening alone in 131 patients with presurgery biopsy confirmed prostate cancer and 121 patients with prostatitis and/or benign prostatic hyperplasia. The overall aim of this research was to develop a reliable platform that will enable the diagnosis of patients with prostate cancer relative to nonmalignant cases. Xie and colleagues (73) found that PSA alone had a sensitivity of 52% and specificity of 79% in all patients, whereas the A+PSA platforms showed a sensitivity of 79% and a specificity of 84% in all patients. The A+PSA platform also had a decreased false-positive outcome of only 16% versus 21% when PSA alone was used. Overall, the accuracy of the A+PSA test platform was as high as 81%, whereas PSA alone only showed an accuracy of 65%. Wang and colleagues (14) used phage protein microarray technology and 119 prostate cancer patient sera and 138 healthy control sera to identify increased autoantibody levels of bromodomain-containing protein 2 (BRD2), eukaryotic translation initiation factor 4 γ 1 (eIF4G1), ribosomal protein L22 (RPL22), ribosomal protein LBa (RPL13a), and hypothetical protein XP_373908 (XP_373908) as the antigens most frequently bound to autoantibodies in prostate cancer patient serum. This microarray displayed 81.6% sensitivity and 88.2% specificity. Except for hypothetical protein XP_373908, these structures are derived from intracellular proteins involved in regulating either transcription or translation and closely resembled autologous proteins. However, when tested, their DNA sequences were not identical to those of genes encoding for autologous proteins (14). Moreover, the autoantibody signature was detected in only five of 14 serum samples from patients who had undergone prostatectomy and in three of 11 serum samples from patients with hormone-refractory disease, suggesting that the autoantibody profile is attenuated on removal of the "immunogen" or after treatment with antiandrogen chemotherapeutic agents, or both. Taken together, these results provide evidence that the above-mentioned autoantibodies are associated with the presence of this cancer (14). A more recent microarray study, which aimed to identify an autoantibody signature to distinguish prostate cancer from benign prostatic hyperplasia in patients who showed increased PSA levels, displayed a sensitivity of 95% and 80% specificity compared with 12.2% sensitivity and 80% specificity of PSA alone. This microarray, tested against the sera of 41 patients with prostate cancer and 39 patients with benign prostate hyperplasia, identified talin-1 (TLN1), TAR DNA-binding protein (TARDBP), LEDGF, Caldesmon (CALD1) and Parkinson disease (autosomal recessive, early onset) 7 oncogene (PARK7) as potential diagnostic autoantibody signature (74).

Breast cancer

Biomarkers such as carcinoma antigen 15-3 (CA 15-3), carcinoma antigen 27–29 (CA 27–29), and carcinoembryonic antigen (CEA) have been accepted for clinical use; however, due to their low sensitivity and specificity they are suggested to be used for the diagnosis of more advanced stages rather than for the early diagnosis of breast cancer (75). In terms of autoantibody biomarkers, antibodies to HER2 (76), tumor protein 53 (p53; ref. 77), Mucin 1, cell surface associated (MUC1; ref. 78), and NY-ESO-1 (79) were first discovered in patients with breast cancer. In fact, antibodies to HER2/*neu* (76) have been detected in patients with early-stage breast cancer but their presence has also been detected in other cancers, limiting their use as a diagnostic biomarker for breast cancer alone (28, 30, 80). An increase to 44% sensitivity and 97.6% specificity in breast cancer detection was achieved through the successive addition of the three TAAs p53, protein 16 (p16), and avian myelocytomatosis viral oncogene homolog (c-myc; ref. 81). SEREX technology was used by Zhong and colleagues (82) to detect three further breast cancer–associated autoantibodies including serine active site containing 1 (SERAC1), receptor expressed in lymphoid tissues (RELT), and ankyrin repeat and suppressor of cytokine signaling (SOCS) box protein 9 (ASB-9). The combined panel of these three biomarkers achieved 77% sensitivity and 82.8% specificity when tested against 87 patients with breast cancer and 87 healthy control sera (82). The SERPA approach was used by Desmetz and colleagues (83) who have identified HSP60 autoantibodies in a cohort consisting of 49 patients with ductal carcinoma *in situ* (DCIS), 58 patients with early-stage breast cancer, 20 patients with other types of cancer, 20 patients with various autoimmune diseases, and 93 healthy controls and the sensitivity of HSP60 autoantibodies as a potential biomarker for the diagnosis of breast cancer was calculated to be 31.8%, whereas its specificity is 95.7%. A study by Chapman and colleagues (84) with a cohort of 94 healthy controls, 97 primary breast cancer sera, and 40 DCIS sera tested for seven antigens, including HER2, c-myc, p53, breast cancer type I susceptibility protein (BRCA1), breast cancer type II susceptibility protein (BRCA2), Ny-ESO-1, and MUC1. The specificity of the assay was found to be as high as 91% to 98%, even when tested for individual markers only; however, the individual autoantigen assay sensitivity was only 3% to 23% in the DCIS sera and 8% to 24% in the primary breast cancer sera. On comparison, the sensitivity increased to 45% in DCIS sera and 64% in primary cancer sera with a specificity of 85% when a combined panel of six of seven autoantigens was tested, which alongside other cancer detection methods, such as mammography, may lead to a significant improvement in breast cancer detection. A study by Hamrita and colleagues (85) used the SERPA method to test sera from patients with more invasive breast cancer. The study found HSP60 autoantibodies in 47.5% of patients with breast cancer and in only 4.7% of healthy control sera. α-2-HS-glycoprotein (AHSG) autoantibodies have also been identified in 79.1% of 81 breast cancer patient samples and only in 9.6% of 73 control samples;

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however, the diagnostic relevance of these autoantibodies remains to be validated (80).

Lung cancer

Lung cancer is notoriously heterogeneous and therefore no diagnostic test for the early detection of this cancer has been established (86).

A study by Pereira-Faca and colleagues (87) used one-dimensional and 2D electrophoresis as well as Western blotting and mass spectrometry to identify the 14-3-3 Θ autoantibody as a potential biomarker for the early-stage diagnosis of lung cancer in a cohort consisting of 45 patients with newly diagnosed lung cancer, 18 patients with prediagnostic lung cancer, and 62 matched healthy controls. This 14-3-3 Θ autoantibody was tested in a panel alongside autoantibodies to PGP 9.5 and annexin I, and together these displayed a sensitivity of 55% and specificity of 95%. Furthermore, reactivity to laminin receptor 1 (LAMR1) has also shown high reactivity to lung cancer patient sera (88). This protein microarray study by Qiu and colleagues tested 85 patients with prediagnostic lung cancer and 85 matched healthy controls against 14-3-3 Θ, LAMR1, and annexin I and achieved a sensitivity of 51% and a specificity of 82% (88). Yang and colleagues (89) analyzed a study cohort consisting of 40 patients with newly diagnosed lung squamous carcinoma, 30 patients with various other types of cancer, and 50 healthy controls and performed 2D electrophoresis (2D-PAGE) and an ELISA to identify triose-phosphate isomerase (TPI) and mitochondrial superoxide dismutase 2 (MnSOD) autoantibodies as potential early-stage lung cancer diagnostic biomarkers with a sensitivity of 47% and a specificity of 90%. Furthermore, research by He and colleagues (90), used a combination of methods including 2D-PAGE, Western blotting, mass spectrometry, and ELISA to identify further reactivity and therefore autoantibody production to α-enolase1 (α-enolase) in 28% of patients with lung cancer. When α-enolase was used in combination with other potential autoantibody biomarkers such as CEA and cytokeratin fragment 21-1 (CYFRA 21-1) in a cohort of 94 patients with non–small cell lung cancer, 15 patients with small cell lung cancer, 10 patients with gastric cancer, 8 patients with colon cancer, 9 patients with *Myobacterium avium* complex infection of the lung, and 60 healthy controls, the sensitivity of this potential diagnostic lung cancer biomarker panel was calculated to be as high as 69.3% with a specificity 98.3% (90). An ELISA panel of potential diagnostic lung cancer autoantibody biomarkers composed of p53, c-myc, Her-2, NY-ESO-1, MUC1, cancer antigen 1 (CAGE), and TAA GBU4-5 (GBU4-5) tested by Chapman and colleagues yielded promising results of 76% sensitivity and 92% specificity in another cohort consisting of 82 patients with non–small cell lung cancer, 22 patients with small cell lung cancer, and 50 healthy controls (91).

Colon cancer

To date, CEA is the only serologic biomarker in clinical use for the diagnosis of colorectal cancer; however, this biomarker is also hindered by its low specificity and sensitivity (92). A study by Liu and colleagues (92) showed an increase in colon cancer detection sensitivity over CEA when an ELISA-based mini-array containing five TAAs, IMP dehydrogenase 1 (Imp1), nucleoporin p62 (p62), K homology domain containing protein over expressed in cancer (Koc), p53, and c-myc, was used. When 46 patients with colon cancer and 58 healthy controls were probed with the above-mentioned mini-array, the sensitivity for the combined panel was 82.6% and its specificity was 89.7% in the patients with colon cancer (92). Autoantibodies to the FAS receptor (Fas/CD95; ref. 93) also show specificity for the early detection of colon cancer. Reipert and colleagues (93) investigated sera from 38 healthy controls, 38 patients with colorectal adenomas, and 21 patients with colorectal adenocarcinoma in their ELISA-based array for reactivity against Fas and did not detect any reactivity with Fas in the sera of healthy controls. Furthermore, the anti-Fas antibody titers were higher in patients with colorectal adenomas compared with colorectal adenocarcinoma patient anti-Fas titers resulting in sensitivity and specificity of this array of 17% and 100% for colon cancer, respectively (93), making this biomarker a good option to confirm negative disease status but not to confirm positive disease status, and thus the search for colon cancer biomarkers is still ongoing. Another marker called Mucin-5AC (MUC5AC), was investigated to increase sensitivity of colon cancer detection. This ELISA-based experiment was performed on 20 patients with colorectal polyps, 30 patients with colorectal cancer, and 22 healthy volunteers and its sensitivity was found to be 54%, however, this marker exhibited a much lower specificity than Fas of 73% (94). Studies have shown that autoantibodies to p53 can help identify individuals at increased risk of developing colorectal cancer as these autoantibodies have been detected in patients with precancerous colorectal cancer lesions. In fact, the screening for these autoantibodies is suggested in addition to colonoscopy screens (95–97). However, antibodies to p53 have also been associated with a range of other cancers, which reduces the specificity of this biomarker for colon cancer.

Another study by He and colleagues (98) has shown increased levels of autoantibodies to HSP60 in the sera of 13 of 25 patients with colorectal cancer relative to one of 15 healthy volunteer sera, which results in 52% sensitivity and 93.3% specificity of this marker for colon cancer diagnosis; however, the same autoantibodies have also been observed in patients with breast cancer, which demonstrates that this biomarker is not specific to colon cancer alone (98). Research by Chen and colleagues (99) investigating the reactivity to nucleobindin 1 (Calnuc) in sera from 52 patients with colon cancer, 39 patients with breast cancer, 16 patients with cervical cancer, 70 patients with esophageal cancer, 73 patients with gastric cancer, 62 patients with hepatic cancer, 104 patients with lung cancer, 14 patients with nasopharyngeal cancer, 17 patients with ovarian cancer, and 82 healthy controls showed no significantly higher Calnuc frequency in various cancer groups

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(4.7%) to healthy individuals (1.2%). When patients with colon cancer were investigated, Calnuc frequency was detected to be 11.5% in patients, which is significantly higher than the frequency mentioned in controls. The same study achieved an increase to 65.4% sensitivity and 93.9% specificity when Calnuc was added to a TAA panel composed of c-myc, p53, G_2/mitotic-specific cyclin-B1 (CCNB1), and G_1–S–specific cyclin-D1 (CCND1; ref. 99).

Stomach cancer

To date, there are no stomach cancer–specific biomarkers although p53 autoantibodies have been identified as being associated with stomach cancer as well as several other cancers (100, 101). Previously, Shimizu and colleagues (101) tested the sera of 40 patients with gastric cancer after gastric resection for the presence of p53, CEA, and CA 19-9 autoantibodies. This ELISA-based assay showed that 15% of the patients were positive for p53 autoantibody but not for CEA or CA 19-9 and 17.5% were positive for CEA only while 10% were positive for CA 19-9 (101). Patients seemed to express either p53 autoantibodies or CEA and CA 19-9 autoantibodies. When all three markers were applied as a panel, a panel sensitivity of 42.5% was achieved, which was deemed too low for the panel to be used in the diagnosis of gastric cancer (101). Three years later, Qiu and colleagues (100) tested 61 preoperative patients with gastric carcinoma and 30 patients with other gastric diseases including 10 patients with gastritis, 10 patients with gastric ulcers, and 10 patients with gastro spasm against a combined panel of CEA and p53 autoantibodies. This panel showed positive reactivity for these two markers in 31 of 61 gastric carcinoma patient sera, indicating a sensitivity of 50.8%, but did not show positive reactivity with sera from any of the other gastric diseases (100). Although this panel yielded higher sensitivity, it is important to keep in mind that this panel was tested against preoperative gastric cancer patients while Shimizu and colleagues (101) tested postgastric resection patients, suggesting once more that the autoantibody profile could have been attenuated on removal of the "immunogen" after treatment. The GastroPanel, used to detect gastric mucosa variations including atrophic gastritis, incorporates the biomarkers serum pepsinogen I (PGA1) and serum pepsinogen II (PGA2), gastrin-17 as well as antibodies against *Helicobacter pylori*. Because most stomach cancers arise from chronic inflammations such as gastritis (102), GastroPanel may aid in the early-stage diagnosis of the cancer or may also aid in the identification of individuals who may be at increased risk of developing stomach cancer once inflammation of their gastric mucosal wall has been confirmed.

Liver cancer

Hepatocellular carcinoma (HCC), the predominant form of primary liver cancer, is diagnosed by the histologic examination of the liver using ultrasonography (103). Although this technology displays a sensitivity of 60% to 80%, a positive predictive value of 78% and a specificity of up to 98% (104), it is nonetheless subject to detection bias as it is an operator-dependent technology and small tumors may be overlooked against a cirrhotic background (105, 106). Therefore, there is a need to support the diagnosis of this cancer on a more molecular level. The search for autoantibodies for the diagnosis of the cancer is therefore of great interest to develop a blood test for hepatocellular carcinoma diagnosis.

α-fetoprotein (AFP), a normal serum protein synthesized during embryonic development, is currently considered to be the best biomarker available for hepatocellular carcinoma diagnosis (107). Elevated levels of AFP are observed in pregnant woman and chronic liver disease patients; however, lower levels of this biomarker are also observed in healthy individuals and nonpregnant woman, implying that AFP cannot be used for the diagnosis of small hepatocellular carcinoma tumors (108). The sensitivity of the biomarker lies between 40% and 65% and its specificity between 75% and 90% while displaying a positive predictive value of only 12% (109). One major study by Zhang and colleagues (110) was performed in China to measure whether a combination of routine ultrasonography screening and an ELISA-based AFP test (cutoff value at 20 μg/L) increases hepatocellular carcinoma detection rates. Out of the 18,816 people with hepatitis B virus (HBV) infection included in this study, 9,373 were randomly selected to be part of the screening group, which was offered an ultrasonography examination and an AFP test combination every 6 months for a period of up to 5 years and the remaining 9,443 people were randomly selected to be part of the control group, which did not receive any extra screening but continued to use health care facilities (110). During this study, 71 cases of hepatocellular carcinoma were detected in the screening group compared with 67 in the control group (110), but this slight increase was not considered to be sufficient evidence to support further use of AFP testing in combination with routine ultrasonography examination and therefore routine ultrasonography examination alone is used during clinical practice (107). In 2006, Farinati and colleagues (109) tested 1,158 patients with hepatocellular carcinoma for AFP levels in their ELISA-based test. AFP levels less than 20 ng/mL were considered normal, whereas 21 to 400 ng/mL were defined as elevated and more than 400 ng/mL were considered as diagnostically significant. With regards to these levels, the group confirmed the low sensitivity of AFP as 54% and did not recommend this marker for utilization in the routine diagnosis of hepatocellular carcinoma (109). Serum levels of des-γ-carboxyprothrombin (DCP), another potential biomarker for hepatocellular carcinoma diagnosis, have been compared with AFP levels in an ELISA-based experiment performed by Marrero and colleagues (111). This research tested sera from 48 healthy controls, 51 patients with noncirrhotic hepatitis, 53 patients with compensated cirrhosis, and 55 patients with hepatocellular carcinoma against DCP and AFP individually and in combination to find the best marker or panel to differentiate patients with

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hepatocellular carcinoma from other patients with non-malignant chronic liver disease. The study concluded that the sensitivity and specificity of AFP levels alone are 77% and 73%, and of DCP are 89% and 95%, respectively, and the combination of the two markers resulted in 88% and 95% sensitivity and specificity (111).

The utilization of SEREX methodology showed the presence of hepatocellular carcinoma–associated antigen HCC-22-5 (HCC-22-5) autoantibodies in 78.9% patients with liver cancer who were diagnosed as AFP-negative and these autoantibodies were not detected in healthy control sera nor in the sera of patients with lung or gastrointestinal cancer (112). In another SEREX-based study, Takashima and colleagues (113) tested 15 patients with hepatocellular carcinoma and 20 healthy control sera against glyceraldehyde-3-phosphate dehydrogenase (GAPDH), HSP70, MnSOD, and peroxiredoxin (Prx) and found that high GAPDH autoantibody levels were present in 33.3% of patients and in 35% of controls, indicating that routine use of GAPDH for hepatocellular carcinoma diagnosis is not recommended, whereas high HSP70 levels were detected in 46.7% of patients and in only 10% of controls (113). In the same study, high serologic autoantibody levels of MnSOD were detected in 40% of patient sera and in only 10% of controls, whereas high PRX autoantibody levels were detected in 33.3% of patients and 0% of controls (113).

Chronic HBV infection and cirrhosis are high-risk factors for the development of hepatocellular carcinoma and TAA autoantibodies can be found in patients with HBV-associated hepatocellular carcinoma (107, 114). SERPA and protein microarray studies have found autoantibodies to proteins, including EEF2, heterogeneous nuclear ribonucleoprotein A2 (hnRNP A2), DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked (DDX3X), apoptosis inducing factor (AIF), prostatic binding protein (PBP), and TIP to be significantly higher in patients with hepatocellular carcinoma than in healthy individuals or patients with chronic hepatitis. The sensitivity of any of the four markers: DDX3X, PBP, EEF2, and AIF was found to be 50% to 85% and increased to 90% when analyzed as a biomarker panel (115).

Potential Future use of Autoantibodies as Diagnostic Cancer Biomarkers

By avoiding the progression of a cancer to an often incurable metastatic stage, early detection of all cancers may lead to increased survival rates and better quality of life. The golden standard diagnostic techniques used today, such as mammography for breast cancer detection, are highly successful, however, they are often subject to detection bias and may result in false-negative diagnosis of a patient whose tumor has been overlooked because of the limitations of current diagnostic techniques. To aid the early detection of all cancers and to ensure that all oncology patients are correctly diagnosed, the focus now lies in finding biomarkers, indicating a positive diagnosis at an earlier stage. This early detection of any cancer will potentially aid health care professionals to choose an appropriate therapeutic intervention, which will target early-stage tumors at their most treatable stage.

Levels of certain autoantibodies have been found to arise prior and during tumor formation, indicating that autoantibodies may serve as highly effective biomarkers for the early diagnosis of cancers. To search for such autoantibodies, several state-of-the art technologies and methodologies have been developed, including SEREX, phage display, protein microarrays, reverse-capture microarrays, SERPA, and MAPPing. These methodologies and techniques have enabled the simultaneous identification of several autoantibodies for different cancers and these are currently being tested for their potential to serve as diagnostic biomarkers for specific cancers. So far, the clinical application of most identified autoantibodies has been hindered by their low sensitivity, specificity, and predictive value percentages as well as poor reproducibility within different experimental designs and applications of the methodology.

Nonetheless, the number of autoantibodies identified that displayed improved sensitivity, specificity, and predictive value percentages has been increasing and several studies have shown increases in sensitivity and specificity scores when the potential autoantibodies are applied in combination as in a diagnostic biomarker panel. As previously stated in this review, PSA was the only marker used for prostate cancer diagnosis and its use has now been discontinued because of low sensitivity scores. Research by O'Rouke and colleagues (74) tested a study cohort of 80 samples for reactivity against PSA alone in comparison with a new biomarker panel including markers TLN1, TARDBP, LEDGF, CALD1, and PARK7. The research showed an increase in sensitivity from 12.2% for PSA alone to 95% for the panel, whereas specificity was calculated to be 80% in both PSA alone and the panel. This research is an example of the discovery of combined panels of markers that show potential as biomarker panels for the diagnosis of prostate cancer. On the other hand, Yi and colleagues (80) discovered a single potential diagnostic biomarker called AHSG for breast cancer diagnosis. This marker yielded the high sensitivity of 79% for breast cancer detection.

Chapman and colleagues (91) also showed that a multimarker panel, analyzed via ELISA, was informative for the early diagnosis of lung cancer. This panel included the markers p53, c-myc, HER2, MUC1, NY-ESO-1, CAGE, and GBU4-5 and resulted in 76% sensitivity and 92% specificity, scores that are far above those achieved by previous lung cancer–associated diagnostic autoantibody biomarker studies. Another panel discovered by Liu and colleagues (92) for the diagnosis of colon or colorectal cancer achieved 82.6% and 89.7% sensitivity and specificity. The panel consists of the markers CEA, Imp-1, p62, Koc, p53, and c-myc. Furthermore, Qiu and colleagues (100) demonstrated an increase in sensitivity and specificity to 50.8% and 100%, respectively, when p53 and CEA were tested in combination for the diagnosis of stomach or

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gastric cancer. Finally, Marrero and colleagues (111) also performed an ELISA and demonstrated that the single marker, DCP, has the highest diagnostic potential for the early detection of liver cancer due to its high sensitivity of 89% and specificity of 95%.

In the future, more diagnostic cancer biomarker studies are required that contain larger cohorts to avoid inter-sample variations. Furthermore, consistent methodologic conditions for autoantibody detection are essential. Further autoantibody biomarker research may provide new knowledge of molecular events in carcinogenesis and cancer progression, thus improving early detection of individuals at risk of disease recurrence.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The authors confirm that this review has not been published elsewhere and is not under consideration by another journal. All authors have approved the review and agree with the submission to *Cancer Epidemiology, Biomarkers & Prevention*.

Authors' Contributions

Conception and design: P. Zaenker, M.R. Ziman
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Serologic Autoantibodies as Diagnostic Cancer Biomarkers––A Review

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