

Mini review

Autoantibodies in breast cancer sera: candidate biomarkers and reporters of tumorigenesis

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Abstract

A plethora of promising breast cancer-associated autoantigens have been cloned by immunoscreening cDNA expression libraries with breast cancer sera or identified using proteomics, yet no biomarkers, whether individual autoantigens or panels of antigens developed using antibody-based methods have been validated and incorporated to routine oncologic practice for the early diagnosis of breast cancer. Recently, the addition of genomics, proteomics and high throughput technology to traditional immunological techniques has revived the interest in this field, and some of the most promising breast cancer autoantigens are in the process of being validated prospectively in large cohorts of patients with breast cancer. In addition, some of the identified breast cancer-associated autoantigens are recognized by T-cells and may prove to have a role in the treatment of breast cancer in the future. Autoantibodies found in breast cancer patient sera provide important clues about their significance. The discovery of breast cancer-associated antigens has provocative implications beyond the quest for novel diagnostic biomarkers, because autoantibodies target molecules involved in signal transduction, cell cycle regulation, cell proliferation and apoptosis, all of them key processes in carcinogenesis. Molecular components of the DNA double-strand break repair machinery as well as several members of the rapamycin-sensitive pathway elicit an autoantibody response in breast cancer. Data obtained by screening cDNA expression libraries of breast cancer antigens with autoantibodies present in breast cancer sera suggest that autoantibodies in cancer sera may be linked to the process of apoptosis. The studies reviewed here, clearly demonstrate the participation of autoimmunity in breast cancer to an extent previously unsuspected, which may have broad implications for the discovery of molecular targets for drug therapy and cancer biomarkers in general.

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1. Introduction

Although there are multiple promising diagnostic biomarkers of breast cancer in the development phase,

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there are at the present time no definitive antibody-based serologic markers for its early diagnosis in the clinical arena [1–3].

Enormous amount of experimental work has led to the identification of hundreds of autoantigens via recognition by autoantibodies present in breast cancer sera [4–27], yet efforts to consistently predict malignant disease based on autoimmunity to individual antigens or to panels of antigens have not resulted in serologic markers with definitive predicting specificity and sensitivity [1–3]. However, it has been shown that the use of panels of autoantigens rather than individual antigens enhances the likelihood of detecting cancer-associated antigens with potential diagnostic value [10,25,26]. The failure of individual antigens or of groups of autoantigens to be adopted for routine use in the diagnosis of breast or other cancers may in part reflect problems inherent to the technology used in detecting the biomarkers as well as to the lack of systematic methods for identifying informative autoantibodies from the scores of antibodies that are either patient-specific or irrelevant to the disease [25,27,28]. Nevertheless, the studies done using genomics and proteomics strongly suggest that autoantibodies have potential as biomarkers.

This review will discuss the rationale for considering autoantibodies as potential biomarkers for the early diagnosis of breast cancer, and will be restricted to the analysis of the tumor-associated antigen-antibody systems thus far reported in breast cancer as well as the implications of detecting autoantibodies in cancer sera for the process of breast carcinogenesis. A prerequisite to consider an autoantibody as a potentially useful candidate for the early diagnosis of cancer is the demonstration that it can be consistently detected before the tumor is diagnosed clinically. In addition it is essential to establish that the autoantigen recognized by the antibody is not only patient-associated but also breast cancer-associated, i.e. recognized by multiple sera from women with breast cancer and not by non-cancer control sera. We will also discuss why these two requisites, though essential, may not be sufficient to graduate a potential biomarker into a real biomarker, useful to solve the diagnostic and prognostic dilemmas posed by breast cancer.

2. Autoantibodies can be detected in the sera of women years before the clinical diagnosis of breast cancer

There is a useful parallel between cancer and the systemic autoimmune diseases which are characterized by a diverse collection of autoantibodies with definite diagnostic specificities [29–31]. A characteristic profile of autoantibodies is found in each of the systemic autoimmune diseases, and autoantibodies frequently precede the onset of the clinical symptoms of autoimmune diseases such as systemic lupus erythematosus by many years [32].

The literature on paraneoplastic syndromes has frequently reported that autoantibodies with diagnostic significance can be detected in the sera of patients with breast and other cancers prior to the clinical diagnosis of cancer [33–35]. Although these paraneoplastic syndromes are rare, they provide clear examples of autoantibodies preceding the clinical diagnosis of breast cancer.

Crawford et al. detected anti-p53 autoantibodies in the sera of women with breast cancer [36], and since then, multiple surveys (reviewed in Refs. [15,37]), have found anti-p53 antibodies in 9–26% of women with breast cancer. Subsequently, anti-p53 antibodies were detected in the sera of subjects who developed lung, prostate or breast cancer, 5–11 months after the demonstration of the antibodies [38]. Anti-p53 antibodies have also been found in 11.1% of women with a positive family history of breast cancer, hinting that certain autoantibodies can have predictive value of risk [39].

In an epidemiologic study, Frenkel et al. analysed the sera of 169 women who were healthy at the time of blood donation for the presence of antibodies to 5-hydroxymethyl-2'-deoxyuridine, an oxidized DNA base, using ELISA. Sera collected 6–72 months before these women were discovered to have breast cancer showed significantly elevated levels of this antibody. They suggested that this autoantibody can potentially serve as a marker for increased risk of breast cancer, because relative high serum levels were also detected in otherwise healthy women with a first degree family history of breast cancer and in women with the diagnosis of benign conditions [40]. More recently, Tomkiel et al. showed that an autoantibody of the IgG isotype, with specificity for the N-terminal

moiety of the 32 kDa subunit of replication protein A (RPA32) was detected at a high titer ($>1:10,000$ serum dilution) in the serum of a woman who 1 year later was discovered to have breast cancer. Subsequently, anti-RPA32 antibodies were found in approximately 11% of patients with breast cancer including ductal carcinoma in situ (DCIS) of the breast [20]. Additional support for the possibility that autoantibodies can precede the clinical diagnosis of cancer was provided by studies done by Tan [41] and Fernández Madrid et al. [20,25,42–44] which revealed the presence of high titer IgG antibodies at the time of the diagnosis of breast and other cancers. Since IgG molecules are relatively long lived, these reports suggested that an established autoimmune response to some autoantigens is already present in cancer sera before the tumor is clinically evident.

The literature cited demonstrates that autoantibodies to tumor-associated antigens develop in patients with breast cancer and can be detected months to years prior to the clinical diagnosis of the tumor and thus are potential candidates as biomarkers for its early diagnosis.

3. There are multiple reports of breast cancer-associated antigens

It has been established that autoantibodies are useful serologic markers in the diagnosis of the systemic autoimmune diseases, such as systemic lupus erythematosus, scleroderma, dermatomyositis and mixed connective tissue disease and that their diagnostic value is related to their immunologic specificity [29–31]. Similarly, the idea that autoantibodies can be markers for the diagnosis and/or prognosis of cancer is not new, and has been pursued unsuccessfully by multiple investigators using traditional immunological techniques for several decades. Since autoantibodies are prevalent in human sera and are part of the normal immune response [45], one important problem inherent to autoantibody-based methods for identifying tumor-related antigens is demonstrating their tumor relevance.

Antibodies to proteins encoded by oncogenes and tumor suppressor genes have been thought to represent prime candidates for biomarkers for the early diagnosis of breast cancer. However,

the multiple studies on the diagnostic or prognostic potential of anti-p53 autoantibodies in the sera of women with breast cancer have yielded conflicting results [15,37]. Numerous autoantibodies found in the sera of patients with breast cancer have been proposed as diagnostic or prognostic markers of the disease [4,8,12,15,37,46–48]. More recently, proteomics, genomics and high throughput technology have made major contributions to the study of autoantigen–antibody systems as serologic biomarkers in breast cancer.

4. Proteomics can identify autoantibodies recognizing tumor-associated antigens

There is great interest in applying proteomics to develop new biomarkers for early diagnosis of cancer, to foster a better understanding of the process of carcinogenesis, and to identify molecular targets for drug therapy [17,22,27,49–52]. Novel proteomic approaches include direct profiling of serum using mass spectroscopy, application of a variety of strategies to harness immunity for cancer diagnosis, protein tagging to capture subproteomes rich in diagnostic markers, the use of microarrays, and of multi-dimensional liquid protein separation technologies for comprehensive profiling of serum and other biological fluids. The technological advances and the applications of expression and functional proteomics to the understanding of cancer pathogenesis and the diverse strategies for early diagnosis and treatment of cancer have been reviewed by Hanash [27]. Multiple proteins that induce autoantibodies that are specific for different types of cancer have been identified using 2D gels to separate tumor proteins, followed by Western blotting and incubation with patient sera [17, 27,49,52]. One positive feature of this approach is its ability to assess the contribution of post-translational modifications of the autoantigens to the autoimmune response. In addition, functional proteomics offers the possibility of analysing multiprotein complexes that could be targeted by autoantibodies.

Using 2D gels and mass spectrometry, Le Naour was able to demonstrate a large number of tumor antigens [49]. Li et al. reported the use of SELDI (surface-enhanced laser desorption/ionization) with immobilized metal affinity protein chip arrays to

screen for potential serum biomarkers for early detection of breast cancer [22]. They selected a panel of three biomarkers based on their consistently significant contribution to the optimal separation of stage 0–1 breast cancer patients from non-cancer controls. Bootstrap cross-validation demonstrated that a sensitivity of 93% for all cancer patients and a specificity of 91% for all controls were achieved by a composite index derived by multivariate logistic regression using the three selected biomarkers [22].

Le Naour et al. using a proteomics approach, screened breast cancer patient sera for IgG antibodies to tumor cell proteins and found restricted activity against a set of three proteins identified by mass spectrometry as isoforms of a novel oncogenic protein designated RS/DJ-1. They demonstrated the presence of this protein in 37% of newly diagnosed women with breast cancer and suggested that the presence of autoantibodies and/or circulating RS/DJ-1 protein in the sera from patients with breast cancer may have clinical utility [17]. The suggestion that RS was identical to the oncogene DJ-1 was subsequently confirmed [52]. Proteomics is an attractive and versatile approach which has generated substantial interest in the field of biomarker discovery for the early detection of cancer because of its capability of uncovering antigen–antibody systems in biological fluids.

5. Contribution of genomics to biomarker development in breast cancer

A large number of studies have suggested an important association between cancer and autoimmunity, clearly indicating that various types of human cancer can trigger an immune reaction to tumor-associated antigens [5–7,9–11,15–20,23,25–27,37,41–44,53–61]. The introduction of SEREX (serologic analysis of recombinant cDNA expression libraries) [5] was a major advancement in immunoscreening that resulted in the identification of a wide array tumor-associated antigens eliciting B-cell responses in breast and other cancer patients against a host of tumor antigens, among others, NY-ESO-1 and SSX2, two cancer-testis antigens [10,11,19,57,59,60,62,63], ING1, a tumor suppressor [11], fibulin-1, a breast cancer-related glycoprotein [53], MAGE-3 and MAGE-6, as

well as the novel gene products NY-BR-62 and NY-BR 85, the latter two found to be overexpressed in breast cancer [19]. A novel breast cancer antigen, NY-BR-1 was found to be expressed only in testis and breast [57,64]. Autoantibodies to NY-ESO-1 as well as tumor expression of this antigen have also been reported in sporadic medullary carcinoma of the thyroid [55] and in a wide array of human cancers including melanoma, bladder and prostate cancer and hepatocellular carcinoma [56]. Of potential significance is the report that NY-ESO-1 expression in a large proportion of benign breast lesions suggesting the possibility that histologically and clinically benign lesions may harbor molecular changes that can be immunogenic [57]. The success of SEREX as a powerful approach to recognizing tumor-associated antigens has been tempered by the failure of hundreds of autoantigens identified by serum antibodies in becoming useful diagnostic and prognostic biomarkers in routine oncologic practice. Nevertheless, SEREX succeeded in identifying a number of important tumor-associated autoantigens recognized by humoral and cellular effectors of the immune system in breast cancer [11,18,19,23,53,57–60,62–66]. Recently, a comprehensive survey of SEREX-defined autoantigens as well as the multiple advantages and some of the inherent problems to this approach have been discussed (reviewed in Ref. [64]). Chen also described some modifications proposed by several laboratories to make the evolving SEREX technology even more effective [64]. Minenkova et al. also attempted to improve the potency of the SEREX approach by combining it with phage display technology using lambda phage as a display vector [61]. They identified several breast cancer-associated antigens including topoisomerase-II-beta and topoisomerase I-binding protein [61]. Using a T7 phage display method, Hansen et al. [16] identified short sequences recognized by IgG antibodies which correlated with breast cancer patient survival. Recently, Fernández Madrid et al. proposed an approach designed to minimize the confounding effects of unrelated autoantibodies and to eliminate screening steps that may prevent the identification of autoantigens commonly recognized by cancer sera [67]. Using cloning sera from women with invasive ductal carcinoma (IDC) of the breast with high titer IgG autoantibodies for biopanning of a T7 phage

breast cancer cDNA display library, they identified a panel of breast cancer-associated autoantigens [25]. Several autoantigens including annexin XI-A [68], the p80 subunit of the Ku antigen [69], the ribosomal protein S6 [70], and other known and unknown autoantigens could significantly discriminate between breast cancer and non-cancer control sera [25]. A partial cDNA sequence encoding a peptide identical to aminoacids 41–74 of annexin XI-A was recognized by 19% of all breast cancer sera and by 60% of sera from women with DCIS of the breast. In addition, partial sequences identical to annexin XI-A, the nucleolar protein interacting with the FHA domain of pKi-67 [71], the KIAA1671 gene product [72], the ribosomal protein S6, cyclin K [73], elongation factor-2 [74], Grb2-associated protein 2 [75], and other unknown proteins could significantly distinguish DCIS from IDC of the breast and appeared to be potential biomarkers for the diagnosis of breast cancer.

As indicated above, several autoantigens identified by serum antibodies have been found to be expressed in breast tumor tissue and their therapeutic potential is presently being explored [11,18,19,23,53,57–60,62–66,76,77]. Although a detailed discussion of this topic is outside the scope of this review, we should mention that the availability of serologically defined tumor antigens in breast and other cancers using SEREX has facilitated the identification of proteins recognized by tumor-specific T lymphocytes and has stimulated the interest in vaccine strategies. The use of SEREX led to the identification of a number of breast cancer-associated autoantigens recognized by cancer patient sera, expressed in tumor tissue and recognized by cellular effectors of the immune system which are potentially relevant for immunotherapy approaches.

6. The future of breast cancer-associated antigens as potential biomarkers

The field of biomarkers is driven by the urgent need to develop reagents discovered by proteomic or genomic technologies, capable of identifying subjects who are at high risk of developing cancer or those who have an early stage of breast or other cancers, and able to help the clinician to formulate an accurate prognosis [78].

The multiple breast cancer-associated autoantigens reported in the literature may augur rapid advances in this field. As discussed above, autoantibodies recognizing cancer-associated antigens can be detected before the clinical diagnosis of cancer, they may be expressed in tumor tissue and some of them may be recognized by autologous T-cells. Although these are valid reasons to be optimistic, it is likely that some or even many of those breast cancer autoantigens commonly recognized by multiple patient sera, and thus considered bona fide candidate biomarkers for diagnosis or prognosis of breast cancer, may never arrive to the real world of clinical medicine. A breast cancer-associated antigen may be patient-specific or it can be rare in expression libraries made from non-autologous cells, and thus useless as a biomarker, or it may be commonly recognized by multiple breast cancer sera. Currently used methodology attempts the difficult task to single out tumor-associated antibodies from the scores of irrelevant patient-specific responses. The strategy for demonstrating tumor-relevance early in the immunoscreening process and to avoid procedures that might exclude potentially important biomarkers has been discussed (reviewed in Ref. [67]).

Pepe et al. noted that many studies of predictive/-diagnostic markers continue to use statistical methods based on probability, despite the fact that such methods are not suited to the task of evaluating classification accuracy [79]. The limitations in using probability and the serious pitfalls of using traditional methods based on parameters in logistic regression models have been recently reviewed [79]. These authors have shown that strong associations between outcome and marker do not necessarily imply that the marker can discriminate between persons likely to have the outcome and those that do not. It has been shown that traditional statistical methods frequently used by epidemiologists to assess etiologic associations are not adequate to determine the potential performance of a marker for classifying or predicting risk for persons [80,81]. Pepe et al. stressed that this important point is not widely appreciated and may explain to some extent the disappointing performance of many identified ‘markers’ when they are used to predict outcome for persons. These authors discussed methods to quantify the classification accuracy of a new marker adequately, and suggested that proposed

markers for classifying or predicting risk in individual subjects must be held to a much higher standard than merely being associated with outcome, and that their sensitivities (true-positive fraction) and specificities (false-positive fraction) must be shown to be adequate through appropriate statistical evaluations [79]. Validation of the plethora of the proposed diagnostic biomarkers of breast cancer requires evaluation of their specificity and sensitivity against the present diagnostic standards, i.e. mammography, in a prospective follow-up of a cohort of women without clinical history of breast cancer. Their prognostic and predictive values should then be evaluated against the accepted prognostic pathologic indicators among incident cases of breast cancer within the same cohort of women.

7. Autoantibodies in breast cancer sera as reporters of breast tumorigenesis

Although autoantibodies are frequently found in the sera of patients with cancer, they have been generally thought to be nonspecific and of uncertain significance [45]. However, the immune recognition of self is emerging as an important theme in the biology of human cancer and the multiple breast cancer-associated antigens identified thus far, have profound implications beyond the quest for novel diagnostic biomarkers of cancer, because autoantibodies in breast and other cancer sera target important molecules involved in signal transduction, cell cycle regulation, cell proliferation and apoptosis, all of them key processes in carcinogenesis.

Autoantigens detected by antibody-based methods are candidates for oncogenic proteins [8,9,17,20,25,41,46]. Fernández Madrid et al. hypothesized that a biomarker useful for the early diagnosis of cancer is likely to be present in the early stages of carcinogenesis, thus probably reflecting molecular changes in signal transduction pathways responsible for cancer development [20,25,42–44]. Eng Tan has also proposed that autoantibodies in cancer sera can be reporters of tumorigenesis [41]. Genomics, proteomics and the use of high throughput technology are providing new information with implications beyond the field of cancer biomarkers. Recent work suggests that the machinery involved in protein synthesis may

provide novel targets for anticancer drugs or for vaccination protocols and that these molecular targets can be identified by strategies designed for biomarker discovery.

Autoantibodies found in cancer patient sera provide important clues on their significance in cancer sera. Using SEREX, Jaeger et al. showed that ING1, a tumor suppressor gene product [11], and a tissue-specific putative transcription factor in breast tissue [18] are recognized as autoantigens by breast cancer sera. Several members of the rapamycin-sensitive pathway elicit an autoantibody response in breast cancer [25]. Increased cell proliferation, a hallmark of aggressive malignant neoplasms, requires a general increase in protein synthesis and a specific increase in ribosomal synthesis. It has been proposed that synthesis of new ribosomes is regulated by phosphorylation of ribosomal protein S6, which is a common effect of mitogenic stimulation of cells [82,83]. The involvement of this pathway in breast cancer pathogenesis is suggested by autoantibodies recognizing ribosomal protein S6, elongation factor-2 and the eukaryotic elongation factor 2 (eEF-2) kinase in the sera of women with breast cancer [25]. The latter enzyme is a calcium and calmodulin-dependent protein kinase regulated through the rapamycin-sensitive mTOR pathway [84]. Since the interaction of the calcium and calmodulin-regulated eEF-2 kinase with heat shock protein 90 has been demonstrated [85], it is of interest that both, eEF-2 kinase and Hsp90 are recognized as autoantigens by breast cancer sera [25,86,87].

Autoantibodies in breast cancer sera recognize components of the DNA double-strand break repair pathway as autoantigens [25]. The heterodimeric Ku protein, composed of 86-kDa (Ku80) and 70-kDa (Ku70) subunits, is a DNA-targeting component of DNA-dependent protein kinase (DNA-PK), which plays a critical role in mammalian DNA double-strand break repair [88,89]. It has been suggested that autoantibodies directed toward the Ku antigen [25] and to RPA32 in breast cancer sera [20], probably indicate that the molecular changes leading to autoimmunity to proteins involved in DNA repair are important in breast carcinogenesis.

Autoantibodies targeting the DNA double-strand repair pathway [25] and the 32 kDa subunit of RPA [20] in breast cancer sera have other implications in

breast cancer. Several lines of evidence suggest that BRCA1 and BRCA2 proteins function in the homologous recombination pathway of DNA double-strand break repair and may be involved in regulation of multiple nuclear processes following DNA damage, including DNA repair and transcription [90–92]. Consistent with its role in DNA repair, BRCA1 physically associates with several DNA repair proteins such as replication protein A among others [93,94]. Wong et al. showed that interaction between BRCA2 and RPA is compromised by a cancer predisposing mutation in BRCA2, suggesting that this interaction is biologically important [92], and Thakur et al. demonstrated by mutational analysis that an intact positive regulatory region (PRR) in the BRCA1 promoter is essential for BRCA1 promoter activity [95]. These authors purified the three subunits of RPA, a specific factor binding double-stranded as well as single-stranded PRRs and suggested the existence of multicomponent transcription regulation through the PRR [95]. RPA is a highly conserved, single-stranded DNA-binding multi-subunit protein complex involved in eukaryotic DNA replication, recombination and repair [96–99]. Besides RPA, they identified a family of proteins which also bind RNA, with a subset of these proteins binding specific DNA sequences and directing transcription [95]. The BRCA tumor suppressor paradox in breast cancer refers to the apparent exception posed by this malignancy to Knudson's two hit model [100]. Indeed, unlike other tumor suppressor genes, no disease-causing dominant BRCA1 or BRCA2 mutations have been detected in sporadic breast or ovarian cancers. Powell and Kachnik suggested the possibility that a BRCA-dependent pathway could be inactivated not just by loss of BRCA1 or BRCA2 but by loss of other components of the BRCA-regulated response network, and that these alterations might play a role in sporadic breast cancer [100,101]. Autoantibodies to several components of the DNA repair machinery found in breast cancer sera [20,25] are consistent with this hypothesis, because these autoantibodies may reflect molecular changes in the genes or gene products that cooperate with the loss of BRCA function required for the full development of breast cancer.

Evidence has accumulated suggesting that molecular changes in autoantigens during apoptosis are

involved in the genesis of autoantibodies in the systemic autoimmune diseases [102,103]. Casciola-Rosen et al. demonstrated that autoantigens in systemic lupus erythematosus cluster in cell surface apoptotic blebs following UV radiation [102,104]. Similarly to the model of the systemic autoimmune diseases, data obtained by screening cDNA expression libraries with autoantibodies in cancer sera [25] suggest that apoptosis may be involved in the autoantibody response in breast cancer.

Defects in DNA damage-induced apoptosis contribute to tumorigenesis and to the resistance of cancer cells to a variety of therapeutic agents [105]. Since double-strand DNA breaks are characteristic of apoptosis and in view of the recognized role of Ku in repairing double-strand DNA breaks, Ajmani et al. proposed the possibility that Ku might oppose the process of apoptosis [106]. It has also been observed that agents which bind to the minor groove of DNA inhibit topoisomerase I, RNA polymerase II, and DNA polymerase and initiate intrinsic apoptotic cell death. The report on autoantibodies to the topoisomerase I binding protein in patients with breast cancer [61] could be consistent with a link between apoptosis and autoreactivity in breast cancer.

It has been recognized that various aspects of apoptosis are influenced by calcium as a mediator of signal transduction [107]. Both, calcium/calmodulin-dependent kinase and ALG-2 (apoptosis-linked gene 2) have been assigned a central role in the apoptotic process [107]. In view of the report that autoantibodies to the N-terminal domain of annexin XI have been detected in a large proportion of women with breast cancer and particularly in the sera from women with DCIS of the breast [25], it is of interest that deletion analysis done on ALG-2 revealed that its gene product interacts specifically with the N-terminal domain of annexin XI [108]. Although the role of annexin XI in breast cancer is unknown, its possible involvement and that of ALG-2 in apoptosis in breast cancer may be worth investigating.

Activation of elongation factor-2 kinase, which has been assigned a pro-apoptotic function in some systems [109] leads to selective phosphorylation of elongation factor 2 and transient inhibition of protein synthesis [110]. It has been demonstrated that the activity of eukaryotic elongation factor 2 kinase is markedly increased in several forms of malignancy

including breast cancer and that nonspecific inhibitors of this enzyme promote cell death [111]. In this respect, autoantibodies to both elongation factor-2 kinase and to elongation factor-2 have been demonstrated in breast cancer sera [25]. Weiser et al. showed that a histone deacetylase inhibitor induced apoptosis preferentially in cultured cell lines from thoracic cancers origin and dramatically induced the expression of NY-ESO-1, a SEREX-defined antigen [66]. Data obtained with experimentally-induced autoantibodies also point to a link between apoptosis and autoantibody formation in cancer. Gautier et al. showed that rats can be cured from induced peritoneal carcinomatosis by injections of apoptotic bodies derived from tumor cells and interleukin 2 [112]. This treatment generated a tumor-specific cytotoxic T-cell response associated with a humoral response consisting of autoantibodies directed against BARD1, originally identified as a protein interacting with the product of BRCA1. They suggested that BARD1 is hydrolyzed by the calcium-dependent cysteine proteases, calpains, and that the highly immunogenic form of cleaved BARD1 could contribute to the antitumoral response mediated by the apoptotic bodies [112]. In aggregate, these data suggest that autoantibodies detected in breast cancer patient sera may be linked to the process of apoptosis.

8. Conclusions

The use of genomics, proteomics and high throughput technology has led to the identification of a large group of breast cancer-associated autoantigens recognized by autoantibodies present in cancer sera. These studies strongly suggest that autoantibodies have potential as biomarkers and may in the future facilitate the early diagnosis of breast cancer. It has been suggested that proposed markers for classifying or predicting risk in individual subjects must be held to a much higher standard than merely being associated with outcome, and that their sensitivities and specificities must be shown to be adequate through appropriate statistical evaluations. Validation of the most promising of the proposed biomarkers for diagnosis and prognosis of breast cancer demands the application of these high

evaluative standards in prospective studies of large cohorts of women with breast cancer.

The old notion that autoantibodies in cancer lack specificity has been disproved by multiple studies demonstrating that they are tumor-associated, that the molecules recognized as autoantigens in cancer sera are frequently overexpressed in tumor tissue and that some of these antigens are also recognized by T-cells. Recent work suggests that molecules recognized by autoantibodies as breast-associated autoantigens are involved in signal transduction, DNA double-strand break repair and apoptosis and may provide novel targets for anticancer drugs or for vaccination protocols and that these molecular targets can be identified by strategies designed for biomarker discovery.

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