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Tumor Microenvironment–Released Peptides: Could They Form the Basis for an Early-Diagnosis Breast Cancer Test?

Eleftherios P. Diamandis[1,2,3*]

Despite expectations that cancer biomarkers could revolutionize cancer diagnosis and treatment, this promise did not bear fruit *(1)*. That is because cancer-specific circulating biomarkers in serum capable of being measured with a simple technique and identifying cancer in noncancer patients with high sensitivity and specificity have not yet been found. The relatively few clinical biomarkers currently used are more useful for patient management than for early diagnosis or population screening *(2)*. Given that some cancers are relatively rare (e.g., ovarian cancer), a screening test for early diagnosis must have an extremely high specificity (e.g., >99%) and a sensitivity sufficient (e.g., >80%) to achieve a reasonable positive predictive value (e.g., ≥10%) *(3)*. Prostate-specific antigen, the most studied screening cancer biomarker for prostate cancer, has recently been reexamined in prospective clinical trials, and the results are controversial *(4–6)*. That is why the US Preventive Services Task Force does not currently recommend routine screening for prostate cancer *(7)*.

Currently available breast cancer biomarkers in the circulation, such as CA15–3 and the associated antigen CA27.29, which recognize different epitopes on the same antigen (mucin 1), have relatively poor characteristics, such as a 30% sensitivity for early-stage disease, 60%–70% sensitivity in advanced cases, and increased values in patients with benign conditions, including ovarian cysts, benign breast diseases, and benign liver diseases. Carcinoembryonic antigen has similar limitations. These biomarkers do not have a place in early-disease diagnosis or in population screening *(8)*.

The most widely used screening method for breast cancer is mammography. This procedure is not recommended for women younger than 40 years. For older women, its use is controversial, because women with an average risk for breast cancer do not appear to gain a significant survival benefit when the cancer is diagnosed by mammography, compared with other means (e.g., self-examination) *(9)*. Mammography may be more useful for patients with an increased risk of breast cancer, but it has its own limitations, such as patient exposure to ionizing radiation and to harm produced by follow-up procedures in patients with false-positive screening results.

This information reinforces the notion that there is a great need for finding new and noninvasive ways for early diagnosis of breast cancer. An ideal test would be one that uses serum samples with a well-validated and quantitative technique and has high sensitivity and specificity. The report by Li et al. in this issue of *Clinical Chemistry (10)* is an attempt to address this problem by combining recent biological and technological advances.

The central hypothesis of this study is that proteolytic activity in the tumor microenvironment (not necessarily originating from tumor cells) may liberate peptides that enter the circulation. Such peptides could then be efficiently isolated and fractionated via the use of chip technology and then analyzed quantitatively by mass spectrometry. Specifically, the authors have shown that an enzyme, carboxypeptidase N (CPN), is produced in higher amounts within the microenvironment of breast cancer than in healthy tissues and that the proteolytic activity of CPN releases at least 6 peptides, one originating from a bradykinin substrate and 5 from the substrate C3f (a complement protein). After fractionation and extraction of these peptides from serum, they are quantified by MALDI-TOF mass spectrometry.

To substantiate their claims, the authors, initially using a mouse model of breast cancer, found increased amounts of CPN in tumor interstitial fluid (compared with normal interstitial fluid) but not in the serum of these animals. They then demonstrated that the 6 aforementioned peptides were increased as early as 2 weeks after tumor implantation. These increased concentrations then progressively decreased until 8 weeks after tumor implantation. For breast cancer patients, Li et al. used immunohistochemistry to show that CPN is low or absent in healthy tissues but that it is increased

[1] Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada; [2] Department of Clinical Biochemistry, University Health Network, Toronto, Ontario, Canada; [3] Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada.
* Address correspondence to the author at: Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 60 Murray St., Rm. L6-201, Toronto, Ontario, M5T 3L9 Canada. Fax 416-619-5521; e-mail ediamandis@mtsinai.on.ca.
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in early and late breast cancer tissues. Then, analogous to their findings with the mouse model, they demonstrated that the 6 aforementioned peptides were increased in early-stage breast cancer, with their concentrations generally decreasing from early- to late-stage disease. On the basis of these data, the authors claimed that this approach may be highly promising for noninvasive diagnosis of breast cancer, with a substantial advantage for detecting early-stage disease, which would presumably allow for earlier therapeutic interventions and, hopefully, lead to better clinical outcomes.

This report is not the first time that peptides have been proposed as candidate biomarkers for cancer detection *(11, 12)*. Villanueva et al. previously suggested that peptidomic panels captured by mass spectrometry in serum (generated by exoproteases during the blood coagulation cascade) can form tumor-specific signatures suitable for diagnostics *(13)*. That study, however, had several limitations, which have been outlined elsewhere *(14)*. Could the approach by Li et al. form the basis for a noninvasive diagnostic test for early breast cancer? A common observation has been for the early enthusiasm for newly proposed cancer biomarkers to decrease over time with subsequent validation studies. This phenomenon arises because many original studies are conducted under hidden biases *(15)*. Additionally, early studies usually are of small numbers of samples, making it difficult to draw generalizations from the data. Li et al. *(10)* studied only approximately a dozen samples per breast cancer stage, low numbers that preclude firm conclusions. The critical data of Li et al. for breast cancer patients presented in their Fig. 6 show both large variation in the concentrations of the informative peptides and substantial overlap between the patient groups (cancer vs. noncancer). The highest concentrations of these informative peptides are observed in early-stage disease. This finding is a potential advantage, but it goes against what we know about cancer markers. Most, if not all, clinically useful cancer biomarkers are correlated with tumor burden, and their concentrations in serum are increased, not decreased, as the disease progresses. Although Li et al. provide some possible explanations for this finding, we should reserve judgment on whether this phenomenon is real or artifactual, given the small number of samples and the wide scatter in the data.

Cancer cells and the tumor microenvironment are well known to overproduce proteases and other molecules that allow tumor cells to migrate and invade surrounding tissues. Thus, the idea of using this increased proteolytic activity as an indicator of tumor presence or metastasis is a reasonable one; however, no specific peptide for cancer cells has been reported thus far. We will have to await further validation of this assay with larger numbers of patients to calculate sensitivities, specificities, positive and negative predictive values of these tests for patients with breast and other cancers. As I have indicated earlier *(16)*, there are 2 major reasons, excluding fraud, for biomarker failings at the clinic. One is that many reported biomarkers represent "false discoveries" in which the original findings cannot be reproduced owing to preanalytical, analytical, or postanalytical shortcomings. Specific examples of false biomarker discoveries are given elsewhere *(17)*. The other reason for biomarker failures is that subsequent validation studies reduce the originally highly promising sensitivities and specificities to levels that are not clinically useful. That is, despite being statistically significant, the data turn out to have no clinical value because they are nonactionable: They will not help with unequivocal diagnosis, selection of therapy, or disease subclassification.

The future will show whether the findings presented by Li et al. will be refined and validated to a degree that justifies their clinical applicability or will follow the fate of a myriad of biomarkers that either were false discoveries or were put on the shelf for lack of clinical value.

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