Clinical Relevance of Autoantibody Detection in Lung Cancer

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Abstract: Sensitive techniques have recently been developed to identify tumoral antigens and to detect tumor-related autoantibodies in the peripheral blood of patients with cancer. Studies of these new methods indicate that the detection of a combination of autoantibodies could be a relevant prescreening strategy for the early detection of lung cancer in patients at high risk. Nevertheless, the clinical utility of autoantibodies for determining prognosis and monitoring response to systemic therapies in lung cancer is less conclusive. This article summarizes the clinical background and the technical aspects of current methods used for the detection and characterization of autoantibodies in blood, with a special focus on the implications of these methods for the clinical management of patients with lung cancer.

Key Words: Lung cancer, Autoantibody, Immune response, Screening.

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Lung cancer is a worldwide clinical problem and a major cause of cancer deaths. More than 150,000 new cases are diagnosed in Europe every year, and overall mortality is increasing, with an overall 5-year survival rate of 6 to 16%.[1] This poor prognosis is primarily related to the propensity of lung cancer for early spread and late diagnosis. More than 75% of patients present with local or distant metastasis at diagnosis and are associated with 21% and 3% 5-year survival, respectively.[2] Lung cancer such as diagnosed during routine medical care is rapidly fatal; even clinical stage I tumors are at high risk of relapses after complete surgical resection.[3]

Screening trials using chest radiography and sputum cytological analysis have thus far been unable to decrease lung cancer mortality in the high-risk population of smoking individuals.[1,4–6] Recently, screening trials using low-dose spiral computerized tomography (CT) have led to a resurgence of interest in the prevention of lung cancer.[7,8] Nevertheless, this technique has poor specificity because of the high prevalence of noncalcified and ground glass opacities for which a large spectrum of different diseases could be evoked (e.g., granulomatous disease, fibrosis, atypical adenomatous hyperplasia, bronchoalveolar carcinoma, adenocarcinoma, or slowly resolving inflammatory lesions). Such suspicious abnormalities may require invasive procedures (including surgical resection in an attempt at diagnosis) and potentially lead to overdiagnosis of slow-growing preneoaplastic lesions or benign lesions.[9] In addition, the spectrum of lung cancer detected as a result of CT screening seems to be different than that of patient diagnosed during routine medical care. Tumors found in CT screening programs have markedly longer doubling times and a substantially greater proportion with very long (>400 days) doubling times.[3] Therefore, CT screening seems to identify a somewhat different cohort of patients with lung cancer that includes many more patients with slow growing tumors.

All these limitations have thus spurred continued interest in identifying reliable tumor markers that can be detected in serum. Because whole blood is considered to provide a dynamic representation of an individual's physiological and pathological status, human serum has been extensively studied in the quest for cancer biomarkers.[10] In small cell lung carcinoma (SCLC), lactate dehydrogenase (LDH) and neuron-specific enolase (NSE) have been identified as independent biological factors.[11] Since 1993, cytokeratin 19 fragment (CYFRA 21-1) has been proposed as an independent prognosis marker in the non-small cell lung cancer (NSCLC) subgroup.[12] CYFRA 21-1 was then validated in a meta-analysis including 2063 patients as a putative covariable in analyzing NSCLC outcome inasmuch as a high serum level is a significant determinant of poor prognosis whatever the planned treatment.[13] Other markers have been studied, such as antigens (CA125, NSE, and tissue polypeptide antigen [TPA]), proliferation markers (KI67), angiogenesis markers (vascular endothelial growth factor [VEGF]),[14] and oncogene or tumor suppressor genes (Ras, Rb, p53, Bcl2, and human epidermal growth factor receptor 2).[15,16] Nevertheless, none of these markers has sufficient predictive value as a screening blood assay. Finally, these markers are mostly used in conjunction with diagnostic imaging, medical history, and physical examination to establish the initial diagnosis (reference value), to subsequently assess the efficacy of treatment and to monitor evidence of relapse. Therefore, it is of high clinical importance to develop an easy-to-perform screening test that

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can identify patients with lung cancer in early curable stage(s).

The search for tumor antigens that induce specific immune responses in patients with cancer is a promising approach in this field. Evidence for a specific humoral response against a number of intracellular and surface tumoral antigens is now established in patients with cancer. In this study, we review the potential and the limitations of currently available methods for the identification of autoantibodies in early-stage lung cancer. Moreover, we discuss the clinical applications of autoantibody detection in the context of detecting patients with early-stage cancer, selecting the most efficient therapy and monitoring these therapies by repeated blood analyses. We also discuss how autoantibody profiling will impact diagnosis and benefit therapy. Finally, autoantibody analysis will contribute to a better understanding of the complex invasion process in patients with cancer, potentially leading to new strategies to eradicate tumor cells or control their outgrowth into life-threatening overt metastases.

ORIGIN OF THE IMMUNE RESPONSE

The role of adaptive immune reactions in cancer has a long history of heated debate. The immunosurveillance theory[17] has long dominated the field based on clinical observations of increased incidence of lung cancers, which are not related to any known viral oncogenes, in immunosuppressed individuals[18] and the development of tumors originating from a transplanted organ when the recipient was subjected to immunosuppressive drugs.[19] The study of immunocompromised mice provided a clear demonstration of the protective role of the immune system against tumors. Animals with specific disruptions of genes involved in adaptive or innate immunity exhibit greater sensitivity to chemically induced and spontaneous tumor appearance.[20,21]

The hypothesis that the immune system not only can protect the host against tumor development but also, by selecting for tumors with lower levels of immunogenicity, has the capacity to promote tumor growth was first proposed by Dunn et al.[22,23] They refined the cancer immunosurveillance theory into a theory of cancer immunoediting and proposed three possible tumor cells outcomes: elimination (i.e., cancer immunosurveillance), when tumor cells are cleared by both the innate and adaptive immune response, as in the host defense against microbial pathogens; equilibration, when lymphocytes and interferon-γ exert potent and relentless selection pressure on tumor cells that is sufficient to contain but not fully extinguish a tumor bed containing many genetically unstable or mutated cells[24]; and finally escape, wherein the immunologically sculpted tumor expands in an uncontrolled manner.[25] In contrast, Willimsky and Blankenstein[26,27] demonstrated that even highly immunogenic sporadic tumors are unable to induce functional cytotoxic T lymphocytes, arguing against the immunosurveillance of spontaneous cancer.

The recent interest in the role of T-cell immunology in cancer promotion and progression has been at the expense of our understanding of the humoral immune response to cancer. Accordingly, tumor-associated antigens (TAAs) can elicit a T lymphocyte response, which directly or indirectly kills tumor cells by recruitment of macrophages, polymorphonuclear cells, and natural killer cells. Nevertheless, TAAs can also elicit a B lymphocyte response, inducing the production of autologous antibodies (autoantibodies). The precise mechanism underlying this B-cell reaction remains essentially unknown. The chief role of B cells is to produce soluble substances known as antibodies. When a B cell encounters its specific or eliciting antigen (along with various accessory cells), it differentiates into a plasma cell, producing one specific antibody that plays a crucial role in a cascade of events called the humoral immune response, ultimately leading to the destruction of the antigen. Finally, it remains unclear whether B cells and their associated antibodies promote de novo carcinogenesis[28] or allow controlled tumor progression, or instead whether autoantibody production only represents an epiphenomenon of specific immunotolerance to TAA production.

Although a wide variety of ubiquitously expressed TAAs have been identified in several cancers,[29] the expression of tumoral proteins is probably not sufficient to elicit a humoral response. Autoantibody production has frequently been related to tumor protein alterations or modifications such as heat shock protein (HSP) binding (HSP70/P53[30]), proteolysis of apoptotic cancer cells,[31] overexpression (p53,[32] and FK506-binding protein 52 [FKBP52][33]), aberrant post-translational processing (mucin 1 [MUC1][34]), and ectopical localization.[35] Interestingly, responses against these TAAs usually fail to reach high titers because of immunotolerance mechanisms.[36,37] In addition, patients with cancer with localized diseases have higher serum antibody titers than those with metastatic disease, suggesting that TAA immunotolerance occurs with cancer progression.[38,39] Finally, the majority of autoantibodies are in the IgG subclass, suggesting that cognate helper T-cell immunity is present and operative in patients with B-cell responses. It is probable that an integrated immune response against TAA exists that involves both $CD8^+$ and $CD4^+$ T cells, and B cells[40] (Figure 1).

AUTOANTIBODY DETECTION

The identification of autoantibodies in the peripheral blood of patients with cancer holds great promise but remains technically challenging. Current approaches are all based on the combination of a tumor antigen source and the immunodetection of corresponding autoantibodies in patients' sera. Serological analysis of recombinant complementary DNA (cDNA) expression library (SEREX) analyses recombinant expression cloning antigens from a cDNA expression library obtained from autologous tumor-extracted messenger RNA[41] and has been widely used to identify new TAAs. Although SEREX has defined more than 2000 antigens, with approximately one third of those representing novel genes at the time of their discovery,[42] most of these antigens have not been validated in large cohorts and are probably not relevant as tumor markers. Indeed, SEREX-defined TAAs are mainly cytoplasmic proteins because of the bacterial library screening method used, whereas membrane proteins such as HER2[43] have been validated as relevant TAAs. In addition,

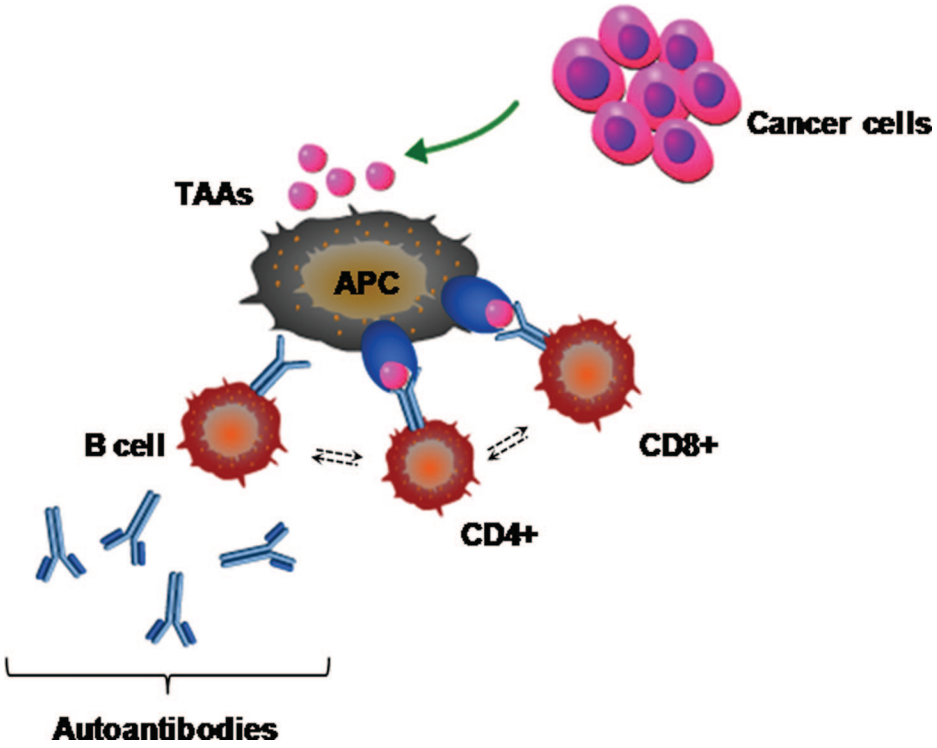

FIGURE 1. Schematic representation of cellular interactions inducing humoral response. Tumor-associated antigens are presented on major histocompatibility complex class I or class II of the antigen-presenting cell (APC) to either $CD8^+$ or $CD4^+$ T cells. TAAs can also elicit a B-lymphocyte response, inducing the production of autologous antibodies (autoantibodies). TAA, tumor-associated antigens.

proteins are not expressed with highly immunogenic posttranslational modifications in bacterial libraries, and the SEREX method misses low abundance TAAs.[44] Finally, despite the proposal of several improvements to the method using yeast eukaryotic models to identify immunogenicity associated with posttranslational modifications,[45,46] the construction of a cDNA expression library for every analyzed autologous serum sample is highly complex and time consuming. To overcome the drawbacks of SEREX, serological proteomic analysis (SERPA) has been proposed, which associates two-dimensional gel electrophoresis with mass spectrometry.[47] This method has been used to describe new TAAs associated with posttranslational modifications or protein overexpression (e.g., HSP60,[48] RHO guanosine diphosphate dissociation inhibitor 2 [RHO-GDI] dissociation inhibitor 2,[49] peroxiredoxin 6 immunogenicity,[50] or heterogeneous nuclear ribonucleoproteins[51]). Nevertheless, similar to SEREX, SERPA only allows for the identification of a limited number of TAAs.

The success of DNA chip approaches for genome-wide analysis has recently inspired research groups interested in the humoral response. Although the conception and initial development of "multianalyte microspot immunoassays" by Ekins[52] and photolithographically generated peptide arrays by Fodor et al.[53] occurred over a decade ago, only recently have several groups reported significant progress toward developing protein microarray technologies. Protein microarrays are based on immobilized antigens followed by analysis of antigen-antibody interactions. Protein microarrays detect a large panel of autoantibodies (thousands) using a one-step strategy and detect unknown proteins as novel TAAs.[54–56] The limitations of protein microarray-based technologies include the fact that only commercially available antigens and native antigens can be analyzed.[57] Newly developed protein array-based detection technologies may circumvent this problem. Similarly to SEREX, phage-display cDNA expression libraries produced from isolated tumor messenger RNA have been successfully used to generate protein microarrays.[58] In addition, identification of antigens containing posttranslational immune modifications can be accomplished using a multidimensional fractionation technique based on liquid chromatography to isolate a mixture of native proteins extracted from cancer cell lines.[59,60]

Therefore, TAAs can currently be enriched and detected through novel strategies. Protein microarray approaches certainly have many advantages for the identification of new tumor biomarkers associated with the humoral response, in particular due to their capacity for high throughput analysis, although the biochemistry of immunomicroarrays must be better characterized and simplified. In the future, additional strategies should be developed that are also able to isolate subpopulations of TAAs with low protein expression. Currently, for the enrichment step, tumor cell fractionation strategies based on the enrichment of subproteome groups such as membrane proteins or nuclear proteins might be feasible approaches for unbiased selection of TAAs.

CLINICAL APPLICATION OF AUTOANTIBODIES IN LUNG CANCER

Early Diagnosis and Single Marker

No current technologies offer early diagnosis. Spiral CT/magnetic resonance imaging generates a false-positive rate (up to 50%). In this context, a screen based on an

TABLE 1. Identification of Autoantibody in Lung Cancer

TAAs	Technique	Antigen Source	No. of Sera	Statistical Values (%)	References
SOX1, 2, 3, 21, and ZIC2	SEREX	NCI-H740 cells	17 LC, and 23 NC	RS: 41, 29, 35, 29, 14	61
Protein gene product 9.5	SERPA	A549 cells	64 LC, 99 OTC, and 71 NC	RS: 14	62
Annexins I and II	SERPA	A549 cells	64 LC, 609 OTC, and 61 NC	RS: 60	63
p53	ELISA	—	133 LC	RS: 23%	64
p53	ELISA	—	115 LC	RS: 19	65
NY-ESO-1	ELISA	—	175 LC, and 51 NC	RS: 23	66
Livin and survivin	ELISA	—	31 LC	RS: 51, 58	67
Alpha-enolase, inosine-5'-dehydrogenase, aldehyde dehydrogenase, 3-PGD, 3-oxoacid CoA transferase, chaperonin, Prx-6, and TPI	SERPA	A549 cells	5 LC, 10 OTC, and 20 NC	—	68
Peroxiredoxin I	ELISA	—	53 LC and 50 NC	RS: 27%	69
TPI and MnSOD	SERPA	—	Screening set: 20 LC and 20 NC; validation set: 40 LC and 30 OTC, and 50 NC	RS: 20, 27	70
Alpha-enolase		NSCLC tumor tissues	109 LC, 29 OTC, and 60 NC	RS: 28	71
Complement factor H	SERPA	Serum	Screening set: 56 LC and 12 NC; validation set: 125 LC and 125 NC	RS: 4.8–26.3	72
Cytokeratin 18 and villin 1	1-DE/WB	LCN1, N231, A549, and RERF-LC-AI cells	2 LC	—	73
Dickkopf-1	ELISA	—	93 LC and 87 NC	Se: 62 Sp: 84	74
TPI	ELISA	—	174 LC and 59 NC	Se: 62 Sp: 66	75
Peroxiredoxin 6	ELISA	—	174 LC and 59 NC	Se: 84 Sp: 75	75

LC, lung cancer; OTC, other types of cancer; NC, noncancer controls; Se, sensitivity; Sp, specificity; RS, reactivity of sera in patients with lung cancer to the TAA; TPI, triosephosphate isomerase; TAA, tumor-associated antigens; ELISA, enzyme-linked immunosorbent assay.

independent test may improve the positive predictive value of these screening tools in smokers at the highest risk for lung cancer. People with a positive biological test would proceed to a radiological test. Positive imaging would confirm lung cancer leading to treatment, whereas negative imaging patients would continue to receive intensive follow-up in specialist clinics. Several autoantibodies have been identified in lung cancer and propose as potential serum diagnosis markers (Table 1). Among them, p53 autoantibodies in lung cancer has been largely investigated and their prevalence occur in approximately 30% of patients with lung cancer.[76] Interestingly, the presence of p53 autoantibodies is highly correlated with p53 gene missense mutations and accumulation of p53 in the tumor (60–70%).[32] Remarkably, p53 autoantibodies appear very early in carcinogenesis, even before the cancer diagnosis.[77,78] Sry-box containing gene (SOX) family proteins have also been proposed as relevant TAAs in lung cancer.[61,79,80] SOX1, 2, and 3, members of the SOXB1 family of proteins, have been shown to be transcriptional activators and to prevent differentiation of neurogenesis,[81] whereas SOX21 represses SOX1, 2, and 3 and promotes differentiation.[82] Recently, SOX autoantibodies have been shown to have diagnostic value in discriminating early-stage lung cancer with Lambert-Eaton myasthenic syndrome from nontumoural Lambert-Eaton myasthenic syndrome.[83] Using SERPA, Hanash's team identified protein gene product (PGP) 9.5 and annexins I and II as immunogenic proteins in lung cancer. PGP 9.5 belongs to a family of ubiquitin COOH-terminal hydrolase isoenzymes that play a regulatory role in the ubiquitin system.[84] PGP 9.5 has been implicated in the removal of ubiquitin from ubiquitinated proteins, thus preventing their degradation by proteasomes. Annexin I is implicated in glucocorticoid-induced inhibition of cell growth, and annexin II is implicated in cell–cell adhesion.[85,86] The New York cosophageal squamous cell carcinoma 1 (NY-ESO-1) gene elicits a humoral response in 15 to 30% of affected patients.[66,87] NY-ESO-1 belongs to an expanding class of immunogenic testicular antigens that are aberrantly expressed in human cancers in a lineage-nonspecific fashion such as cancer-associated gene protein (CAGE) or GBU4-5, a protein of unknown function that encodes a DEAD box domain.[43] More recently, a panel of new autoantibodies including livin and survivin,[67] triosephosphate isomerase and manganese superoxide dismutase (MnSOD)[70,75] alpha-enolase,[71] complement factor H,[72] and cytokeratin 18 and villin 1[73] have been shown to be capable of inducing autoantibodies in patients with lung cancer. Finally, large validation studies estimating the sensitivity and specificity of each autoantibody as early diagnostic lung cancer markers have been investigated only relatively recently in lung cancer. Thus, Dickkopf-1,[74] peroxiredoxin 6, and triosephosphate isomerase[75] exhibited sensitivities and specificities of approximately 60% and 80%, respectively.

TABLE 2. Identification of Autoantibody Signature for Early Lung Cancer Detection

Panel of TAAs	Technique	Antigen Source	No. of Sera	AUC	Se	Sp	*References*
Panel of autoantibodies including ubiquitin	Protein microarray	—	Screening set: 125 LC and NC validation set: 122 LC and NC	0.79	85	86	*88*
14-3-3 theta, annexin I and PGP 9,5	1-DE/WB	A549, H522, and H23 cells	Screening set: 19 LC, and 19 NC validation set: 26 LC and 24 NC		55	95	*89*
14-3-3 theta, annexin I and LAMR1	Protein microarray	A549 cells	85 prediagnostic LC	0.73	51	82	*10*
c-myc, p53, cHER-2, MUC1, NY-ESO-1, CAGE, and GBU4–5	ELISA	—	109 LC, and 50 NC		76	92	*43*
Seven amino-acid peptides	Phage cDNA expression library	—	121 LC and NC	0.98	70	73	*90*
Six clones and one identified as olfactomedin 1	*Phage cDNA expression library*	—	*Screening set: 40 LC, and 10 NC validation set: 30 LC and 90 NC*		*92*	*92*	*91*

LC, lung cancer; OTC, other types of cancer; NC, noncancer controls; Se, sensitivity; Sp, specificity; RS, reactivity of sera in patients with lung cancer to the TAA; TAA, tumor-associated antigens; ELISA, enzyme-linked immunosorbent assay.

Early Diagnosis and Combination of Autoantibody Detection

When considered alone as diagnostic tools, most autoantibodies show insufficient sensitivity and/or specificity to be proposed as complementary or alternative strategies for early detection of lung cancer. Indeed, most of them are present in approximately 30% of patients with lung cancer (Table 1). Moreover, autoantibodies can be present in different types of cancer and thus lack specificity. To achieve the highest degree of sensitivity and specificity and to encompass the histological heterogeneity of lung cancer, combining multiple autoantibody reactivities into a panel assay test has been proposed as relevant screening tests and validated in several independent populations of patients including healthy controls, noncancer chronically diseased patients, and patients with cancer (Table 2). Hanash and coworkers[89] proposed for the first time a panel of autoantibodies for early detection of lung cancer. The combination of one newly identified TAA (14-3-3 theta) using SERPA plus two previously identified TAAs (annexin 1[63] and PGP9.5[62]) gave a sensitivity of 55% with 95% specificity and an area under the curve of 0.838. Chen et al.[88] developed a high-density peptide microarray derived from biopanning of a lung cancer phage display library to identify and characterize an autoantibody signature for lung adenocarcinoma patients. A total of 2304 phage-peptide clones were randomly selected from the biopanned phage libraries to generate phage-peptide microarrays. A set of 22 discriminating peptides derived from a training set of 125 serum samples from lung adenocarcinoma patients and control subjects was found to predict cancer status with 85% sensitivity and 86% specificity in an independent test set of 125 sera. Interestingly, one immunoreactive phage-peptide clone was identified as ubiquilin 1 and exhibited an area under the curve value of 0.79 in an independent validation set including 62 lung adenocarcinomas and 60 controls.[88] Robertson and coworkers[43] demonstrated that the combination of p53, c-myc, HER2, NY-ESO-1, CAGE, MUC1, and GBU4-5 autoantibody detection by enzyme-linked immunosorbent assay yielded a biological test with a sensitivity of 76% and a specificity of 92%. Very recently, sensitivities of 36%, 39%, and 37% and specificities of 91%, 89%, and 90% were observed in three independent cohorts of patients with lung cancer based on the detection of p53, NY-ESO-1, CAGE, GBU4-5, annexin 1, and SOX2 autoantibodies.[92] Also very recently, a phage cDNA expression library was used to identify a six-phage peptide detector allowing for discrimination between patients with NSCLC and healthy controls with a sensitivity and specificity of more than 92% in an independent set of 90 patients with NSCLC and 90 matched healthy controls, 30 patients with NSCLC with chemotherapy, and 12 chronic obstructive pulmonary disease patients.[91] Finally, the use of an autoantibody signature before cancer reaches the clinical stage has been investigated in a high-risk cancer population of smokers. Natural protein microarrays based on extensive protein fractionation followed by spotting of aliquots from individual fractions was performed to determine the occurrence of autoantibodies to annexin I, laminin receptor 1 (LAMR1), and 14-3-3 theta reactivity in 85 patients within 1 year before a diagnosis of lung cancer and 85 matched controls.[10] A significant correlation was observed between the presence of antibodies and cancer development, confirming that autoantibody production precedes the onset of symptoms and the diagnosis of NSCLC.

In summary, early detection of cancer is probably the most exciting application of autoantibodies detection. Clinical trials have been initiated testing various TAAs in early-stage lung cancer detection, and the results of these trials suggest that autoantibody analyses can provide important information about the risk of lung cancer development in smokers that cannot be obtained by current methods (e.g., measurements of radiographic changes by CT). Although these data are very promising, large-scale multicenter trials with robust autoantibody detection assays and well-defined cutoffs are now warranted to substantiate the claim that autoantibody detection will contribute to the future clinical management of patients with cancer.

Monitoring Systemic Antilung Cancer Therapies

Most of the current emphasis is being placed on cancer autoantibodies as diagnostic biomarkers, but it is likely that autoantibodies can also be used as markers of prognosis or monitors of response to therapy. Although many studies on cancers of the head and neck, breast, and gastrointestinal tract have shown that patients who developed serum p53 autoantibodies have a worse prognosis, the prognostic significance of serum p53 autoantibodies in lung cancer has remained controversial. Winter et al.[93] first reported that patients positive for p53 autoantibodies have a (borderline) better survival rate in SCLC. This result was later confirmed by Lai et al.[94] In contrast, Rosenfeld et al.[95] reported that the presence of antip53 autoantibodies in the sera of patients with newly diagnosed SCLC was not associated with any clinical characteristics or prognostic markers. The number of autoantibody detected in patients with lung cancer as prognostic biomarker is low. Several cancer/testis antigens expressed in normal adult tissues solely in the testicular germ cells of normal adults and in various cancers have been investigated for their potential prognosis value in lung cancer. Among them, NY-ESO-1,[96] melanoma-associated antigen 3 (MAGE-A3),[96] and MUC1[97] have been reported as independent markers of a worse prognosis in patients with NSCLC.[96,97] Nevertheless, although these efforts have provided significant associations in metastatic lung cancer, the prognostic and predictive value of autoantibody detection in metastatic lung cancer has not been proven by large studies, and autoantibody signatures do not seem to outperform conventional imaging methods.[91]

TAAs and Immunotherapeutic Target

Finally, based on the recognition by humoral or cellular immune responses in the autologous human host, tumor antigens have provided the cancer researcher with powerful tools to identify attractive new targets for vaccine-based therapies.[98,99] Cancer immunotherapy is largely based on the use of peptide antigens derived from amino acid sequences of tumor antigens. Current cancer immunotherapy approaches propose the combination of tumor antigens, including TAAs, with a protein immunologic adjuvant system, creating a novel class of compounds called antigen-specific cancer immunotherapeutics (ASCIs). This combination is supposed to induce the immune system to produce an effective T-cell response against antigens found exclusively on the tumor but not (or at low levels) on normal cells. Based on these promising phase II data,[100] a randomized, double-blinded phase III study known as MAGRIT (MAGE-A3 as adjuvant NSCLC immunotherapy) trial including 33 countries in Europe, North and South America, Asia, and Australia has been initiated. The MAGRIT study is the largest-ever trial in the adjuvant treatment of NSCLC. The objective of the MAGRIT trial is to investigate the efficacy of MAGE-A3 ASCI in preventing cancer relapse, when given after tumor resection in patients with MAGE-A3-positive stages IB, II and IIIA NSCLC.[101] The study will also evaluate potential side effects of MAGE-A3 ASCIs, to confirm the promising tolerability seen in phase II.

MUC1, which is overexpressed and aberrantly glycosylated in NSCLC, has also been proposed as a relevant immunotherapeutic target. L-BLP25 (Stimuvax; Biomira, Alberta, CA) is a liposome vaccine targeted to the extracellular core peptide of MUC1.[99] Preclinical studies confirmed that the vaccine could elicit antigen-specific T-cell proliferation and interferon-γ secretion, and initial phase 1 and phase 2 trials showed that L-BLP25 had a favorable safety profile.[102] A randomized phase II trial of maintenance L-BLP25 versus best supportive care in patients with stage IIIB/IV NSCLC has been reported. Updated survival analysis have shown a survival trend favoring patients randomly assigned to the L-BLP25 group (median survival, 30.6 versus 13.3 months for L-BLP25 and best supportive care, respectively) in a subgroup of patients with locoregional stage IIIB disease.[103] These promising results is currently tested in a phase III placebo-controlled trial, named START (Stimulating Targeted Antigenic Responses to NSCLC), in patients with stage III NSCLC after response to primary chemo-radiotherapy in more than 30 countries. The primary end point of START is progression-free survival.

CONCLUSIONS

As autoantibodies can report malignant transformation before standard clinical signs, research groups have focused on the clinical value of autoantibody analyses for early detection of lung cancer, in particular in high-risk populations. Nevertheless, TAAs seem to hold in the range of 10 to 30% patients, and single autoantibody detections have low diagnosis value to be used as serum diagnosis test. Promising data from early disease patients come from the development of robust quantitative multiplex technologies allowing concomitant detection of a multitude of autoantibodies, but large clinical autoantibodies trials are necessary before propose a robust autoantibody profile for early diagnosis of lung cancer. Autoantibody detections have also be investigated for the real-time monitoring of the efficacy of systemic therapies. Nevertheless, studies are hampered by the decrease in autoantibody levels in later disease stages, which probably occur due to the reduction in immune competence as the disease progresses. Finally, biomarkers of susceptibility and short-term risk are likely to provide insight into the biology of tumors that develop, leading to new interventions to support prevention. It is probable that the detection and characterization of autoantibodies will provide new insights into the complex biology of the humoral response to lung cancer and will have important implications in the future for the clinical management of patients with lung cancer.

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