Editorials

Genome-Wide Views of Cancer

NEARLY coincident with the publication of the article by Hedenfalk et al.[1] in this issue of the *Journal* are reports of the complete DNA sequence of the human genome.[2,3] This remarkable point in biomedical history marks the beginning of an era in which we expect to learn the molecular basis of all human diseases. How long this will take is uncertain, but the boundaries of the problem are now clear. The increasing understanding of molecular medicine will shift clinical practice from empirical treatment to therapy based on a molecular taxonomy of disease. Physicians will be prescribing rationally designed drugs that have increased efficacy and reduced toxicity.

The need for such new treatment is particularly evident in oncology. Although the clinical and pathological heterogeneity of cancer has long been recognized, the mainstay of cancer therapy remains nonspecific cytotoxic drugs that are effective only in some patients, yet cause side effects in most.

Historically, the molecular pathogenesis of cancer has been elucidated one gene at a time. Now we have entered a new phase, wherein methods of analyzing thousands of genes at a time can be brought to bear on the challenges of diagnosis and treatment. This evolving strategy will be able to determine whether variations in gene sequences correlate with the response to chemotherapy. In addition, new methods using DNA microarrays now permit the simultaneous measurement of the level of expression of thousands of genes in a tumor sample (transcriptional profiling). Recent studies have demonstrated the use of DNA microarrays to subclassify leukemia, lymphoma, melanoma, and breast cancer according to the innate gene-expression profile of the tumor.[4-7]

In this issue of the *Journal,* Hedenfalk and colleagues report the use of transcriptional profiling to obtain the molecular signatures left by mutant *BRCA1* and *BRCA2* genes in patients with breast cancer.[1] A substantial proportion of cases of early-onset, familial breast cancer are due to a mutation of *BRCA1* or *BRCA2,* whereas such mutations are rare in cases of sporadic breast cancer. Nevertheless, it is possible that these sporadic cancers arise from disturbances of molecular pathways in which *BRCA1* and *BRCA2* participate. The finding of histopathological differences and differences in the expression of estrogen and progesterone receptors between tumors with a *BRCA1* mutation and tumors with a *BRCA2* mutation supports the idea that these two classes of breast cancers are molecularly distinct.

Hedenfalk et al. used complementary DNA (cDNA) microarrays containing probes for 5361 genes to explore the patterns of gene expression in samples of 22 primary breast tumors that had mutations in *BRCA1, BRCA2,* or neither gene. The method entails a comparison of the binding of flourescent labeled cDNA generated from messenger RNA (mRNA) — the product of expressed genes — from a reference tissue and the tumor to an array of thousands of different cloned cDNA molecules on a glass chip (the cDNA microarray). This method allows the identification of active genes and silent genes in the two tissues. To visualize the 22 samples in 5361-dimensional space, the investigators used a form of data reduction known as multidimensional scaling to decrease the complexity of the data set. Multidimensional scaling demonstrated that the patterns of gene expression among the *BRCA1*-mutation–positive tumors, the *BRCA2*-mutation–positive tumors, and the sporadic tumors were largely distinctive.

This study generated an enormous amount of data (22 samples × 5361 genes = 117,942 data points). However, although the number of genes analyzed was large, the number of samples was quite small, creating a statistical challenge common to microarray-based studies such as this. Searching through a list of 5361 genes to find 1 or more that correlate with an element of interest (e.g., the distinction between tumors with a *BRCA1* mutation and tumors with a *BRCA2* mutation) is equivalent to testing 5361 hypotheses without correction for multiple-hypothesis testing. To deal with this statistical problem, the authors first performed random permutations of the class-membership labels (i.e., positivity or negativity for a *BRCA1* mutation and positivity or negativity for a *BRCA2* mutation) and found that the correlation of these random patterns with the gene-expression profiles was not as strong as the correlations found with the actual data, indicating that the observed results could not be explained by chance alone.

The authors also evaluated whether the gene-expression patterns in tumors with *BRCA1* mutations and tumors with *BRCA2* mutations could be used to identify the status of each sample in the data set. To answer this question, they used a cross-validation method whereby 1 of the 22 samples was left out and the results for the remaining 21 samples were used to predict the status of the withheld sample. This process was repeated for each of the 22 samples. With the use of this approach, most of the samples were correctly classified.

With respect to identifying *BRCA1* mutations, only a single error was made. In this case there was no *BRCA1* mutation, but the tumor displayed the expression profile of a tumor with a *BRCA1* mutation. Examination of this patient's germ-line DNA revealed that, in fact, the transcriptional activity of her *BRCA1* gene was silenced as a result of abnormal methylation of the gene's promoter region. Thus, the results of the cDNA microarray analysis of her tumor were

actually correct: the function of *BRCA1* was abrogated, but by a mechanism that was not revealed by the sequence of the *BRCA1* gene.

The study by Hedenfalk et al. illustrates the potential of genome-wide views to influence the diagnosis of cancer. Complex patterns of gene expression can serve as proxies for abnormalities in entire molecular pathways, without the need to identify the particular gene that causes the disturbance. It is likely that in the future, the integrity of functionally important pathways in tumors will be evaluated by transcriptional profiling rather than by the sequencing of individual genes within the pathway, most of which are still unknown. The study by Hedenfalk et al. also illustrates the way in which the difficulty of sequencing large genes like *BRCA1* can be partially overcome through the use of transcriptional profiling based on DNA microarrays.

There are other important implications of this investigation and others like it. First, we can now have sufficient confidence in genomic techniques to begin incorporating them into the design of clinical trials. Evaluations of the efficacy of investigational drugs will be greatly facilitated by analyses involving the entire genome. In patients with lymphoma, for example, transcriptional profiling of tumor-biopsy specimens obtained at diagnosis can be used to predict the response to chemotherapy.[5]

What barriers could impede the routine clinical implementation of DNA-microarray–based diagnosis of cancer? Issues such as the high cost and the complexity of the techniques are easily surmountable even in cases in which the entire genome, rather than a fraction of it, is screened. Rather, the main roadblock is the time that will be required to perform the requisite carefully controlled, large-scale studies to confirm these findings. In addition, the probable shift toward gene-based diagnosis makes the education of patients imperative. The successful implementation of personalized gene-based medicine will require informed physicians who can critically evaluate this new type of clinical trial and who are prepared to counsel their patients when these methods become routinely available.

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Hypothermia for Traumatic Brain Injury — A Good Idea Proved Ineffective

TRAUMATIC brain injury is an important cause of death and disability in both civilians and military personnel.[1] In areas with organized trauma care systems and adequate critical care, the mortality from severe traumatic brain injury appears to have been lowered from approximately 50 percent in the 1970s to 30 percent more recently. More important, this reduction in mortality has been associated with an increase in the proportion of survivors with relatively normal cerebral function. However, this remarkable achievement is not widely recognized. These improvements can be ascribed to the more rapid transportation of patients to emergency departments, the avoidance of hypotension and hypoxia, more effective methods of resuscitation, early brain imaging, prompt surgical intervention, and fastidious intensive care, including the monitoring and control of intracranial pressure.

Some of the neurologic injury that occurs at the moment of traumatic impact is probably irreversible. However, the injury then sets in motion a series of biochemical processes that worsen the ultimate outcome. To inhibit or reverse these processes has been the goal of neuroscientists for many years. To date, there have been about a dozen clinical trials of drugs such as free-radical scavengers, glutamate antagonists, and calcium-channel blockers that might reduce the injury to the brain in patients with head trauma. Although much has been learned about the pathophysiology of traumatic brain injury and the factors that affect outcome, none of these drugs have proved to be effective. Nonpharmacologic approaches to the treatment of patients with traumatic brain injury have focused largely on preventing intracranial hypertension and maintaining adequate cerebral perfusion.

The multicenter clinical trial of hypothermia in patients with severe traumatic brain injury reported by Clifton et al. in this issue of the *Journal*,[2] although disappointing, represents a landmark achievement. In 1938, Temple Fay, a neurosurgeon at Temple University School of Medicine, pioneered the clinical use of hypothermia that was induced with the use of bathtubs filled with ice water and open windows in winter.[3,4] Since then, laboratory studies and small trials have suggested that hypothermia is effective. In