Future for Ovarian Cancer Screening: Novel Markers From Emerging Technologies of Transcriptional Profiling and Proteomics

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The completion of the human genome project and the burgeoning use of genomic, transcriptional profiling, and proteomic technologies to investigate DNA, RNA, and protein levels in tumors, serum, plasma, and urine offer the exciting potential of identifying novel and effective cancer-specific screening markers. If the markers are secreted or released from tumor cells and migrate to serum, plasma, urine, or other accessible sites, they may be useful as screening markers either alone or in combination with currently identified tumor markers or screening approaches. Thus, these technologies potentially provide for a rapid advance in the identification of novel tumor markers comparable to that initiated by the introduction of monoclonal antibody technology.

In this issue of the Journal, Mok et al. (1), using transcriptional profiling of ovarian cancer cell lines, found that messenger RNA (mRNA) levels of prostatin (a serine protease, previously identified in prostatic secretions), osteopontin (a secreted bone morphogen), and creatine kinase B (a marker for renal and lung cancers) are higher in ovarian cancer cell lines than in normal ovarian epithelial lines. Prostatin mRNA and protein levels subsequently were found to be markedly elevated in ovarian cancer cell lines and in ovarian cancer tissue. The preliminary data presented by Mok et al. (1) suggest that prostatin is released from ovarian cancer cells, migrates to the bloodstream, and may be a novel marker for the diagnosis or management of ovarian cancer. This is exciting evidence that global, nondirected screening approaches at the level of DNA, RNA, or protein may identify a series of markers complementing those found previously by antibody-based or candidate gene approaches.

Prostatin has low sensitivity in early-stage disease and lower sensitivity than CA 125, the best characterized marker for advanced epithelial ovarian cancers. Similar to CA 125 (2–4), prostatin levels were frequently elevated in serum from patients with serous epithelial ovarian cancers, with limited increases in serum from patients with mucinous tumors or serous borderline tumors. A lack of correlation between CA 125 and prostatin suggested that combining the two markers could increase the sensitivity. Indeed, in a screen of 37 patients and 100 control subjects, Mok et al. (1) reported that this combination resulted in 92% sensitivity (95% confidence interval [CI] = 78.1% to 98.3%) and 94% specificity (95% CI = 87.4% to 97.7%), compared with a sensitivity of 64.9% (95% CI = 47.5% to 79.8%) for CA 125 and of 51.4% (95% CI = 34.4% to 68.1%) for prostatin at a specificity of 94% for prostatin in the same set of samples.

Prostatin is found in eight normal human tissues (5,6). Whether the prostatin level will be elevated in serum from patients with other cancers remains to be determined. Indeed, a number of new markers identified by genomics, transcriptional profiling, proteomics, and candidate gene approaches may be elevated in multiple types of cancers. Such markers that would indicate a high likelihood of cancer without identifying its location would also create many challenges.

Fourteen other candidate tumor markers have been found through transcriptional profiling or the related serial analysis of gene expression, subtractive hybridization, and differential display technologies (1,7–13). For example, mesothelin and NES1 were identified by monoclonal antibody and candidate gene approaches, respectively (14,15). Mesothelin is elevated in the serum of 76% of ovarian cancer patients and may complement CA 125 (14). NES1 is elevated in the serum of 56% of ovarian cancer patients (15). Thus, transcriptional profiling, when used alone or in combination with other markers, will probably identify more markers for ovarian cancer, increasing the prospect of detecting ovarian cancer at an early, curable stage.

Ovarian cancer and breast cancer demonstrate similar stage-by-stage cure rates (16). In contrast to breast cancer, where most cancers are detected at an early stage, only 25% of ovarian cancers are diagnosed in stage I, when cure rates approach 90% (16). Cure rates for ovarian cancer when diagnosed at an advanced stage are less than 20%. Thus, early detection should improve the outcome in patients with this devastating disease.

Where screening for cancer has apparently decreased death rates, the effective screening approaches contain at least two stages, prescreening followed by a biopsy of accessible tissue. For example, in breast cancer, prescreening is a clinical breast examination and mammography, which have reasonable sensitivity but relatively low specificity, followed by biopsy. The location of the ovary deep in the pelvis makes biopsy difficult, requiring laparoscopy or laparotomy. A positive predictive value approaching 10% (one cancer for each 10 surgical procedures) will be needed. Because ovarian cancer is relatively uncommon in the average population, with a prevalence of 40 cases per 100 000 for women older than 50 years, a specificity of more than 99.7% will be required to achieve a positive predictive value of 10%, with a sensitivity of 67% (3). Because of the low prevalence of ovarian cancer, the positive predictive value of a screening test is essentially not affected by changes in sensitivity. Increasing the sensitivity to 100% would not substantially alter the specificity needed to achieve a positive predictive value of 10%. Thus, it is important to maintain high specificity, which limits the potential use of multiple markers to increase sensitivity, if the increased sensitivity is achieved at the cost of a decreased specificity.

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Ovarian cancer, fallopian tube cancers, and primary peritoneal carcinomatosis, as well as non-gynecologic tumors, occur at an increased frequency in individuals with a strong family history of breast and ovarian cancers or with proven abnormalities in BRCA1 and/or BRCA2 genes. This higher prevalence may allow the use of screening approaches with a lower specificity to obtain the desired 10% positive predictive values. Women, who are likely to undergo prophylactic oophorectomy, may also be much more tolerant of lower positive predictive values. In these women, ovarian cancers are more likely to be polycystic, and primary peritoneal carcinomatosis is much more frequent (17–19). Primary peritoneal carcinomatosis may prove particularly difficult because CA 125 and transvaginal ultrasound examinations can be normal (17). Although the long preclinical interval for ovarian cancer in low-risk individuals may be amenable to screening, the rapid progression and the very short preclinical interval tumors in women with BRCA1 or BRCA2 mutations may not. Indeed, several intra-abdominal cancers have been found in women who received normal screening results a few months before the cancer was detected clinically (17).

At present, CA 125 is the standard to which new screening markers for ovarian cancer must be compared (3,4). With a cutoff of 30–35 U/mL, which results in a sensitivity of 50%–60% for early disease, CA 125 specificity approaches 99% in apparently healthy postmenopausal women (3,4). The specificity is lower in premenopausal women and in women with benign gynecologic conditions, including ovarian cysts, endometriosis, and uterine fibroids, which are part of the differential diagnosis for ovarian cancer (3). CA 125 levels are elevated in women with hepatic disease, renal failure, and pancreatitis, which may alter clearance of the antigen or irritate the serosa of the peritoneum or pleura (3). It is important to note that a sensitivity of 99% for CA 125 is far lower than the 99.7% required for a positive predictive value of 10%.

Proteomics can identify diagnostic markers if post-translational modification is required for activity (20–22). Such markers would be missed by transcriptional profiling. Furthermore, proteomics can be used as a high-throughput, nonbiased global discovery approach that can use patient serum, plasma, urine, or other sources of secretions, obviating prior knowledge of which proteins are secreted or released from tumor cells at sufficient levels. Surface-enhanced laser desorption ionization time-of-flight (SELDI-TOF) analysis and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) technology have the potential to identify patterns or changes in thousands of small proteins (<20 kd). When combined with matrices that selectively absorb certain serum proteins, these approaches can globally analyze almost all small proteins in complex solutions, such as serum or plasma. In a preliminary study, when linked to powerful heuristic computer algorithms, SELDI-TOF could identify a pattern of protein changes in serum with 100% sensitivity with 94% specificity for both early and late ovarian cancer in a limited set of samples (23). Maturation of proteomics should add additional markers or patterns of markers that can predict ovarian cancer.

The National Cancer Institute’s Early Detection Research Network (EDRN) has established the following five-phase criteria for the development and evaluation of biomarkers (24): 1) a preclinical exploratory phase to identify promising directions, 2) a clinical assay and validation phase to evaluate the ability of the assay to detect established disease, 3) a retrospective/longitudinal phase to determine the putative biomarker’s ability to detect preclinical disease and to define a “screen positive” rule, 4) a prospective screening phase to identify the extent and characteristics of disease detected by the test and the false-positive rate, and 5) a definitive trial (prospective randomized trial) to determine the impact of screening on reducing the burden of disease in the general population. Prostate, along with other putative biomarkers, must undergo rigorous testing with the use of these criteria before the results of these studies can be generalized to the low-risk population as a whole.

References


NOTE

Editor’s note: Dr. Robert C. Bast, Jr., receives royalties for helping to invent the CA-125 assay.