Letters to the Editor

Identification of Serum Amyloid A Protein As a Potentially Useful Biomarker for Nasopharyngeal Carcinoma

To the Editor: I read with interest the paper by Cho *et al.* (1), which demonstrates that serum amyloid A protein (SAA) may be a potentially useful biomarker for nasopharyngeal carcinoma. The authors discovered this biomarker by using serum proteomic profiling with the so-called SELDI-TOF-MS technology (surface-enhanced laser desorbtion ionization time-of-flight mass spectrometry). SELDI-TOF-MS has recently been proposed as a powerful method for cancer diagnostics as well as for cancer biomarker discovery (2, 3). Examples of using this method for diagnosis of ovarian, prostate, breast, bladder, and other cancers have already been reported (2–4). However, the ability of this technology to facilitate discovery of new cancer biomarkers has recently been questioned (5–7).

After the publication of the first successful report for ovarian cancer (4), I indicated that this method is biased towards identifying high abundance molecules that are likely not cancer derived (8). I further proposed that the most likely source of such putative biomarkers is the liver and that most of these molecules are acute-phase reactants (8). The report by Cho *et al.* fully confirms these predictions. First, SAA was found in serum at relatively huge concentrations (~0.2–2 g/L), levels that are many thousand-fold higher than classical cancer biomarkers (such as carcinoembryonic antigen, prostate-specific antigen, CA125, etc.), which originate from tumor cells. Recently, I have compiled a list of positively identified putative cancer biomarkers by this technology. These molecules are present in serum at concentrations similar to those of SAA (in the gram-per-liter range); they are also derived from the liver, and many of them are acute-phase reactants (6, 7). I have also previously proposed that such biomarkers (acute-phase reactants) are not likely to be specific for any type of cancer and would be expected to be elevated in other malignant diseases and in inflammatory diseases (6, 7). To their credit, Cho *et al.* admit that SAA was previously reported to be elevated in many different malignancies, such as cancers of the kidney, colon, prostate, and so forth, and in leukemias and lymphomas (1). Moreover, they have shown that this biomarker does not originate from the cancer cells but is released into the circulation by the liver (1). In accordance with my previous suggestions, this report confirms that serum proteomic profiling by SELDI-TOF-MS identifies high abundance proteins that are not tumor derived and confirms that many are elevated in serum as acute-phase reactants, thus representing cancer epiphenomena. As I have indicated before, these epiphenomena are unlikely to be of much clinical use in our future efforts to more effectively diagnose and monitor cancer.

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In Response: We appreciate the useful comments by Dr. Eleftherios Diamandis on our article. Although we fully agree with his opinion pinpointing the possibility of encountering abundant molecules such as acute-phase reactants in the serum proteomic analysis, the doubt in surface-enhanced laser desorption ionization time-of-flight mass spectrometry (SELDI-TOF-MS) technology in the discovery of new cancer biomarkers should be clarified. SELDI-TOF-MS is a powerful separation technology capable of rapid binding and resolution of thousands of biomarkers in a large number of serum and/or plasma samples. However, abundant proteins present in the serum and plasma samples will also be faithfully reflected in the SELDI-TOF-MS retentate map. Hence, it is not surprising to see acute-phase reactants as the major components if they are, in fact, elevated among the cancer patients. Therefore, it is not a question of SELDI-TOF-MS itself being unable to facilitate discovery of new cancer biomarkers but a question of what type of sample one uses, how one enriches the desired biomarkers, and what control subjects are included. For instance, if tumor tissue lysate instead of serum or plasma is analyzed by SELDI-TOF-MS, acute-phase reactants should not be a problem at all. To identify cancer-associated biomarkers that are in low abundance in serum or plasma, one might need to perform preliminary fractionation or enrichment procedures prior to SELDI-TOF-MS instead of just using the crude samples. Furthermore, to better differentiate the cancer-associated biomarkers from the elevated acute-phase reactants, we shall go one step farther to include other patients, with bacterial infections, viral infections, inflammations, or arthritic diseases, as controls as well.

As far as tumor specificity and cross-reactivity are concerned, it

is not uncommon to find that biomarkers initially thought to be specific to certain organs or disease states are later shown to cross-react with other organs or other disease states to a different extent when more control tissues are tested subsequently. For example, when more and more studies had been made of the well-known tumor suppressor protein p53, it was found that it is, in fact, a stress protein the expression of which can be stimulated by various genotoxic stresses (1, 2). Nevertheless, its important roles in the cancer progression process through its functions in cell cycle arrest and apoptosis remain unchanged. Another example that parallels the "epiphenomena" is the widely accepted use of C-reactive protein in risk stratification for cardiovascular disease. C-reactive protein is also a typical acute-phase protein; its original relevance to cardiovascular disease was questioned and ridiculed until the role of inflammation in thrombosis was confirmed. Therefore, the message we would like to bring forward is that, although serum amyloid A protein (SAA), like many other acute phase reactants, is not cancer specific, its elevation during cancer relapse is so tremendously high that its role in disease monitoring in cancer patients is worth further investigation. This is also supported by previous studies demonstrating that interleukin 1 (IL-1) and interleukin 6 (IL-6) can stimulate tremendous elevation of serum or plasma SAA by thousands-fold rapidly (3). Concurrently, other investigations also showed an abundant presence of IL-1 or its receptors in nasopharyngeal cancer (4), breast cancer (5), head and neck squamous cell carcinoma (6), and gastric carcinoma (7). Together with many more studies, this suggests a strong relationship of cancer progression with the process of inflammation. So it will be interesting to examine whether the rapid production of SAA in the liver or in the epithelia of many different organs (8) could be related to the stimulation by cytokines abundantly present in the tumor cells. It is also important to investigate the mechanism of how SAA is produced at different stages of clinical manifestation in cancer patients bearing in mind that SAA was much elevated in patients, preferentially at the time of distant metastases when compared with patients with primary local tumor lesions, as shown in our joint study (9).

It is important to reiterate that we have never claimed that using SAA alone is sufficient as a marker for the diagnosis of nasopharyngeal carcinoma. It is just one of the markers among many that we have identified by SELDI-TOF-MS. What we are suggesting is that SAA, probably coupled with serum-circulating EBV DNA, with other SELDI-TOF-MS–identified biomarkers, and with clinical parameters, could be useful in delineating the relapse of patients who have already received a diagnosis of nasopharyngeal carcinoma. This is exactly what we are working at in our laboratory in collaboration with Ciphergen Biosystems Incorporation right now. Tumor markers with absolute specificity are rare or perhaps nonexistent. As high-throughput bioinformatics analyses become more mature and more clusters of cancer-associated biomarkers of intermediate specificity are found, we anticipate that future cancer diagnosis, prognostication, and monitoring will rely on using computer algorithms of these biomarkers (derived by neural network or artificial intelligence training) with intermediate but well-defined specificities.

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