

## Cathepsin D: Autoantibody profiling as a diagnostic marker for cancers

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this report, we focused on the possibility to use anti-procathepsin D autoantibodies as a diagnostic and/or predictive marker for cancers.

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### Abstract

Current diagnostic assays for many cancers are antigen-based and rely on the detection of circulating proteins that are associated with a particular cancer. These assays depend on the expression, synthesis, and release of specific proteins by cells (*e.g.*, tumor cells) through either active secretion or shedding, or as a consequence of cell death (either necrosis or apoptosis). As such, these antigenic proteins must "escape" the primary site of disease, saturate the antigen-processing capacity of the individual's immune components, gain access to the circulation, and reach a sufficient steady-state concentration to be detected by enzyme- or radiolabel-based immunoassays. These events usually occur after the initial establishment of disease. Thus, and despite the fact that certain specific antigenic epitopes exhibit common recognition among patients with the same tumor types, the use of these antigen-based cancer assays has not been widely accepted in clinical practice, and many individual countries differ in the use of these potential diagnostic factors. Lately, an increasing number of studies demonstrated that procathepsin D secreted from cancer cells, acts as a mitogen on cancer cells and stimulates their pro-invasive and pro-metastatic properties. In

### INTRODUCTION

It is a well-established fact that early diagnosis significantly influences prognosis of diseases. If breast cancer is diagnosed and treated while it is still confined to the breast, the cure rate can approach 100%<sup>[1]</sup>. However, the five-year survival rates in breast cancer are very low in those patients diagnosed in later stages as compared to those diagnosed in early stages<sup>[2]</sup>.

Currently, biomarkers in breast cancer lack reliability for screening. The only validated serum biomarkers for breast cancer, including carcinoembryonic antigen, cancer antigens (CA)27.29 and CA15.3, are used primarily to monitor advanced diseases and do not have sufficient clinical sensitivity for early detection<sup>[3,4]</sup>. Therefore, lack of a reliable, highly sensitive and specific screening diagnostic test is truly an unmet medical need for overwhelmingly prevalent breast cancer, resulting in a high mortality/morbidity in women in the United States and worldwide.

Cancer patients frequently develop autoantibodies. These autoantibodies (AAb) produced by the patient's own immune system upon exposure to tumor-associated antigens (TAA) or tumor-related molecules are emerging as promising biomarkers for the early detection of

cancers<sup>[5,6]</sup>. AABs are specific, secreted in large quantities despite the presence of a relatively small amount of the corresponding antigen<sup>[2,3,7]</sup>. AABs are present in the serum before the antigens can be detected and are secreted prior to the first clinical signs<sup>[7]</sup>. AABs are also expected to have persistent concentrations and long half-lives ( $t_{1/2}$  between 7 and 30 d) in blood due to limited proteolysis and clearance from the circulation, making sample handling much easier<sup>[7]</sup>.

Although AABs are proposed as early indicators of cancers, not all antigens are capable of eliciting adequate autoimmune response<sup>[3]</sup>. For instance, the sensitivity of detection of AABs to a panel of 6 TAAs in breast cancer ranges from 20% to 73% (55%, 62% and 73% in grades 1, 2 and 3 primary invasive breast cancers, respectively; 20%, 62% and 41% in early, intermediate and high grade ductal carcinomas *in situ*, respectively)<sup>[1]</sup>. Clearly, these levels of sensitivities of AABs to individual or panel of breast cancer TAAs are clearly not sufficient to build a reliable screening/diagnostic test<sup>[3,7]</sup>. To increase the predictive value of tumor-specific antibodies for use as immunodiagnostics, several groups have begun testing multiple antigens in parallel<sup>[3]</sup>. Therefore, it is necessary to identify and validate AABs against a tumor specific antigen/s with a high sensitivity.

## PROCATHEPSIN D

Numerous clinical studies reported an association between procathepsin D/cathepsin D levels and prognosis, incidence of metastasis, tumor aggressiveness and a degree of chemoresistance in a variety of solid tumor types<sup>[8]</sup>. In the last two decades, an increasing number of studies demonstrated that procathepsin D (pCD), secreted from cancer cells, acts as a mitogen on both cancer and stromal cells and stimulates their pro-invasive and pro-metastatic properties<sup>[9-13]</sup>. Studies dealing with pCD diagnostic and prognostic value in cancer are complicated by the fact that there are several forms of cathepsin D in a cell at the same time: pCD, intermediate enzymatically active cathepsin D and mature two-chain cathepsin D. It is highly probable that tumor-promoting function of secreted cathepsin D is specific for only zymogen form of it. On the other hand, most of anti-cathepsin D antibodies recognize all forms, making prognostic evaluation difficult to interpret.

Recently, a new possibility to use the current knowledge of procathepsin D-cancer association appeared using anti-pCD autoantibodies as a promising marker<sup>[14,15]</sup>. Research performed in our laboratory has demonstrated the presence of anti-pCD autoantibodies<sup>[16]</sup>. As these antibodies are specific to pCD and do not recognize mature CD<sup>[17]</sup>, they represent a fine target for comparison of the pCD secretion with cancer progression. It is possible that the level of anti-pCD autoantibodies correlates with the stage of several solid tumors, thus offering development of a non-invasive screening test. We prepared an enzyme-linked immunosorbent assay for evaluation of the presence of anti-pCD antibodies using a specifi-

cally modified synthetic activation peptide as an antigen assay. Employing multiple antigen peptide, we were able to measure the level of anti-pCD autoantibodies in patient serum<sup>[5,18]</sup>.

We hypothesize that the amount of the pCD in the patient's serum will change with the progress of the cancer disease, thus corresponding with the increased number of pCD-releasing cancer cells. This hypothesis configures well with our preliminary findings on breast cancer and clearly shows higher levels of antibodies in more advanced stages. These preliminary data define the high clinical potential of this assay.

Different approach was suggested by Luo *et al*<sup>[15]</sup>. This group separated proteins from a lung adenocarcinoma cell line and then immunoblotted them with serum samples from patients diagnosed with lung cancer. When compared with autoantibody profiles from three years prior to the appearance of cancer, several immunoreactive spots were found to be cathepsin D. Detailed studies showed that the majority of patients had a most intense reaction against pCD.

Although both studies are somewhat preliminary and used only a limited number of patients, these data strongly suggest that the search for correlation between pCD secretion and cancer development or cancer detection promises to find a clinically relevant possibility to diagnose and/or screen for cancers. In order to identify the optimal types of tumors and the best technique, it is first needed to analyze a larger number of samples.

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