



EPIGENETICS

Unravelling the cancer code

There's more to the genetic causes of cancer than sequence mutations. This added complexity could offer scientists an opportunity to tackle cancer even earlier.

BY VICKI BROWER

What causes a cell to turn cancerous? Ever since the discovery of oncogenes in 1989, the prevailing theory is that mutated genes drive the process: a predisposition to cancer is somehow written in our genetic code. This depressing portrait might have made cancer prediction easier, but hindered attempts at cancer prevention. Fortunately, the story became a lot more complicated.

It was geneticist Bert Vogelstein at Johns Hopkins University in Baltimore, Maryland, who clarified the role of that first oncogene as well as several others that followed; he is one of the originators of the notion of cancer as a genetic disease. Recently, while examining biopsies from ovarian cancer patients, he discovered that more than half of the tumour samples had mutations in the *ARID1A* gene¹. Yet *ARID1A* does not directly stimulate the formation of tumours. “What we did not expect,” says Vogelstein, “was that this gene is involved in determining the epigenetic changes that can lead to tumours.” That is, the carcinogenic effect of *ARID1A* occurs by encouraging changes in gene expression levels and not in DNA sequence.

Vogelstein's findings were the tip of the iceberg. The Cancer Genome Project, an international effort to sequence the genomes of a number of different cancer types, has also found that “an incredibly high number of mutations actually affect epigenetics and

epigenetic regulators”, says Jean-Pierre Issa, an epigeneticist at the MD Anderson Cancer Center in Houston, and a researcher on the project. And Vogelstein's insight into *ARID1A* is a clue to the pathways that connect genetics and epigenetics in cancer. “This closes the loop, as we find that even genetic lesions are controlled by epigenetic mechanisms,” explains Issa.

Such findings are leading scientists to realize that the root cause of cancer is more complicated than inherited or acquired genetic mutations. “We used to think of cancer in binary terms — genetic mutations, or no mutations — but that's not the case anymore,” says David Sidransky, an oncologist at Johns Hopkins. Researchers are finding that epigenetic changes frequently precede and can induce genetic mutations that cause cancer. If these early epigenetic alterations can be detected and reversed, it might be possible to prevent certain cancers.

BEYOND THE HUMAN GENOME

The best known epigenetic modification is methylation — whereby a methyl group (CH₃) attaches to a portion of DNA. Methylation of a gene reduces or stops its expression (see DNA methylation patterns, page S13). Patterns of methylation can be inherited from the mother or acquired during life. If the genetic code is the hardware for life, the epigenetic code is software that determines how the hardware behaves — and as such it can be rewritten.

The Human Genome Project (HGP) aimed

to decode life's hardware. Scientists hoped that one result would be discoveries of disease-causing genetic mutations. But mutations like those to *BRCA1/2*, and are highly predictive of cancer risk, were found to be the exception rather than the rule. Indeed, the HGP helped confirm that underlying most common diseases are hundreds if not thousands of genetic mutations that vary from person to person. “Even before the rough draft of the HGP was completed in 2000, it had become clear that it is impossible to understand the genetics of cancer without epigenetics,” says Issa.

The HGP drew attention away from the nascent study of cancer epigenetics, which began in the mid-1980s at Johns Hopkins in the lab of cancer biologist Stephen Baylin. He noticed that cancer cells contained regions of DNA with increased methylation and hypothesized that if a tumour suppressor gene was hypermethylated, its activity would decrease or stop entirely — just as if it were a genetic mutation — allowing the tumour to flourish. In other words, Baylin reasoned, this epigenetic change would produce the same result as a genetic mutation.

Firm evidence came in 1994. Baylin and his colleague, oncologist James Herman, were investigating renal cell carcinoma (RCC), the most common type of kidney cancer in adults. Around 60% of RCCs are caused by an inherited mutation in the von-Hippel Lindau

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tumour-suppressor gene (VHL), which hobbles the gene's ability to express the tumour suppressing protein. Baylin and Herman showed that 20% of the remaining patients with the non-inherited form of RCC did not have a mutation in VHL. Their genes were silenced not by a mutation but rather by hypermethylation².

The following year, in collaboration with Sidransky's lab at Johns Hopkins, Baylin and Herman showed that human cancers commonly arise when a particular tumour suppressor gene, known as p16, is hypermethylated. Moreover, in many cancers including RCC, epigenetic and genetic mutations often work in tandem: one of the two copies of a tumour suppressor gene is inactivated by genetic mutation, while the other is hypermethylated. This finding "convinced us that epigenetic abnormalities could play an important driving role in cancer — and we and many others have been pursuing this possibility ever since," says Baylin.

The move from a purely genetic to an epigenetic model is crucial for prevention strategies. As numerous gene therapy trials have shown, it is very difficult to treat a genetic disease by re-activating the dormant, mutated gene or by replacing it with a non-mutated one. "Epigenetic changes are reversible, and therefore have an edge over genetics," says Mukesh Verma, an epigeneticist at the National Cancer Institute's division of cancer control and population sciences in Bethesda, Maryland. Furthermore, epigenetic changes in cancer occur

before genetic mutations. "If you can prevent methylation of those tumour suppressor genes, you might have a valuable prevention strategy," says Baylin.

THE ENVIRONMENTAL LINK

Epigenetics has also provided clues that link environmental factors with cancerous genetic changes. Changes in methylation can be detected in the blood of cancer-free individuals who smoke and eat high-fat diets, and these changes have been shown to precede genetic mutations³. More recently, Karl Kelsey, a molecular epidemiologist at Brown University in Providence, Rhode Island, has uncovered independent associations between epigenetic patterns in breast cancer tumours, the tumour size, alcohol consumption and folate intake⁴.

A prime candidate at the interface of environment and genetics is chronic inflammation, which is known to precede the development of numerous types of precancerous lesions — and indeed certain cancers themselves, including oesophageal, liver and colon cancers. Inflammation has been linked with increased DNA methylation in otherwise healthy looking tissue. Issa calls chronic inflammation "a truly epigenetic phenomenon".

Long-term inflammation may result from infection with *Helicobacter pylori* or hepatitis C virus, or from autoimmune diseases such as ulcerative colitis (a form of inflammatory bowel disease). People with ulcerative colitis often develop colon cancer at a younger age — for example in their 50s — than the 60 to 70 year average age of onset. "All of the epigenetic changes that occur in colon cancer, specifically DNA hypermethylation and gene silencing, are accelerated — and appear in the inflamed tissue prior to actual cancer," says Baylin.

Half the world's population is infected with inflammation-causing *H. pylori*, yet stomach cancer afflicts barely 0.03% of those. "There must be something in the body itself that makes it react differently to infection," says Emad El-Omar, a gastroenterologist at the University of Aberdeen in Scotland. El-Omar is investigating whether genetic variation can influence this response

Genetic polymorphisms are normal genetic variations within a population that can subtly raise or lower each person's levels of a particular protein. While everyone within the normal population produces the same pro- and anti-inflammatory chemicals (or cytokines) an individual's particular levels vary according to genetic make-up. Certain polymorphisms, El-Omar hypothesized, might tip the balance towards chronic inflammation and then to cancer.

El-Omar found that polymorphisms in the inflammation-related IL-1B and transforming growth factor (TNF)-α genes determine the levels of circulating IL-1B and TNF-α pro-inflammatory cytokines. People with a genotype disposed to higher levels of these

cytokines have an increased risk of developing stomach cancer following *H. pylori* infection. El-Omar has made similar discoveries in other cancers. In colorectal cancer, for example, he discovered there is an inflammatory environment in and around premalignant lesions. Within this region he found nine differentially expressed genes linked with inflammation, including those responsible for IL-8 and the CXCL- family of cytokines⁵.

Others researchers are making similar connections. For example, there is evidence that pro-inflammatory prostaglandin endoperoxide synthase⁶ and TGF-β⁷ are both significantly associated with increased risk of colon cancer. Although the evidence linking inflammation to epigenetics and cancer is mounting, the underlying mechanism of the association and the value of screening for these polymorphisms remain less clear.

POTENTIAL FOR REVERSAL

Drugs and dietary substances that alter epigenetic pathways are currently being tested. During his research on RCC, for example, Baylin and colleagues were able to reverse hypermethylation of the VHL gene with the drug 5-azacytidine. Trials of demethylating drugs as adjuvant treatments to prevent lung cancer recurrence are underway. If successful, prevention trials are the next logical step. "We need five- and ten-year survival data with current drugs to be sure there are no secondary effects before we give them to reasonably healthy people for prevention," says Issa. He sees a different source for the first wave of preventive medications. "I would bank on discovering more 'gentle' approaches to epigenetic manipulation for cancer prevention — be they natural products, existing drugs with a good safety records, or even vitamins or diet."

Epigenetic changes also have potential utility as biomarkers (see Portents of malignancy, page S19). Being able to read the methylation pattern of certain genes could give scientists a heads-up for people on the brink of developing cancer.

Slowly the importance of the epigenome in cancer development is being appreciated. "Geneticists are hugely more aware of the importance of epigenetics in the development of cancer," observes Baylin. When it comes to cancer prevention, the future could lie in arresting the reversible epigenetic changes before irreversible mutations take hold. ■

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