

# Molecular Technologies for Personalized Cancer Management

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CANCER IS ONE OF THE MOST SIGNIFICANT CAUSES OF MORBIDITY and mortality in the United States, accounting for nearly a quarter of all deaths in 2003.<sup>1</sup> Despite advances in understanding the etiology of cancer, its clinical management remains challenging. Many cancers are detected at advanced stages, when invasion of surrounding tissues and metastasis to distant sites have already occurred, rendering standard treatment modes relatively ineffective. Patients whose tumors are classified at similar stages often demonstrate variable clinical outcomes, making it difficult to accurately assess prognosis, select appropriate interventions, and communicate disease severity to patients. Standard treatment modes including chemotherapy and radiotherapy have consistent toxicities, but do not benefit all patients. An effort is under way to translate advances in molecular biology, bioinformatics, and computing into tools that may improve the diagnosis, staging, and treatment of patients with cancer.<sup>2</sup> These tools range from tests that evaluate single genes for unique interindividual differences to chips that profile the expression of entire sets of genes or proteins. These emerging genomic and proteomic technologies may help to individualize and improve the clinical management of cancer.

Assessment of genotypes at single loci may contribute to evaluation of cancer risk, diagnosis of malignancy, and prediction of disease progression. Several studies, for example, have linked mutations in the *BRCA1* and *BRCA2* genes to susceptibility to both breast and ovarian cancers. Given this risk, otherwise healthy individuals who test positive for *BRCA1/2* mutations may opt to undergo prophylactic bilateral mastectomy and oophorectomy.<sup>3</sup> Similarly, mutations in the *APC* gene contribute both to hereditary and sporadic colorectal cancers, making screening for mutations in this gene a possible component of colorectal cancer risk assessment and clinical management.<sup>4</sup> For prostate cancer, mutations in the *p53* gene are associated with metastasis and may serve as a marker for progression.<sup>5</sup>

The evaluation of genotype at single loci may also enable clinicians to identify patients who are most susceptible to certain toxic effects of chemotherapeutic agents. A genetic polymorphism in thiopurine methyltransferase (TPMT), an enzyme that metabolizes a class of chemotherapeutic agents called thiopurines, may predict treatment outcome in patients with leukemia. Patients who have the genetic trait for TPMT deficiency are at risk of potentially fatal hematological toxic effects because of the accumulation of toxic metabolites, with the degree of toxicity correlating directly with the number of mutant TPMT alleles.<sup>6</sup> Genetic polymorphisms in other metabolic enzymes have also been shown to affect drug levels and toxic effects of commonly used chemotherapeutic agents, while polymorphisms in the

gene for a drug transporter called P-glycoprotein appear to be linked to drug resistance.<sup>7,8</sup>

Profiling the expression of thousands of genes using microarray technology may also contribute to the clinical management of cancer. Microarrays have helped to improve cancer diagnosis by identifying both specific genes that contribute to cancer susceptibility and patterns of gene expression that distinguish different hematological malignancies.<sup>9,10</sup> A recent study of 151 patients with breast cancer without lymph node involvement demonstrates that genomic profiling may also contribute to the assessment of prognosis.<sup>11</sup> The authors identified a specific gene expression pattern that was significantly associated with metastasis and poor prognosis in patients whose clinical characteristics, such as tumor grade, were not predicative of poor outcomes.

The use of genomic profiling has also led to an improved understanding of cancer pathogenesis, an important step in the development of novel targeted therapies. Identification of specific gene products in certain leukemias, as well as breast and ovarian cancers, has led to development of specific drugs that target these tumors with relatively little toxicity to normal tissue.<sup>12</sup> These drugs target proteins that are uniquely found or are relatively abundant in tumor cells, allowing for more specific targeting of tumor with less toxicity to normal tissues and, hence, fewer adverse effects. In addition to contributing to new drug development, genomic profiling has been used to identify patterns of gene expression that may predict the potential efficacy and toxicity of existing chemotherapeutic agents in individual patients.<sup>13</sup>

Profiling of proteins—the end products of expressed genes—is an emerging discipline known as proteomics and may also contribute to an improved understanding of cancer pathogenesis and to advances in cancer management. Clinical proteomics seeks to determine how changes in information flow at the cellular level, represented by alterations in complex patterns of protein activation and expression, relate to disease pathogenesis, and whether certain changes are predictive of disease development, progression, and response to specific treatment modalities.<sup>2</sup>

Advances in clinical proteomics currently depend on 2 different technologies. First, surface-enhanced laser desorption and ionization time-of-flight mass spectrometry (SELDI-TOF) may be used to generate spectra of ion mass-to-charge ratios from a sample such as blood serum. These spectra do not identify proteins in the sample but instead demonstrate the relative abundance of proteins at each mass-to-charge ratio value. By analyzing SELDI-TOF spectra of samples from both healthy individuals and patients with cancer, a clustering algorithm can be trained to recognize unique features of spectra indicative of malignancy. SELDI-TOF spec-

tra of serum samples from undiagnosed patients in both high-risk groups and the general population can then be analyzed using this model to predict disease status.<sup>14</sup>

Researchers have recently demonstrated that such proteomic pattern analysis may be used to facilitate early detection of ovarian cancer. With the malignancy still confined to the ovary, treated stage I ovarian cancer has a 5-year survival rate of 95%. Unfortunately, most patients are currently diagnosed when their cancer has reached advanced stages, and their 5-year survival is no more than 35%.<sup>15</sup> By analyzing serum samples from women with ovarian cancer, healthy women with significant risk factors for ovarian cancer such as family history, and women with unrelated medical conditions, the investigators detected stage I ovarian cancer in the latter 2 populations with a sensitivity of 100%, a specificity of 95%, and a positive predictive value of 94%.<sup>15</sup> In comparison, the most widely assessed biomarker for ovarian cancer today, cancer antigen 125, exhibits a positive predictive value of less than 10% alone and approximately 20% when assessed in conjunction with ultrasound screening.<sup>15</sup>

A second proteomic technology being developed for clinical applications is the protein microarray. Protein microarrays are a series of immobilized spots, each containing a bait molecule such as an antibody, a nucleic acid, a drug, or a recombinant protein or peptide, to which the patient's tissue or fluid is applied. If proteins from the sample bind to the bait molecule, they may be detected using antibody probes. Reverse-phase protein microarrays, in which the patient sample is fixed to the slide and the probe is applied to the microarray, have demonstrated improved reproducibility and analytical sensitivity vs conventional protein and DNA microarrays in profiling studies that use small samples such as those typically procured from patient biopsies in clinical trials.<sup>16</sup>

Proteomic profiling with protein microarrays has been used in the research setting to assay levels and activation states of key signaling proteins with the goal of reclassifying human tumors to more accurately reflect the degree of malignancy and metastatic potential, and to determine molecular signatures indicative of early cancer.<sup>17</sup> One study using protein microarrays, for example, demonstrated that activation of Akt, a pro-survival signaling molecule, is a critical early step in the progression of prostate cancer and hence a potential target for rational therapeutic intervention.<sup>16</sup> Researchers are also attempting to determine whether protein microarrays may be used to identify key proteins whose activities are altered in individual patient tumors, with the goal of informing the selection of effective and safe treatment regimens.<sup>18</sup> A conceptual example of this approach is combinatorial cancer therapy, in which a series of inhibitors acting on specific proteins in a signaling pathway is administered in combination to achieve improved efficacy at lower doses of each agent, thus yielding a less toxic drug regimen.<sup>2</sup>

Current evidence suggests that genomic and proteomic technologies may facilitate early cancer detection, provide accurate molecular characterization of a patient's tumor, and

permit the rational selection or design of therapeutics that maximize efficacy and minimize toxicity, all from samples procured via routine blood tests and tissue biopsies. While promising, these technologies face a number of technical challenges in the course of their development into practical clinical applications. Established standards are needed in the production of genomic microarrays and implementation of techniques in the clinical laboratory setting.<sup>19</sup> Large-scale clinical use of protein microarrays requires thorough validation of antibody probes, and expansion of the currently limited number of available antibodies relative to potentially relevant intracellular targets.<sup>20</sup> Advanced bioinformatics and computing tools are needed to reliably and reproducibly harness the information embedded within complex proteomic profiles. Validation of these profiling techniques is also a concern. Large reference laboratories have begun to evaluate the potential implementation of proteomic pattern diagnostics, and several National Cancer Institute-based clinical trials are planned that may bring the goal of personalized cancer care closer to a reality.

#### REFERENCES

1. American Cancer Society. Cancer Facts and Figures 2003. Available at: <http://www.cancer.org/downloads/STT/CAFF2003PWSecured.pdf>. Accessed December 11, 2003.
2. Baak JP, Path FR, Hermsen MA, Meijer G, Schmidt J, Janssen EA. Genomics and proteomics in cancer. *Eur J Cancer*. 2003;39:1199-1215.
3. Haber D. Prophylactic oophorectomy to reduce the risk of ovarian and breast cancer in carriers of BRCA mutations. *N Engl J Med*. 2002;346:1660-1662.
4. Traverso G, Shuber A, Levin B, et al. Detection of APC mutations in fecal DNA from patients with colorectal tumors. *N Engl J Med*. 2002;346:311-320.
5. Cheng L, Leibovich BC, Bergstralh EJ, et al. P53 alteration in regional lymph node metastases from prostate carcinoma: a marker for progression? *Cancer*. 1999;85:2455-2459.
6. McLeod HL, Krynetski EY, Relling MV, Evans WE. Genetic polymorphism of thiopurine methyltransferase and its clinical relevance for childhood acute lymphoblastic leukemia. *Leukemia*. 2000;14:567-572.
7. Lu Z, Zhang R, Diasio R. Dihydropyrimidine dehydrogenase activity in human peripheral blood mononuclear cells and liver. *Cancer Res*. 1993;53:5433-5438.
8. Shirota Y, Stoehlmacher J, Brabender J, et al. ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. *J Clin Oncol*. 2001;19:4298-4304.
9. Mariadason JM, Augenlicht LH, Arango D. Microarray analysis in the clinical management of cancer. *Hematol Oncol Clin North Am*. 2003;17:377-387.
10. Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer. *Science*. 1999;286:531-537.
11. van de Vijver MJ, He YD, van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med*. 2002;347:1999-2009.
12. Workman P, Clarke PA. Innovative cancer drug targets: genomics, transcriptomics and clinomics. *Expert Opin Pharmacother*. 2001;2:911-915.
13. Sotiropoulos C, Powles T, Dowsett M, et al. Gene expression profiles derived from fine needle aspiration correlate with response to systemic chemotherapy in breast cancer. *Breast Cancer Res*. 2002;4:R3.
14. Wulfkuhle JD, Liotta LA, Petricoin EF. Proteomic applications for the early detection of cancer. *Nat Rev Cancer*. 2003;3:267-275.
15. Petricoin EF, Ardekani AM, Hitt BA, et al. Use of proteomic patterns in serum to identify ovarian cancer. *Lancet*. 2002;359:572-577.
16. Pawelz CP, Charboneau L, Bichsel VE, et al. Reverse phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. *Oncogene*. 2001;20:1981-1989.
17. Liotta LA, Petricoin EF. Molecular profiling of human cancer. *Nat Rev Genet*. 2000;1:48-56.
18. Wulfkuhle JD, Aquino JA, Calvert VS, et al. Signal pathway profiling of ovarian cancer from human tissue specimens using reverse-phase protein microarrays. *Proteomics*. 2003;3:2085-2090.
19. Ntzani EE, Ioannidis JP. Predictive ability of DNA microarrays for cancer outcomes and correlates: an empirical assessment. *Lancet*. 2003;362:1439-1444.
20. Templin MF, Stoll D, Schrenk M, Traub PC, Vohringer CF, Joos TO. Protein microarray technology. *Trends Biotechnol*. 2002;20:160-166.