How Are We Going to Discover New Cancer Biomarkers? A Proteomic Approach for Bladder Cancer

A handful of cancer biomarkers are currently used routinely for population screening, disease diagnosis, prognosis, monitoring of therapy, and prediction of therapeutic response. Unfortunately, most of these biomarkers suffer from low sensitivity, specificity, and predictive value, particularly when applied to rare diseases in population screening programs. Thus, for the classic cancer biomarkers much is left to be desired in terms of clinical applicability. We need new cancer biomarkers that will further enhance our ability to diagnose, prognose, and predict therapeutic response in many cancer types. Because biomarkers can be analyzed relatively noninvasively and economically, it is worth investing in discovering more biomarkers in the future. The completion of the Human Genome Project has raised expectations that the knowledge of all genes and proteins will lead to identification of many candidate biomarkers for cancer and other diseases. These predictions still need to be realized. The prevailing view among specialists is that the most powerful single cancer biomarkers may have already been discovered. Likely, in the future we will discover biomarkers that are less sensitive or specific but could be used in panels, in combination with powerful bioinformatic tools, to devise diagnostic algorithms with improved sensitivity and specificity. These efforts are currently in progress *(1)*.

Most of the currently used cancer biomarkers were discovered after development of novel analytical techniques such as immunologic assays and the monoclonal antibody technology. Animals were immunized with extracts from tumors or cancer cell lines, followed by screening of hybridomas for monoclonal antibodies that recognize "cancer-associated" antigens. More recently, and with the completion of the Human Genome Project, many researchers have hypothesized that the best cancer biomarkers will likely be secreted proteins *(2)*. Approximately 20–25% of all cell proteins are secreted. However, this is not an absolute requirement because many classic cancer biomarkers, such as carcinoembryonic antigen (CEA) and Her2/neu, are bound to cell membranes, but their extracellular domains can be found, through shedding, in the circulation. Other groups are using bioinformatics such as digital differential display and in silico Northern blotting to compare gene expression between healthy and cancerous tissues to identify overexpressed genes *(3)*. Although one of the prevailing hypotheses in new biomarker discovery is that the most promising biomarkers should be overproduced proteins, this is not generally true for some of the best-known cancer biomarkers, such as prostate-specific antigen (PSA) *(4)*. Overexpressed genes are now identified experimentally by use of microarrays. Some of these genes have been proposed as candidate cancer biomarkers *(5, 6)*. Despite this reasonable hypothesis, very few cancer biomarkers have been discovered by use of this approach.

Another approach, followed by our group, is based on the hypothesis that if a molecule is already a known cancer biomarker, members of the same family of genes/proteins may also constitute novel biomarkers. We have since shown that kallikreins, a group of serine proteases with high homology at both the DNA and protein level (this family includes PSA), are candidate biomarkers for ovarian, prostate, and breast cancers *(7)*.

A novel approach that has been introduced recently is the use of proteomic technologies for new biomarker discovery *(8)*. In fact, serum proteomic patterns, identified by the so-called surface-enhanced laser desorption/ionization-time-of-flight mass spectrometry (SELDI-TOF) technology, have been proposed as highly accurate predictors for presence of cancer, and they are currently under clinical evaluation *(9)*. The same technology has been used to identify molecules that may constitute novel biomarkers *(9)*. Aspects of this technology have been criticized recently *(10)*.

Some cancers are more amenable than others to early diagnosis by biochemical testing. For example, it has previously been suggested that certain biological fluids, such as sputum, urine, pancreatic juice, and cerebrospinal fluid, may be the fluids of choice for early diagnosis of lung, bladder, pancreatic, and brain tumors. Indeed, one would expect that biochemical testing of urine should be able to diagnose early bladder carcinoma because candidate informative molecules could be excreted into the urine during cancer development. Proteomic profiling of urine has been suggested as a diagnostic test for bladder carcinoma *(11)*. In addition, many other biochemical molecules or genetic markers have been discovered that could be used to diagnose bladder carcinoma with fair sensitivity and specificity. Such molecules (or methods) include, but are not limited to, the following (the approximate diagnostic sensitivities and specificities are in parentheses): BTA stat (68%; 66%); BTA-TRAK (71%; 62%); NMP22 (64%; 71%); telomerase (74%; 89%); HA-HAase (91%; 86%); Immunocyt (68%; 79%); F/FDP (68%; 86%); multicolor fluorescence in situ hybridization assays (84%; 90%); cytokeratins (76%; 84%); metalloproteinases (60%; 80%); and p53 mutation (32%; 100%) *(12, 13)*. The most common noninvasive test, however, is voided urine cytology (VUC), which has a sensitivity of ~50% and a specificity of 97% *(12)*. This test has higher sensitivity for higher grade tumors.

Bladder cancer is very common, ranking second only to prostate cancer for cancers of the urinary tract. Approximately 54 000 new cases of bladder cancer are diagnosed and ~12 000 people die from this disease every year in the United States alone. Most patients are diagnosed with superficial tumors, which can be completely resected. However, two-thirds of these patients will experience recurrence within 5 years, and almost 90% will have a recurrence by 15 years. Early diagnosis leads to better

clinical outcomes, underscoring the importance of finding new ways for screening the general population. Currently, potential bladder tumor markers can be used in various clinical scenarios, including *(14)*:

- Serial testing for earlier detection of recurrence;
- Complementary testing to urine cytology to improve the detection rate;
- Providing a less expensive and more objective alternative to the urine cytology test; and
- Directing the cytoscopic evaluation of patient follow-up.

The gold standard for the detection of urothelial neoplasia is cytologic examination of urothelial cells from voided urine, urinary bladder washings, and urinary tract brushing specimens in combination with cystoscopic examination *(12, 13)*. Because cystoscopy is an invasive procedure and urinary cytology suffers from low sensitivity and specificity, particularly for lower grade tumors, it is desirable to identify novel biomarkers for this cancer. Biochemical testing of urine is a noninvasive and less expensive procedure for diagnosing and monitoring this disease. Because none of the markers mentioned above has sufficient sensitivity and specificity, the quest for identifying additional bladder cancer biomarkers continues.

In this issue of *Clinical Chemistry*, Kageyama et al. *(15)* propose proteomic analysis of urine as a new way to identify bladder cancer biomarkers. Previously, Celis et al. *(16)* used two-dimensional gel electrophoresis and developed a comprehensive database for bladder cancer profiles of both transitional and squamous cell carcinomas. Through their studies, Kageyama et al. *(15)* were able to identify a potential tumor marker, calreticulin, which is found in the urine of patients with bladder carcinoma. The authors used a differential display method of bladder cancer vs healthy urothelial tissue and mass spectrometry to identify proteins that are increased in cancer tissue. In addition to calreticulin, an endoplasmic reticulum chaperone, they found nine other candidate proteins that could constitute new biomarkers for bladder carcinoma. The authors confirmed their data with quantitative Western blot analysis, immunoprecipitation, and immunohistochemistry. Their reported sensitivity and specificity were 73% and 86%, respectively, similar to the values reported for other biochemical bladder markers (see above). However, the diagnostic accuracy of their test was vulnerable to urinary tract infections *(15)*.

The main question surrounding bladder cancer and urinary biomarkers is how these molecules can be used in clinical practice. Clearly, these tests are not useful for population screening because of their low sensitivity and specificity. In addition, none of the available tests is sufficiently accurate to replace cystoscopy in the investigation of a patient with a possible bladder tumor. VUC has relatively low sensitivity, especially for low-grade tumors, but it is currently the most specific test for bladder carcinoma. Consequently, when VUC is positive, it indicates a high-risk tumor that requires definitive treatment. VUC is currently used for monitoring of patients with known high-risk disease, and positive cytology with negative cystoscopy may indicate malignancy of the prostate or upper urinary tract.

Currently, patients with bladder cancer are followed up with frequent cystoscopic examinations. Cystoscopy is invasive and expensive, and a urinary bladder test that is simple, quick, and inexpensive could be invaluable in this clinical scenario. Current guidelines suggest that low-risk patients should be surveyed once a year with cystoscopy and high-risk patients at 3-month intervals. Currently, cystoscopy is always combined with VUC. Because, as mentioned earlier, new urinary bladder tests such as BTA or NMP22 could detect lower-grade disease recurrence with higher sensitivity than VUC, it could be worthwhile to consider including one or more of these tests in the routine follow-up of patients with bladder carcinoma. However, large prospective studies will be necessary to test the clinical utility of these assays against cytology. Such trials could show the value of these new tests in reducing the frequency of cystoscopy and in contributing to the earlier and more sensitive detection of disease recurrence, leading to earlier therapeutic interventions and, fortunately, to improved clinical outcomes.

In conclusion, bladder cancer biomarkers have proliferated more than any other class of cancer markers over the last 10 years. We now have at hand a multitude of molecules that can be measured with automated, inexpensive, quantitative assays in urine. These markers may aid in the monitoring of patients with bladder carcinoma and have the potential to reduce the number of follow-up cystoscopies, thus reducing healthcare costs and patient discomfort and, at the same time, detecting relapsing disease more effectively than VUC. It is time to test these new possibilities with prospective clinical trials.

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