Clinical Proteomics

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Editorial

Cancer Proteomics

New Opportunities for Old Molecules?

Daniel W. Chan[1,] and Shu-Ling Liang[1]*

[1]Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD

"Old" molecules, such as enzymes, inhibitors, and related proteins, are being rediscovered under the new light of proteomics research. Proteomic biomarkers have become a major focus, especially in the area of cancer research, because most of the functional information of the cancer-associated genes resides in the proteome. Because cancer is a complex disease, it might require a panel of multiple biomarkers to achieve sufficient clinical efficacy. Serum/plasma is the most accessible biological specimen collected from patients. The development of serum proteomic diagnostics could be the most promising new test for cancer.

Why a Single Biomarker is Not Sufficient as Cancer Diagnostics

The human proteome is an exceptionally complex biological system involving proteins with posttranslational modifications and dynamic intermolecular collisions. These protein complexes can be regulated by signals from cancer cells, their surrounding tissue microenvironment, and/or from the host. Some proteins are secreted and/or cleaved into the extra-cellular space and may represent valuable serum biomarkers for diagnostic purpose. It is estimated that the cancer proteome may include more than 1 million proteins as a result of posttranslational modifications. Therefore, finding the elusive single disease-related protein would require the laborious separation and identification of every protein biomarker ***(1–3)***.

Until recently, the low molecular weight range (<15 kDa) of the circulatory proteome and metabolome went largely uncharacterized. However, this collection of low molecular weight molecules promises to contain a rich source of previously undiscovered biomarkers ***(4)***. This is because of biological processes that give rise to cascades of biomarker fragments from enzyme-generated proteolytic activity. The rich information archive can arise within the unique disease-tissue microenvironment. These low molecular weight molecules exist below the range of detection achieved by

*Author to whom all correspondence and reprint requests should be addressed:
Daniel W. Chan, Department of Pathology, Room 3M05, CRB2 Building, Johns Hopkins Medical Institutions 1550 Orleans St., Baltimore, MD 21231
E-mail: dchan@jhmi.edu.

conventional two-dimensional gel electrophoresis, because they cannot be efficiently separated by gel-based techniques. As a result, investigators have turned to mass spectroscopy, which exhibits its optimal performance in the low molecular weight range. Such complexity clearly highlights the need for ultra-high resolution proteomic technology for robust quantitative protein measurements and data acquisition ***(5)***.

Finding New Cancer Biomarkers

The search for new cancer biomarkers consists of three steps: discovery, validation, and translation. Discovery research to find candidate biomarkers is relatively easy. Many potential biomarker candidates could be discovered using powerful technologies, such as protein microarrays and mass spectrometry. However, validation of biomarkers with consistent analytical performance and clinical significance in a general population is difficult. Furthermore, the translation of these biomarkers into clinical diagnostics with the performance characteristics of a clinical assay is not easy and labor intensive. It also requires hundreds, if not thousands of samples to be analyzed and generates enormous amount of data to be processed ***(6,7)***.

The approach for cancer biomarker discovery and validation in our laboratory starts with the selection of the right technologies to discover biomarkers. We use protein chips and mass spectrometry. Then, we identify well-characterized clinical specimens, followed by the development of bioinformatics tools for data analysis and multiplexing of biomarkers because most diseases (e.g., cancer) are heterogeneous. The design of multicenter case control study with extensive clinical validation is important to minimize the impact of possible confounding variables. Finally, we believe that proteomic profiling is not sufficient. The goal is to identify biomarkers with biological and clinical significances.

Prostate-Specific Antigen: New Application for an "Old" Enzyme?

Prostate cancer is the leading cause of cancer in the United States. The diagnosis of prostate cancer is most often suspected after finding an elevated serum prostate-specific antigen (PSA) or abnormal findings on digital rectal examination (DRE).

PSA is a protease. It is a 27-kD glycoprotein that is produced by the secretory cells that line the prostate glands (acini). PSA is released from the prostate epithelial cell as a proenzyme (pPSA) with a 7-amino acid peptide chain attached to it ***(8)***. As it is secreted into the lumen, the propeptide is removed by human kallikrein 2 to generate active and mature form of PSA. This molecule then undergoes proteolysis to generate inactive PSA, which enters the bloodstream and circulates in an unbound state (free PSA). A small amount of active PSA diffuses into the circulation and is rapidly bound by protease inhibitors, including alpha-1-antichymotrypsin (ACT) and alpha-2-macroglobulin ***(9)***. Serum PSA that is measurable by current clinical immunoassays was found to exist primarily as either the free form (free PSA) or bound to ACT.

Free PSA in serum is now known to be composed of at least three distinct molecular forms of inactive PSA: pPSA, bPSA, and iPSA. Of particular interest is the proenzyme, pPSA, and its association with cancer. A second form of PSA, termed benign PSA (bPSA) is an internally cleaved or degraded form of PSA that is more highly associated with benign prostatic hyperplasia (BPH) ***(10)***. Preliminary research indicated that pPSA could improve the diagnostic utility of PSA ***(11)***.

Enzyme-Related Proteins as Biomarkers for Ovarian Cancer

Ovarian cancer is one of the most deadly diseases. Early detection remains the most promising approach to improve long-term

survival of patients. In a recent multi-center serum proteomics study, more than 500 patients with invasive epithelial ovarian cancer, benign pelvic masses, and healthy controls were included ***(12)***. Data from ovarian cancer patients and healthy women at two centers were analyzed independently and the results cross-validated to discover potential biomarkers. The results were validated using the samples from two of the remaining centers. After protein identification, biomarkers were tested on samples from the fifth center, which included healthy women, women with ovarian cancer, and patients with breast, colon, and prostate cancers. Three biomarkers were identified as follows: (1) apolipoprotein A1 (downregulated in cancer); (2) a truncated form of transthyretin (downregulated); and (3) a cleavage fragment of inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4) (upregulated). In the independent validation to detect early stage invasive epithelial ovarian cancer from healthy controls, the sensitivity of a multivariate model combining the three biomarkers and CA125 (74%) was higher than that of CA125 alone (65%) at a matched specificity of 97%. When compared at a fixed sensitivity of 83%, the specificity of the model (94%) was significantly better than that of CA125 alone (52%). These biomarkers demonstrated the potential to improve the detection of early stage ovarian cancer.

Among the three biomarkers, the cleaved form of ITIH4 is the most interesting. Several proteolytically derived fragments from the proline-rich region (PRR) of human ITIH4 have been identified by surface-enhanced or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS or MALDI-TOF-MS) as potential disease markers ***(13)***. The ITIH4-related fragments were first immunocaptured by beads coupled with peptide-specific antibodies. The eluates were analyzed by SELDI-TOF-MS. In addition, freshly collected and immediately processed serum and plasma samples were used to analyze the ex vivo stability of these ITIH4 fragments. Human serum ITIH4 was shown to be extensively proteolytically processed within the PRR, and its fragmentation patterns were closely associated with different disease conditions. Fragmentation patterns were generally consistent with cleavages by endoprotease followed by exoprotease actions. Observed fragments changed little under different assay conditions or blood collection and processing procedures. The fragmentation patterns within the PRR of human serum ITIH4 are associated with different disease conditions and may hold important diagnostic information.

Conclusion

The future of cancer diagnostics will be based on a panel of proteomic biomarkers. They could be used to detect cancer at an early stage, to predict and to direct therapies. Enzymes and related proteins are important biological molecules, which could serve as cancer biomarkers. These biomarkers could be intact or fragments of proteins. The challenge is to be able to find and validate these potential biomarkers as clinical diagnostics. With the advances in proteomic technologies, we are closer than ever to find these "new" enzyme molecules or fragments. The translation of newly discovered biomarkers could provide an opportunity to revolutionize the era of personalized medicine.

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