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Timothy Ley has found that 'epigenetic' changes that alter DNA expression offer a possible approach to tackling leukaemia.

EPIGENETICS

Reversible tags

Enzymes that modify gene expression without changing the DNA sequence are now viewed as central to the development of leukaemia — and may lead to new drugs.

BY JESSICA WRIGHT

Conversations about cancer often focus on genetics, with the blame placed on gene mutations that lead to rampant cell growth or stop tumour cells from dying.

But recent discoveries in leukaemia have shifted the focus away from genetics to epigenetics — small chemical changes to DNA or its associated proteins that alter the level of gene expression. Researchers have found that the sources of many leukaemias can be traced back to mutations in the enzymes that add or remove these chemical tags.

"A couple of years ago, if I had said that to somebody they would have laughed me out of the room," says Timothy Ley, a geneticist at Washington University in St Louis, Missouri. "There just was no clear notion that epigenetic modification would be so critically important for the pathogenesis of the disease."

Epigenetic tags regulate gene expression by acting as gatekeepers, blocking or allowing access to a gene's 'on' switch. These chemical tags (such as methyl or acetyl groups) are added directly to DNA or onto histones, the large spool-like proteins around which DNA is tightly wound. DNA methylation masks certain regions on the genome, whereas modifications to histones can loosen or tighten the DNA reel, altering which genes are exposed.

Epigenetic changes can either be inherited or accumulated throughout a lifetime. But importantly for cancer research, they are reversible. Researchers can tinker with the enzymes that add or remove the chemical tags, restoring the gene's normal role — an ability that makes these enzymes attractive targets for leukaemia drugs (see 'Target practice', page S8).

"It's easy to make inhibitors of enzymes," says Scott Armstrong, a paediatric oncologist at the Memorial Sloan-Kettering Cancer Center in New York. "Everyone sees this as a potential opportunity."

Among the more promising candidates are inhibitors of DOT1L, an enzyme that adds methyl groups to histones, and molecules that may block mutant forms of IDH1 and IDH2. These mutations lead to increased methylation of DNA. About ten drugs targeting epigenetic mechanisms are currently in development, but Armstrong predicts that the number may soon rival that of tyrosine kinase inhibitors, by far the most successful class of leukaemia drugs.

Epigenetics is likely to have a role in cancers beyond leukaemia too. "We're finding out that every other cancer's just like it, but I would argue that leukaemia has been one of the proving grounds," says Ross Levine, an oncologist at the Memorial Sloan-Kettering Cancer Center.

This is in part because it is easier to take a specimen to study leukaemias — by taking blood or sampling bone marrow — than it is for solid tumours.

Leukaemia researchers have seen hints of the role of epigenetics in the disease for decades, with odd patterns of methylation being observed in people with leukaemia since the 1960s. Less clear were the specifics: which epigenetic pathways were disrupted, and through which enzymes? "This was the $64,000 question," says Ley.

MANY MUTATIONS

As the cost of sequencing has fallen precipitously in the past five years, pieces of the puzzle have started to fall into place. Sequencing technology enables researchers to scan exomes — the protein-coding portions of genomes — or even entire genomes and reveal "the complete panoply of mutations present in these patients", Ley says. The data are making it clear that enzymes involved in epigenetics are prime players in leukaemia.

For example, in a study published in May 2013, Ley and his colleagues sequenced the whole genomes of 50 adults with acute myeloid leukaemia (AML) and the exomes of another 150. More than three-quarters of these individuals turned out to have a harmful mutation in an epigenetic enzyme[1]. That is a significant correlation, even without knowing how

common the mutations are in people without AML, Ley says.

One of these enzymes, DNMT3A, adds methyl groups to DNA and was found to be mutated in 51 of the 200 patients. It had already caught Ley's attention. In a 2010 study, his team sequenced it in 281 individuals with AML and found that 62 of them — 22% — carried harmful mutations in it[2]. These individuals were found to have a much shorter survival than AML patients without a DNMT3A mutation.

It is still unclear how these DNMT3A mutations contribute to leukaemia. On their own, they do not seem to change patterns of methylation or gene expression. However, they tend to co-occur with mutations in two other genes, *NPM1* and *FLT3*, and cells with mutations in all three genes have less methylation than controls do. This combination effect "is an important clue that we need to study in detail", says Ley.

Ley's new study also found a strong link with leukaemia and two enzymes, IDH1 and IDH2, which are essential to the Krebs cycle through which cells produce the energy they need to survive. Mutations in these enzymes ramp up DNA methylation, triggering gene expression changes that ultimately lead to cancer. Indeed, Ley and his team found mutations in *IDH1/2* in 39 of the 200 AML genomes they studied.

An *IDH1* cancer connection first surfaced back in 2008, when several research teams found mutations in *IDH1*, along with elevated methylation, in glioblastoma and astrocytoma brain tumours. The following year, Ley found an *IDH1* mutation in the genome of an individual with AML[3].

The initial *IDH1* discoveries "made no sense" because *IDH1* was not known at the time to be involved in epigenetics, says Maria Figueroa, a pathologist at the University of Michigan in Ann Arbor, who helped reveal that role. "How would the Krebs cycle affect the epigenome?"

The answer would emerge only after Figueroa joined forces with others in the field.

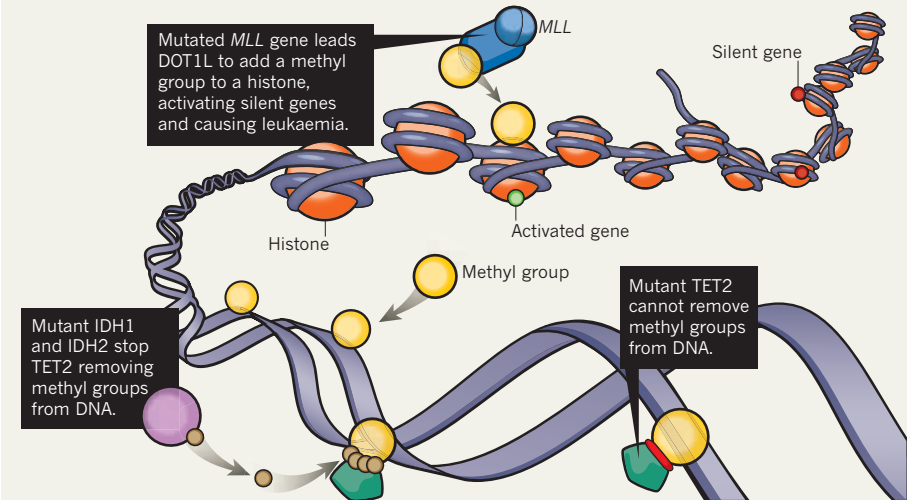
LOOKING FOR PATTERNS

Levine's group at the Memorial Sloan-Kettering Cancer Center was studying *TET2*, a leukaemia-linked gene that removes methyl tags from DNA. He noticed that people with leukaemia never seemed to have mutations in *IDH1* and *TET2* at the same time. The obvious explanation, he says, is that both mutations affect the same pathway, so a cell only ever needs one of them to become cancerous (see 'An on–off switch for genes').

Applying their skills in genetics, epigenetics and biochemistry, Levine's group and Figueroa worked together to show that mutant forms of the IDH1 and IDH2 enzymes stop TET2 from removing methyl groups from DNA[4]. The mutations in the *IDH1* and *IDH2* genes that code for the enzymes cause a change in their function, rather than simply stopping them from being expressed. This makes them good drug targets, Figueroa says.

AN ON–OFF SWITCH FOR GENES

Mutations affecting enzymes that add or remove chemical tags to DNA or its associated proteins can change which genes are expressed, leading to leukaemia.



Mutations in *IDH1* and *IDH2* also seem to be involved in epigenetic modifications to histones. Histone modifications are another rich source of drug targets for leukaemia. Epizyme, a pharmaceutical company based in Cambridge, Massachusetts, has developed a small-molecule inhibitor of DOT1L, which adds methyl groups to histones.

The DOT1L connection to leukaemia can be traced back to 2005, when researchers found that mutations in this enzyme are associated with a fusion gene that is one of the oldest known risk factors for leukaemia[5]. The fusion gene forms when chromosome 11 swaps a large chunk of genetic material with any of a number of other chromosomes, as a portion of the *MLL* gene, located on chromosome 11, is joined to one of more than 60 possible partner genes.

"It's easy to make inhibitors of enzymes. Everyone sees this as a potential opportunity."

Because this fusion is large enough to be visible under a microscope, researchers have been aware of its involvement in AML and acute lymphoblastic leukaemia since the 1990s. What was less clear, however, was how exactly it leads to the disorder.

In 2011, Armstrong and his team showed that many *MLL* fusions activate genes via DOT1L's histone-modifying activity[6]. Histones bind DNA throughout the genome, so tinkering with an enzyme that modifies them would be expected to have broad effects on gene expression. Surprisingly, however, removing the *DOT1L* gene from mice seems largely to affect those genes that the *MLL* fusion modifies, Armstrong found. And Epizyme's DOT1L inhibitor kills only those cells that have the *MLL* fusion gene. This sort of specificity, says Armstrong, is "central to the idea that epigenetics is likely to be a therapy".

It may also make it possible to tailor epigenetic treatments for individuals based on their specific set of mutations. To that end, researchers are trying to pin down mutations and methylation patterns that delineate people into disease subgroups. In 2010, for example, Figueroa and her colleagues looked at how methyl tags are scattered across the genomes of 344 people with leukaemia[7]. They identified 16 distinct patterns of methylation and linked many of them to known leukaemia mutations. Individuals with *MLL* mutations, they found, have fewer methyl groups in a subset of genes than people in the other subgroups. People with *IDH* mutations, in contrast, have excessive methylation.

The technique researchers use to identify methylation patterns — screening the whole genome on a diagnostic microarray — is likely to be too complicated for clinics. But Figueroa's team is developing simpler methods to detect methylation status, which may help clinicians predict prognosis and decide on treatment options. "These are things we can do today, without waiting for novel therapies," she says.

The ultimate goal is a designer drug for each subgroup of methylation patterns. In the meantime, "there is still a tremendous amount of work to be done," says Armstrong. "We are just starting to understand who the players are, what the players do, and how they work together." ■

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