Silencing the Critics: New Studies Move Closer to Answering Epigenetic Questions

The role of epigenetics in cancer has been debated since the early 1980s, but results of several recent experiments have prompted even the naysayers to consider that epigenetic changes are not just a consequence but a cause of cancer. And now the field is exploding with the first drug approval, numerous clinical trials, questions about the consequences of such therapies, and even discussions of a federally funded, large-scale epigenome project.

The Smoking Guns

Epigenetics is the study of heritable changes in gene expression such as methylation and loss of imprinting that occur without a change in genetic code. For cancer, the big question is how epigenetic changes affect tumorigenesis. Several experiments have recently been published that some experts in the field think finally provide the answer.

For example, in two of those experiments, researchers at St. Jude Children’s Research Hospital in Memphis, Tenn., and the Whitehead Institute for Biomedical Research in Cambridge, Mass., independently showed that they could reverse the neoplastic phenotype of murine cancer cells by transplanting a nucleus to a mouse oocyte. The resulting embryos supported apparently normal growth through blastocyst development. Because the genetic mutations in these nuclei did not change but their phenotypes did, the investigators concluded that epigenetic changes were partially responsible for the original cancer and that those changes were reversed in the oocyte.

Additionally, in two studies that focus on humans, researchers found that epigenetic changes can predispose individuals to cancer. In one such study, researchers found that loss of imprinting at the insulin-like growth factor II gene (IGF2) was detectable in both normal and cancerous mucosa in 30% of colon cancer patients but in just 10% of the healthy population. Similarly, scientists identified two individuals with germline epigenetic silencing in the DNA repair gene MLH1, which predisposes them to colon cancers.

“MLH1 was the absolute clincher,” said Peter A. Jones, Ph.D., director of the University of Southern California Norris Comprehensive Cancer Center in Los Angeles. Because the change occurred in the germline, MLH1 silencing had to precede any mutation in the progression to cancer, and this finding decisively illustrates the importance of epigenetics, he said. “That really changed the whole ballgame.”

Although there is more work to be done on these systems and others, new research comes out every week that adds to the field, which is rapidly gaining ground in the laboratory and the clinic.

If epigenetic changes play a role in the carcinogenic process, then the logical conclusion is that reversing such changes would help control the disease. Already numerous inhibitors of methylation and histone deacetylase (HDAC) activity are in clinical trials. On May 19, azacitidine became the first such epigenetic therapy to receive approval from the U.S. Food and Drug Administration for cancer treatment. The drug was used to treat 268 patients with myelodysplastic syndrome (MDS) in randomized and nonrandomized trials. About 15% of patients had a partial or complete response to the drug, which is thought to work by demethylating tumor suppressor genes and allowing the cell to undergo either cell death or differentiation.

Jean-Pierre Issa, M.D., a leukemia specialist at the University of Texas M. D. Anderson Cancer Center in Houston, and his colleagues are testing a related compound, decitabine (5-aza-2'-deoxycytidine), in several trials. Like azacitidine, which was originally developed as a cytotoxic agent, decitabine induces demethylation when used at lower doses.

However, decitabine’s exact mechanism of action is not clear, said Issa. With regard to recent trials testing the drug in leukemia patients, he said, “we don’t know exactly why they respond, but we do know that hypomethylation is required for a response to occur.” In other words, only those patients whose cells show a reduction in methylation have a clinical response to therapy. Whether that demethylation reactivates some sort of checkpoint is not obvious, although Issa said the cells seem to die off rather than differentiate.

Since few single-agent therapies are curative in cancer, Issa and others are testing several decitabine combinations. In one strategy, the team plans to pair two epigenetic therapies, decitabine and the HDAC inhibitor valproic acid, for the treatment of MDS. Preliminary patient responses have been good enough that the team has designed a two-arm randomized trial to compare the combination to decitabine alone in 40 or 50 patients.

A second strategy that the team is pursuing is to combine the demethylating agent with a traditional cytotoxic chemotherapy, cytarabine, in patients with chemotherapy-resistant leukemia. In theory, demethylation of the cellular DNA may make the cancer more susceptible to drugs that interfere with the cell cycle and might help induce apoptosis in response to cytarabine.

Mechanism of Action Obscured

Although the trials sound promising and preliminary patient data look good, a study in the October issue of Cancer Cell suggests that even the proposed hypotheses for the mechanism of action...
underlying these epigenetic drugs do not quite explain how they really work.

Previous studies have focused on the ability of epigenetically active compounds to reactivate specific target genes, either by causing demethylation or by decreasing chromatin compaction. But studies that look at the global effects of these compounds have been lacking until now. “It raises alarm bells about what is being done in clinical trials,” said Andrew Feinberg, M.D., from the Johns Hopkins School of Medicine. “It is amazing, that nobody has systematically examined what happens to gene expression in the clinical studies.”

To get a broad view of how different compounds affect gene expression, Feinberg, along with David Gius, M.D., from the National Cancer Institute, and colleagues compared three strategies: using gene knockouts to silence DNA methyltransferases, which hook methyl groups onto DNA; using the methyltransferase-inhibiting drug decitabine; or using trichostatin A, which alters chromosome compaction.

In Feinberg’s view, two results were most striking. First, the effects of the two drugs were more similar to each other than were the effects of decitabine to those of the gene knockouts, despite the fact that decitabine and the methyltransferase gene knockouts are thought to work through the same basic mechanism of demethylation. Second, as many genes were downregulated as were upregulated in response to demethylation, which is contrary to the field’s working hypothesis that demethylating agents work by reactivating silenced gene promoters.

In addition, the team found that a cell’s response to the addition of a drug was determined by its genetic state. Thus, cells lacking a functional copy of one or the other methyltransferase genes responded differently to the addition of decitabine or trichostatin A than did cells that lacked both methyltransferase genes.

“The system is both more complicated and more specific than we previously thought, and we need to rethink how we use these drugs,” Gius said. Trials need to be constructed in such a way that scientists and clinicians can determine why one patient responded and another didn’t, he added.

Nancy Davidson, M.D., an oncology professor at Johns Hopkins School of Medicine who studies the effects of methylation and histone acetylation on breast cancer, has come to much the same conclusion about the need to find out why some patients respond and others do not.

She is currently awaiting approval for a trial designed to test the effects of SAHA (suberoylanilide hydroxamic acid), an HDAC inhibitor, on breast cancers. As planned, the trial will enroll patients with newly diagnosed cancer who will be treated with SAHA for three days prior to surgery. The research team will then compare the original biopsy samples and the tumor samples recovered from surgery for changes in gene expression patterns by microarray analysis and by looking at specific gene targets, such as the estrogen receptor.

While the different epigenetic markers, including DNA methylation, imprinting, and histone acetylation, are all familiar in and of themselves, researchers do not yet know how common such modifications are across the genome and what is normal in different tissues. To address this issue, scientific teams in different locations are trying to assemble broader studies of the epigenome.

In June, Johns Hopkins School of Medicine received a $5 million grant from the National Institutes of Health to establish a center devoted to understanding epigenetics on a genome-wide scale. Although initially the researchers will focus on complex neurological diseases, the tools they develop will likely be applicable to cancer.

For example, they plan to put substantial effort toward developing new tools to look quantitatively at methylation and allele-specific gene expression. They are also devising statistical methods to include such information in genetic models of risk and to explore the interplay between genetic traits and epigenetic ones.

Benjamin Tycko, M.D., Ph.D., at Columbia University in New York, is also working to develop readily usable gene chips that would provide reproducible, quantitative data on methylation with the intent of comparing methylation states between different tissue or cell samples. “But we need to emphasize that while all of these methods will uncover target genes in a more or less comprehensive way, none will achieve complete nucleotide coverage of every base,” said Tycko.

That limitation, he continued, is why there needs to be a federally funded epigenome project to comprehensively look at a few critical samples at the level of every nucleotide.

Although that idea may sound far-fetched, it is already being discussed at the NCI. Earlier this year, NCI held a meeting with 18 leading scientists in the field. The major recommendation from that meeting, according to an internal white paper that is currently making its way through NCI, is that discussions should start on how to approach an epigenome project.

Such a project would more closely resemble a proteomic project than the genome project because the epigenome is going to differ between cell types, tissues, and the age of the organism under study. For this reason, said Tycko, choosing the samples for such a large-scale project would be absolutely critical.

It is not yet clear, of course, whether the government will undertake such a grand project, and there are mixed responses within the field. Some researchers would prefer to see more targeted work continue, while others emphasize the need to develop unbiased methods to gain unbiased results. Feinberg, a strong advocate of the epigenome concept, said that, without such genome-wide information, researchers are missing the opportunity to develop truly targeted, rational epigenetic therapies and are left with therapies that have broad—and sometimes unpredictable—effects.

—Rabiya S. Tuma