Switching on Epigenetic Therapy

Reversible epigenetic changes that alter gene expression are a characteristic of many cancers and other diseases. Biotech companies are taking note and are starting to develop new drugs to reverse such pathogenic “epimutations.”

It was the mysterious transformation of precancerous cells into muscle cells that got Peter Jones involved in epigenetics, the study of heritable information that does not affect the sequence of DNA. “It was a Fleming-like experience,” says Jones, currently the director of the Norris Comprehensive Cancer Center at the University of Southern California. Back in 1980, an assistant spotted what appeared to be mold in a culture dish of immortalized mouse embryonic cells treated with the chemical 5-azacytidine. “It was the most massive cell colony ever. It was so big, that it looked like a mold,” reminisces Jones. 5-azacytidine had transformed the precancerous cells into muscle cells, but how?

It took Jones and others a decade to figure it out. Azacytidine is able to replace the base cytosine in DNA but cannot carry epigenetic marks such as methyl groups. Methyl groups are added to certain cytosines by the enzyme DNA methyltransferase, rendering genes inaccessible to transcription factors and silencing their expression. During tumor formation, DNA methylation can be misregulated. This may lead to spreading of methyl marks across the genome and the silencing of tumor suppressor genes as well as other genes encoding, for example, MyoD, which regulates the differentiation of muscle cells. In Jones’ experiment, treating precancerous cells with azacytidine resulted in demethylation of the MyoD gene followed by its reactivation and the differentiation of these cells into muscle cells. Jones realized that azacytidine could be used to treat tumors by reversing pathogenic epigenetic marks or “epimutations” and reactivating the expression of tumor suppressor genes. In 2004, azacytidine (brand name Vidaza) made by Pharmion (now owned by Celgene) became the first epigenetic drug on the market, with worldwide sales of $165 million in 2007. Vidaza is currently used to treat patients with myelodysplastic syndrome (MDS), who have few therapeutic options and develop acute leukemia. “5-azacytidine is the first breakthrough for MDS patients,” says Ulrich Mahlknecht at Saar University, one of the first German physicians to treat cancer patients with azacytidine. A worldwide phase III clinical trial is currently underway to test a related drug 5-aza-deoxycytidine or Dacogen (developed by the biotech company SuperGen and marketed by the Japanese pharmaceutical company Eisai) in elderly patients with acute myeloid leukemia. Mahlknecht is optimistic that “epigenetic cancer therapy might be a good choice to treat slow growing cancers and could be integrated into conventional chemotherapy protocols.”

Investors are taking note of the epigenetics field, and big pharma are buying up biotech companies that are developing promising epigenetic therapies. The latest deal, worth $375 million and announced last month, is between the UK pharmaceutical giant GlaxoSmithKline and SuperGen. The biotech companies Constellation and Epizyme, in Boston, Massachusetts, have raised $49 million and $42 million, respectively, since their founding last year. They aim to develop drugs to block new classes of enzymes (histone methyltransferases and demethylases) that methylate and demethylate histones rather than DNA. In 2008, Eisai paid $3.9 billion for MGI Pharma in Bloomington, Minnesota, who had acquired the worldwide exclusive rights for Dacogen from SuperGen in 2004. Meanwhile, Syndax in Waltham, Massachusetts, has raised $40 million to develop entinostat for treating lung cancer, a new epigenetic drug that blocks another class of enzymes, called histone deacetylases (HDACs), involved in epigenetic processes. And in late 2007, Pharmion, who make Vidaza, was acquired by the Swiss pharma company Celgene for $2.9 billion. In 2004, Merck acquired Aton Pharma for $125 million in order to further develop their drug Vorinostat (Zolinza), an HDAC inhibitor used to treat advanced lymphoma. “Epigenetic cancer drugs might be able to reach blockbuster status,” says Frank Lyko of the German Cancer Research Center in Heidelberg, because the phenomenon of misregulation of tumor suppressor genes and oncogenes due to epimutations can be found in a variety of cancer types. “Epigenetic changes are responsible for at least 50% of gene inactivating events in cancer,” notes Jones. “They are potentially reversible, whereas genetic changes are not.”

The current boom in the development of epigenetic therapies partly reflects the realization that elucidating the pathogenesis of diseases with an environmental component requires understanding the epigenetic mechanisms involved. This year, the US National Institutes of Health (NIH) in Bethesda, Maryland, launched the 5 year $190 million Epigenetics Roadmap Project, with the goal of mapping the entire human “epigenome”—that is, all of the epigenetic marks within a cell of a given tissue type and developmental stage. One of the Reference Epigenome Mapping Centers of the NIH program is at the University of California, San Diego, where Joseph Ecker (also at the Salk Institute) leads a research group. Ecker has compared the DNA methyl marks across the genomes of human embryonic stem cells and fetal fibroblasts and has found marked differences. These and other methylomes should help to define the differences between normal and pathogenic epigenetic changes and whether drugs can reverse the latter without interfering with the former. “Unless we know how these drugs affect the entire epigenome, we don’t really understand their full mechanism of action,” says...
Ecker. The NIH has also invested about $16.8 million over 5 years in the Center for Epigenetics of Common Human Disease at the Johns Hopkins University in Baltimore, Maryland. Here, Andrew Feinberg and his team are searching for pathogenic epimutations that may cause cancer or other diseases such as autism or depression. Meanwhile, Jones has received $9 million from the US charity Stand up to Cancer to develop epigenetic cancer therapies. And in the UK, the Wellcome Trust has spent $4.1 million as part of a public-private partnership—the Structural Genomics Consortium—that includes GlaxoSmithKline, the NIH’s Chemical Genomics Center in Bethesda, Maryland, and the Departments of Chemistry and Biochemistry at the University of Oxford. The goal is to develop small-molecule inhibitors against 25 proteins involved in epigenetic pathways. Within its Framework 6 program, the European Union has funded the Epigenome Network of Excellence (NoE) comprising 81 European research groups with €12.5 million between 2004 and 2009. This program has been extended until August next year and will likely be continued thereafter but with a focus on “epigenetics systems biology,” according to Jörn Walter of the University of Saarbrücken, Germany, who leads one of the epigenetic research teams.

Lack of Specificity?
There are a number of different mechanisms for the epigenetic regulation of gene expression. In addition to DNA methylation/demethylation, the histones of chromatin may have methyl marks or acetyl groups added or removed. In general, histone acetylation enables genes to be expressed, whereas histone methylation silences genes. HDAC inhibitors such as Vorinostat or entinostat, by blocking removal of acetyl groups from the lysine residues of histone tails, alter the packaging of genes in chromatin such that those that are pathogenically silenced are switched back on. Vorinostat—approved by the US Food and Drug Administration (FDA) to treat lymphoma in 2006—counteracts the deacetylation and repression of vital genes such as the tumor suppressor p53. Another approved HDAC inhibitor is valproic acid (Depakote), which has long been used to treat bipolar disease, epilepsy, and most recently migraine. The investigational HDAC inhibitor entinostat is in several phase II clinical trials to treat Hodgkin’s lymphoma, leukemia and lung cancer. One major issue with HDAC inhibitors is that they target not only the lysines in histones but also those in other proteins, leading to unwanted side effects. “We need to know much more about the mechanism of action of the different classes of HDACs,” says Kapil Bhalla, an oncologist at the Georgia Cancer Center in Augusta, Georgia.

But lack of specificity is also an issue with other epigenetic drugs. Azacytidine, for example, results in demethylation of many regions of the genome and not only pathogenically repressed tumor suppressor genes. “It’s a mixed blessing,” says Rudolf Jaenisch at the Whitehead Institute in Cambridge, Massachusetts. “If you generally inhibit the methylationtransferase, then you protect one tissue but you induce cancer in another.” Experiments in Jaenisch’s lab show that widespread demethylation of the genome could prevent mice from developing colon cancer while increasing their chance of developing tumors of the thymus. Timothy Bestor of Columbia University in New York points out that: “The drugs under development and in trials have genomewide effects, and this lack of specificity and the lack of known targets mean that side effects are likely to be severe.” In Bestor’s view, “convincing evidence of a beneficial effect of DNA methylation inhibitors in cancer has not appeared despite much effort.” He thinks that “the extent of pathogenic gene silencing by DNA methylation may have been greatly overestimated.”

Combining Epigenetic Drugs
Jones sees the lack of specificity in a different light. “The thing that you’re actually targeting, is an abnormal chromatin state,” says Jones. “It seems like the abnormal state responds most effectively to these kinds of drugs.” Normal epigenetic marks—like those that inactivate the second X chromosome in females—are not affected by azacytidine or other demethylating drugs, potentially because there are several mechanisms of epigenetic regulation at work. “Abnormal chromatin states may respond to two drugs, which both affect different parts of the pathway and reactivate a gene more specifically,” says Jones. Jones, together with Stephen Baylin at the Johns Hopkins University in Baltimore, Maryland, is developing a combination antitumor therapy comprising an HDAC inhibitor and a DNA methylation inhibitor. “It is possible,” says Jones, although he does not yet have proof of this, “that there are combinations in the pathologically silenced genes, which will respond to double treatments.” In his view, normal genes do not have these miscombinations such that the drugs leave them untouched. “That’s the way I would see the future for getting more specificity,” says Jones.

Epigenetic drug combination therapy is starting to garner interest. Kapil Bhalla and his team have treated an acute myeloid leukemia cell line with a combination of the histone methyltransferase inhibitor DZNep (deazaneplanocin A) and the HDAC inhibitor panobinostat (developed by Novartis). Each drug alone reactivated pathogenically methylated genes such as the tumor suppressor p16, inducing apoptosis of 20% to 30% of the cancer cells. But treatment with both DZNep and panobinostat induced apoptosis of 75% of the leukemic cells. In a clinical trial, Bhalla’s team added the HDAC inhibitor entinostat twice during a 5-azacytidine treatment regimen and observed slowed cancer growth in 46% of patients. Despite this encouraging finding, Bhalla points out: “We don’t really know what’s the molecular mechanism of action of these drugs.” His team has demonstrated that the epigenetic drug combination induced reactivation of certain tumor suppressor genes but has struggled to correlate this with the predicted DNA demethylation pattern. “It might be,” he says “that we did not look at the critical genes that become demethylated and reactivated, and induce differentiation or apoptosis.” Because epigenetic drugs usually take several weeks to show their effects, Bhalla speculates that they may be acting on only a very small stem cell-like population in the tumor.

Designer Epigenetic Drugs of the Future
Frank Lyko at the German Cancer Research Center has taken a different approach. Starting with the crystal structure of the human DNA methyltransferase
DNMT1, he has used rational computer design to come up with a direct inhibitor that fits into and blocks the active domain of the enzyme. Because this molecule (RG108) inhibits only one of the four human DNA methyltransferases, the pattern of demethylation it generates in the genome is different from that of azacytidine. “RG108 is very interesting because it does not require entry into DNA to do the job and therefore might have fewer side effects,” comments Jones. The charity Cancer Research UK is developing RG108 together with the Swiss pharma company Roche. To increase specificity, Lyko and his team are now searching for new small-molecule inhibitors of each of the four human DNA methyltransferases, as part of a 2 year £3.5 million alliance between the German Cancer Research Center and the pharma company BayerShering. Lyko is also developing an azacytidine variant called CP-4200—in which conjugation of a lipid chain to azacytidine accelerates its cellular uptake and activity—with Clavis Pharma in Oslo, Norway.

Biotech entrepreneurs are also starting to become interested in the next wave of epigenetic drugs that alter histone methylation. In 2004, Yang Shi at Harvard Medical School in Boston, Massachusetts, discovered the first histone demethylase, an enzyme that removes two methyl groups from the tail of histone H3. “There was a dogma that histone methylation is not reversible,” says Shi, “but we showed that it is dynamically regulated.” Shi, at the same time as Yi Zhang at the University of North Carolina, then identified other groups of histone demethylases and methyltransferases acting on different histone residues. In 2008, Yang Shi and Yi Zhang helped to found the biotech companies Constellation and Epizyme, respectively. Another founder of Epizyme, Bob Horvitz of MIT in Cambridge, Massachusetts, got interested in epigenetic therapy through his genetic studies with the nematode. Looking at the consequences of mutations in a variety of genes encoding histone methyltransferases or demethylases, he observed that they were “quite distinguishable.” He concluded that “distinct methyltransferases are not themselves all doing the same thing, but rather have distinct biological roles.” This suggested that histone methyltransferases and demethylases may have more biological specificity than HDACs, which are more limited in number and act in a more general way. “Developing inhibitors to different histone methyltransferases, you should have more targeted therapies,” says Horvitz.

The specificity of a single enzyme within a big class is important from the investors’ standpoint, says Mark Levin, the founding CEO of Constellation. “If we want to build a broad platform company around this biology, we want to have many potential targets, so that we have a lot of options,” he says. Both companies are still searching for the appropriate small molecules that block histone methyltransferases. Last year, Constellation finished three high-throughput screens for such small-molecule inhibitors and plans to complete eight this year. These assays are “quite unique,” says Yang Shi. “We have developed ways to really reconstitute some of the activities, using nucleosomes and complexes.” Compared to DNA methylation, histone methylation may turn out to be a more flexible target, but as Frank Lyko notes, “not a single histone methyltransferase target has yet been validated in a mammalian model system.” When people first began to develop drugs to block kinases, they were laughed at, notes Horvitz, because no one could see how one kinase inhibitor would not block all kinases. But it turned out that the active sites of kinases could be targeted by specific small molecules, thus enabling kinase inhibitors to be developed successfully. And the histone methyltransferase field with only a handful of enzymes seems to look “even better than the early days of kinases,” says Horvitz. However, “preclinical and clinical studies have to be done very thoroughly, especially if one’s dealing with a new target class,” he says. “But on the other hand, a new target class opens a whole universe of new possibilities for curing people.”

Sascha Karberg
Berlin, Germany
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