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Original Research

Antiprocathepsin D autoantibodies correlate with the stage of breast cancer

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ABSTRACT

Despite recent advances in surgical techniques and therapeutic treatments, survival rates from breast cancer remains disappointing. Current biomarkers are not sufficiently predictive of prognosis or early diagnosis and would benefit from additional support. We have identified the anti-procathepsin D autoantibodies as a potential indicator of breast cancer outcome and have shown that the level of autoantibodies correspond with the progression of the disease. The significant differences in the level of autoantibodies between individual stages demonstrated the significant promise as a new biomarker.

KEY WORDS: Procathepsin D; Antibodies; Cancer; Breast cancer

INTRODUCTION

Breast cancer represents a major health problem worldwide with more than 1 million new cases every year (1) and the second leading cause of cancer deaths among women. It is well established that early diagnosis can significantly affect prognosis. If breast cancer is diagnosed and treated while it is still confined to the breast tissue, the success rate will approach 100% (2). However, five-year survival rates in breast cancer are low. The benefits of estimating a patient's risk of developing metastases or to have a fast and non-expensive diagnostic marker are clear.

The current situation in biomarkers in breast cancer is not satisfactory. The only validated serum biomarkers including carcinoembryonic antigen, CA15.3 and CA27.29, do not have sufficient sensitivity for detection in early diagnoses (3,4). Validation of several suggested markers such as BC1, BC2, BC3 or inter-alpha trypsin inhibitor H4 or M/z 4292 gave contradictory results (5-7). In addition, the use of SELDI does not really represent an easy and inexpensive assay. Similarly, a directed mass spectrometry recently used for biomarker verification (8) remains rather complicated technique.

The existence of autoantibodies in cancer patient serum is well established (9). These autoantibodies produced by the patient's immune system upon exposure to tumor-related molecules are emerging as promising biomarkers for the early detection of cancer (10). These antibodies are specific, secreted in adequate quantities despite the presence of a relatively small amount of the corresponding antigen (11) and most of all, are present even before the first clinical signs (12). In addition, these antibodies have persistent concentrations and long half-lives. However, attempts to use them as diagnostic markers were mostly unsuccessful (13), most possibly due to the fact that the sensitivities to these autoantibodies to individual tumor-specific antigens are not sufficient to establish a reliable diagnostic test (3). However, although autoantibodies are proposed as early indicators of cancer, not all antigens are capable of eliciting adequate autoimmune response and the levels of sensitivities of autoantibodies are, at least in breast cancer, often not sufficient to build a reliable screening test (3).

Research conducted in our laboratory revealed the formation of anti-pCD autoantibodies (14), which was confirmed by other laboratories (15). These autoantibodies are specific to pCD and do not interact with the mature enzyme CD, making it easier to distinguish between these two molecules. In the current study, we focused on the hypothesis that anti-pCD autoantibodies correlate with the stage of breast cancer, thus offering a possibility to develop a non-invasive screening test.

MATERIAL AND METHODS

Samples

All samples of patient sera were purchased from Asterand (Detroit, MI, USA). The company offers all necessary information without revealing patient's identification.

Peptide

A MAP peptide based on activation peptide fragment 36-44 SQAVPAVTE (16) was prepared by Vidia, Vestec, Czech Republic.

Elisa assay

A solid phase ELISA was designed to measure the anti-procathepsin D antibodies in serum. As an antigen, 1 μg of peptide/well was used. An anti-human Ig-AP antibody (Sigma, St. Louis, MO) at 1:8,000 dilution in PBS-Tween was used.

Statistical analysis

Statistical analysis was performed by one-way ANOVA using Statistica 10 Program and Student t-test. The data were presented as mean ± SD.

RESULTS

Multiple antigenic peptides (MAPs) are peptides that are branched artificially, in which Lys residues are used as the scaffolding core to support the formation ≤8 branches with varying or the same peptide sequences. MAPs have been used to produce antibodies for use in immunological studies. First, we evaluated the differences between normal peptide and the MAP version. Using five randomly chosen patients, we measured the level of antibodies using irrelevant peptide, a 36-44 AA peptide or corresponding MAP peptide. In all cases, the MAP peptide showed consistently and significantly higher detection (Figure 1).

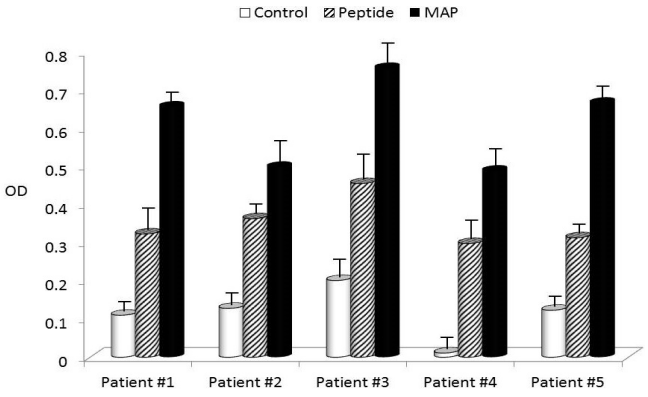

Figure 1. Differences in level of detection of anti-pCD autoantibodies based on the use of activation peptide or is multiple antigenic form.

Demographical data on tested patients are summarized in Table 1. Within the group of 264 patients bearing primary breast carcinoma, serum anti-procathepsin D antibodies showed average values from 0.328 (Stage I) to 0.893 (Stage IV). Control levels were tested on 87 samples of healthy individuals and reached 0.179 ± 0.099. When we compared the levels of antibodies among individual tumor stages, we found that the levels were increasing with the increased stage (Table 2). A similar trend was also found when we correlated the antibody levels with IUCC stage and lymph node stage.

Table 1. Demographic data of the studied subjects

Total number	**264**
Age (years)	56.2 ± 5.9
Maximum	33
Minimum	86
Alcohol	50.8 %
Smoking	15.9 %
Tumor stage	
I	17.4 %
IA	7.9 %
IB	3.9 %
IIA	34.9 %
IIB	6.3 %
III	1.6 %
IIIA	1.6 %
IIIB	24.9 %
IV	18.9 %
UICC Stage	
T1	28.9 %
T2	41.4 %
T3	7.5 %
T4	22.2 %
Lymph node	
N0	39.1 %
N1	17.9 %
N2	17.8 %
N3	14.3 %
N4	10.9 %
Chemotherapy	44.4 %
Radiation	7.9 %

The level of antibodies was significantly lower in patients undergoing chemotherapy (0.499 vs. 0.746) or irradiation (0.512 vs. 0.782). Smoking resulted in significantly increased level of antibodies (0.723 vs. 0.544). No correlation was found with respect of age (Table 2).

DISCUSSION

Breast cancer is the most significant worldwide health problem, affecting one in eight women (17). Therefore, it is crucial to identify biomarkers to predict prognosis and treatment response, and elucidate novel therapeutic targets of breast cancer. Numerous tissue, genetic and serum markers are used in present diagnosis, but none of them can diagnose breast cancer in an early stage (for review see 18).

Recently, a new possibility to use the current knowledge of association of the procathepsin D with various types of cancer emerged. The major advantage is the presence of procathepsin D in the plasma of breast cancer patients (19). As procathepsin D is, under normal physiological conditions, found only inside the cells (20), parts of this molecule are considered non-self and able to induce antibody response. One of the promising tumor markers in breast cancer is procathepsin D (pCD). Many clinical studies revealed an association between procathepsin D/cathepsin D (pCD/CD) levels and prognosis, incidence of metastasis, and aggressiveness in a variety of solid tumor types, with most

research done on breast cancer (21). Major studies and one meta-analysis found that pCD/CD levels in tumor homogenates represent an independent prognostic factor (22,23). Fully mature enzyme CD, originally suggested as a prognostic/diagnostic marker (24) was later found to have questionable value, as several forms of CD – inactive precursor pCD, enzymatically active intermediate (single chain) CD and mature (two chains) CD are simultaneously present in and around cancer tissue, with detecting antibodies not distinguishing among them. In addition, several forms are also present in stromal cells resulting in questionable pCD/CD quantification in tumor tissues and consequently its prognostic significance.

Table 2.

Diagnostic characteristics of serum anti-procathepsin D antibodies	
Parameter	OD (mean ± SD)
Control	0.179 ± 0.099
Tumor stage	
I	0.328 ± 0.035 *
IA	0.415 ± 0.078 *
IB	0.502 ± 0.112 *
IIA	0.656 ± 0.117 *
IIB	0.665 ± 0.120 *
III	0.717 ± 0.109 *
IIIA	0.893 ± 0.154 *
IIIB	0.913 ± 0.133 *
IV	0.893 ± 0.158 *
UICC Stage	
T1	0.459 ± 0.089 *
T2	0.661 ± 0.165 *
T3	0.720 ± 0.201 *
T4	0.734 ± 0.178 *
Lymph node	
N0	0.220 ± 0.056
N1	0.443 ± 0.099 *
N2	0.548 ± 0.111 *
N3	0.725 ± 0.187 *
N4	0.852 ± 0.199 *
Chemotherapy .	
Yes	0.499 ± 0.065 * **
No	0.746 ± 0.112 *
Radiation	
Yes	0.512 ± 0.117 * ***
No	0.782 ± 0.188 *
Smoking	
Yes	0.723 ± 0.115 * ****
No	0.544 ± 0.099 *
Age (years)	
Less than 65	0.666 ± 0.201 *
Over 65	0.679 ± 0.198 *

Data represent mean +/- SD.
* Results between cancer and control groups are significant at P < 0.05 level.
**Results between Chemotherapy YES and NO groups are significant at P < 0.05 level.
***Results between Radiation YES and NO groups are significant at P < 0.05 level.
****Results between Smoking YES and NO groups are significant at P < 0.05 level.

Cathepsin D is an intracellular aspartic proteinase of the pepsin superfamily. Numerous clinical and experimental studies reported significant association between procathepsin D level and prognosis of various cancer types (21,24,25, for review see 26). Many studies also demonstrated that pCD secreted from cancer cells affects multiple stages of tumor progression, suggesting the possibility of using pCD as an indicator of clinical outcome (27) or pCD suppression in clinical practice. However, most studies focused on expression of pCD/CD in cancer (28). In our laboratory, we have developed a model of pCD action and possible inhibition employing either monoclonal antibodies or gene therapy (16). The hypothesis evaluating the anti-pCD autoantibodies is based on these studies (29).

The affinity of anti-procathepsin D antibodies is significantly higher to the entire pCD molecule than to its activation peptide. However, pCD is a 52 kDa peptide, making its use in clinical practice unfeasible and expensive. The mitogenic effects of some individual fragments of activation peptide were already established (16,30), with peptides 27-44 and 36-44 being the most active.

Based on the well documented overexpression of pCD, approaches to use its presence in plasma of cancer patients occurred. Over 20 years ago, pCD was found elevated in plasma of breast cancer patients (19), but the possible use as a prognostic indicator was only suggested. Even closer to our model was the study of Taylor et al. (31), which found epitope recognition of anti-CD autoantibodies in endometrial cancer patients. However, the focus of this study was opposite – to use the autoantibodies to find the circulating forms of cathepsin D and not to find a marker.

The advantage of our model is the use of a multiple antigenic peptide, which significantly increased the antibody binding and allowed better detection at lower levels. According to our results, the level of tested autoantibodies increased with tumor stage, lymph node positivity and lack of treatment.

CONCLUSION

Anti-procathepsin D autoantibodies demonstrated significant promise as a new biomarker of breast cancer. Our data supported the hypothesis that an activation peptide of pCD plays an important role in breast cancer progression (32).

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DECLARATION OF INTEREST

The authors declare no conflict of interest

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