HITTING THE MARK IN CANCER EPIGENETICS

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In 2013 Stefan Pfister, a paediatric neuro-oncologist at Heidelberg University Hospital, had to deliver devastating news to the family of a 12-year-old girl. She had been diagnosed with glioblastoma, a fast-growing and potentially lethal brain cancer. Even though she would receive radiation and chemotherapy, Pfister did not expect her to live more than 18 months. But he was wrong. Three years after her treatments, the young girl was thriving.

Pfister, who also directs the German Cancer Research Center on the hospital campus, was able to determine why. A genomic scan of the girl's tumour revealed epigenetic methylation patterns consistent with low-grade glioma, which is slow-growing and curable, and hard to distinguish from glioblastoma on the basis of histology. The memory of that diagnostic error sticks with him. "If we had known she had a glioma, we wouldn't have told her family that she had little chance of a cure," he says. "We usually put these patients on a watch and wait scheme. We could have spared her all that therapy."

Pfister's experience illustrates the growing potential of genomic technologies in epigenetics to contribute to understanding, diagnosing and treating cancer. Epigenetic modifications to DNA and RNA regulate how and when genes are turned on or off, and they play crucial roles in normal cell differentiation. When epigenetic processes

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are dysregulated, however, these modifications can cause gene expression to go awry and potentially spawn tumours. Tools such as genomic arrays and next-generation sequencing can reveal previously unseen epigenetic marks such as DNA methylation, histone modifications and chromatin remodelling, allowing scientists to "gain a better understanding of cancer and how it evolves", says Manel Esteller, director of Josep Carreras Leukaemia Research Institute (IJC), in Barcelona. In turn, these insights will lead to new opportunities in diagnosis and treatment.

◂ Epigenetic marks are what allow the same DNA in each cell to be transcribed into different proteins across the body. Disrupted epigenetic processes can contribute to cancer.

Methylation Arrays

DNA methylation — the process by which methyl or hydroxymethyl groups attach to the C5 position on the cytosine ring — is the best understood epigenetic marker today. Methylation typically silences gene transcription in patterns that are characteristic to specific cell types and tissues. But when it silences tumour suppressor genes, cancer may appear.

The first epigenetic technologies — methylation arrays — emerged about 15 years ago with the launch of the National Cancer Institute's Cancer Genome Atlas project. These tools are designed to probe heavily methylated regions on DNA, including CpG islands dominated by the presence of cytosine-guanine sequences. (see 'How to decode epigenetic factors'). And they're still workhorses in the field.

Using an array that covers 450,000 genomic sites to scan tumour samples from just over 2,800 patients, Pfister and his colleagues achieved a major advance in the diagnosis of central nervous system (CNS) cancers. Published in 2018, the research revealed 82 distinct tumour classes, only some of which were already known[1]. Freely available online, and now used in more than 10 countries, "the new classifier allows for standardized tumour diagnostics informed by DNA methylation patterns distinguishing the different brain tumour entities", Pfister says. These patterns are reflective of the cell type that each of the different tumours emerged from, as well as their differentiation states when the malignant transformations occurred. According to Pfister, this cellular context predicts how a tumour is likely to look and behave. Illumina's methylation array covers 850,000 sites on the genome, and analyses with it, he says, "are sufficient to capture the cancer cell's basic identity".

Esteller points out that methylation arrays have many positive aspects, such as low-cost and amenability to formalin-fixed, paraffin-embedded tissue samples. In his own research, he has used arrays to identify which patients with non-small-cell lung cancer will probably benefit most from anti-PD-1 immunotherapy, and to find out where metastatic cancers of unknown primary origins (CUP) come from.

Sequencing Moves to the Fore

Methylation arrays, however, only interrogate limited regions of DNA for target alterations.

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Epigenetic sequencing, on the other hand, provides an opportunity to study much larger swaths of the cancer epigenome — including how regulatory elements in tumorigenesis evolve. And as costs fall steadily, epigenetic sequencing is making steady inroads in cancer research. "We see applications for sequencing across the board in epigenetics," says Phillip Febbo, Illumina's chief medical officer. "The lion's share of scientists who look at methylation patterns are now switching to these methods."

Andrew Feber, a cancer geneticist at UCL Cancer Institute in London, is one of those scientists. He aims to find epigenetic traces of bladder cancer in urine, an endeavour he says is particularly well suited to sequencing since it can read the often fragmented arrangements of DNA letters in liquid specimens. "We can use it to identify patients with bladder cancer earlier, and also to interrogate aspects such as inter-tumour heterogeneity and subclonal epigenetic alterations that occur in only a fraction of the tumour cells in a sample," he explains.

Among the various approaches, bisulfite sequencing is the most widely adopted. The process involves treating a DNA sample with sodium bisulphate, which has the effect of making methylated cytosines more visible. That's because the chemical treatment converts unmethylated cytosines on DNA to uracil, while leaving the methylated forms of cytosine — 5-methylcytosine and 5-hydroxymethylcytosine — unchanged. Using specialized software, scientist can then map out where the methylated bases are, and hunt for epigenetic variants indicative of cancer. Feber and his colleagues relied on the bisulfite method to find epigenetic markers for bladder cancer that have since been incorporated into a diagnostic test being evaluated in a clinical trial with more than 16,000 patients[2].

This test could provide a non-invasive alternative to

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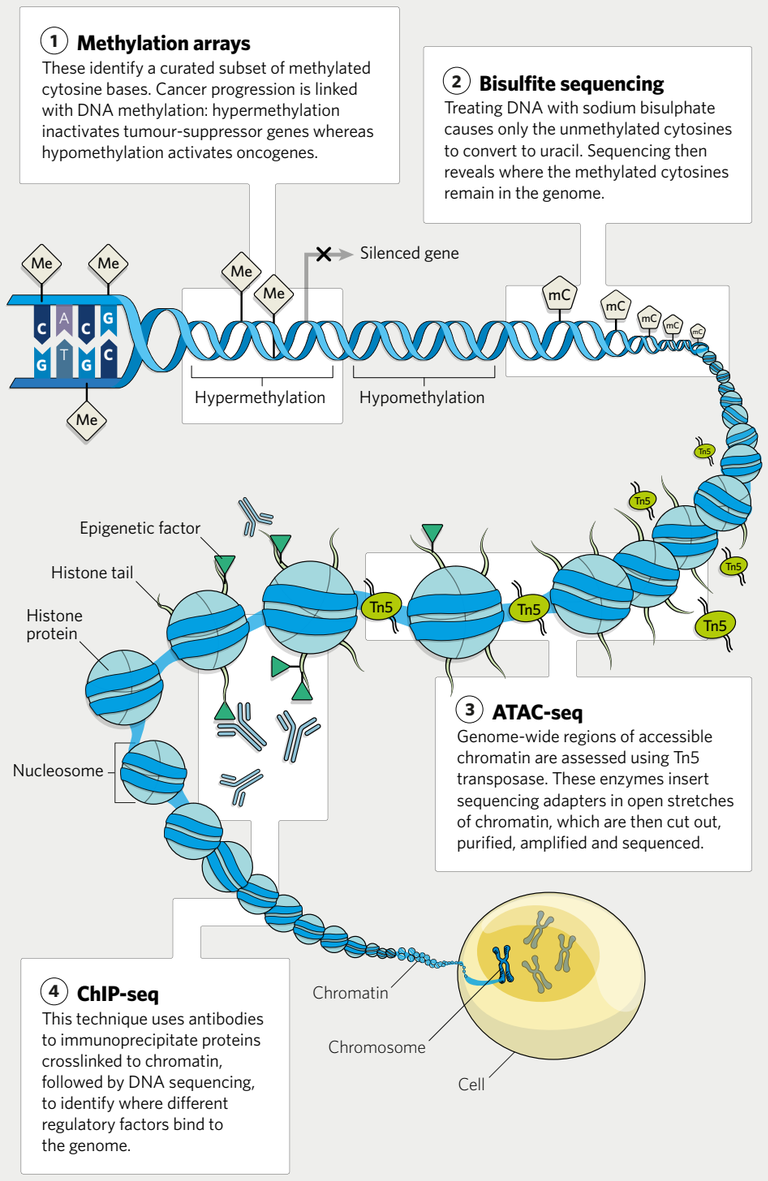
cystoscopy, which poses an infection risk. The test was developed with targeted bisulfite sequencing, which zeroes in on specific genomic regions. However, scientists can also use whole genome bisulfite sequencing to discover entirely new epigenetic pathways in cancer. Though it's currently expensive and difficult to perform, whole genome bisulfite sequencing amounts to the most comprehensive approach for discovering new epigenetic patterns so far.

While it's powerful, bisulfite sequencing has its drawbacks: one being that the chemical treatments are highly damaging to DNA, making it difficult to amplify long fragments for analysis. Febbo says non-destructive bisulfite-free alternatives are now emerging, and he singled out one, in particular, as being especially promising. TET-assisted PIC-borane sequencing (TAP-seq) was developed by computational biologist Benjamin Schuster-Boeckler and his colleagues at Oxford University's Ludwig Institute for Cancer Research. According to Boeckler, TAP-seq relies on milder reactions, detects a wider range of epigenetic modifications, and generates wider coverage at potentially lower costs[3]. "There is a patent pending on the chemistry, but the materials for TAP-seq are all freely available and being used already by numerous groups," says Boeckler, who describes the new method as a "full replacement for bisulfite sequencing".

HOW TO DECODE EPIGENETIC FACTORS

DNA can be modified in several different ways that alter gene expression. Here are four of the common technologies that researchers are using to probe the epigenetic modifications.



Meanwhile, scientists can also obtain complementary epigenetic information by combining sequencing with other approaches. One of them, known as ChIP-seq, reveals how proteins interact with different sites on the genome. Performed by combining chromatin immunoprecipitation (ChIP) with parallel sequencing, ChIP-seq identifies binding sites for transcription factors, and provides additional information about specific epigenetic changes based on the chromatin modifications targeted for analysis.

ChIP-seq has been used by researchers to identify deregulated genes in breast, head and neck, and other cancers[4,5]. But epigenetic modifications can also drive cancer by affecting regulatory elements located far from target genes on the continuous stretch of DNA. That's because, when it comes to regulating gene expression, it's not just the linear proximity of genes, promoters, and enhancers that matters, but also their proximity in three-dimensional space. Methods that report the shape of DNA as it is packaged can home in on these long-range interactions by analysing how chromatin domains are spatially organized within the nucleus of the cell. At the Dana-Farber Cancer Institute in Boston, Massachusetts, scientists are now combining ChiP-seq with a specific chromosome conformation capture technique known as HI-C, which allows for high-throughput, high resolution analyses of the interaction of regulatory elements and their target genes[6]. "We come at epigenetics from the standpoint of transcriptional regulation and chromatin modification, and how they change with cancer progression," says Myles Brown, director of Dana-Farber's Center for Functional Cancer Epigenetics. "And these methods allow us to understand higher-order interactions within the genome."

However, for the transcription machinery to work, chromatin needs to be open and accessible — if it's tightly packed together, gene expression is blocked.

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By using a method called ATAC-seq, scientists can now identify regions of chromatin accessibility, and study how they vary between normal and cancerous cells. According to UCL's Feber, ATAC-seq offers a way to connect chromatin accessibility with DNA methylation changes and their influence on cancer processes. He uses it to study varied chromatin states in bladder cancer epithelial cells. And in a 2018 study, researchers at Stanford University used ATAC-seq to assess tumour- and tissue-specific regulatory elements for 23 different types of cancer, providing what the authors claimed was a "wealth of information on the susceptibility, mechanisms, prognosis, and potential therapeutic strategies," pertaining to these diseases[7].

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Future Opportunities

Where will the arrays and workflows used in epigenetics take the field? Experts interviewed unanimously predicted that epigenetic patterns in the DNA of single cells and in liquid biopsies will emerge as key focus areas in cancer research. Every individual cell has its own epigenetic profile. And enabled by improving technologies, scientist will begin to understand how the epigenome drives differentiation at a cellular level without the need to infer that process from studies of bulk tissues. Sheng Li, a computational biologist who studies cancer epigenetics at Jackson Laboratories in Farmington, Connecticut, says single cell applications will advance her own efforts at finding epigenetic biomarkers in blood that predict how patients respond to specific treatments for leukemia. When it comes to single-cell research, there's still a cost barrier to contend with. "After we break that cost barrier, we'll routinely be able to look at the tumour epigenome with single-cell resolution." Li says.

Similarly, Pfister says single-cell approaches could at some point allow clinicians to diagnose tumours of the CNS by assessing epigenetic markers in cerebro-spinal fluid, potentially eliminating the need for a biopsy. Although there's still lots to learn, cancer epigenetics has come a long way in a short time. And Pfister is still in touch with his glioma patient. "She's recently graduated high school and doing great." ■

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