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GENETICS

Below the surface

Cancer-genome analysis has kick-started a revolution in the diagnosis of glioma.

BY SARAH DEWEERDT

In 2016, the World Health Organization (WHO) announced a fresh approach to classifying glioma, the predominant form of brain cancer in adults. Rather than offering a diagnosis mainly on the basis of the appearance of cells in a tumour, as the previous WHO guidelines had done, the updated rubric relies on analysis of the tumour genome.

The WHO scheme sorts people with glioma into diagnostic groups according to the presence of two genetic alterations. One is a mutation in genes belonging to a family that encodes the enzyme isocitrate dehydrogenase (IDH), which helps cells to produce energy. The other is the loss of two specific chunks of the genome, a phenomenon known as a co-deletion. Together, these alterations provide powerful information about a patient's prognosis (people with both an *IDH* mutation and the co-deletion have the best outcome, whereas those with neither are the worst off), as well as clues about which treatments might be suitable.

"Gliomas are a great example of where molecular, genetic diagnosis has really made a difference in patient care," says Robert Jenkins, a cancer geneticist at Mayo Clinic in Rochester, Minnesota. "Knowledge of the different subtypes is way ahead in brain tumours compared to other cancers."

Thanks to numerous large-scale genome-sequencing studies, knowledge of brain-cancer genetics actually goes far beyond the two diagnostic alterations. Mutations in hundreds of genes have been identified in gliomas. Now, the challenges include unravelling the biological mechanisms that cause these changes, understanding which of the changes affect a person's prognosis and response to therapy, and how, and working out why the alterations tend to occur in certain patterns. "It's not enough to just catalogue mutations," says Benjamin Deneen, a cancer biologist at Baylor College of Medicine in Houston, Texas. "It's important to decode what it all means. And we're now in the era of decoding."

CULPRITS IDENTIFIED

Even before molecular analysis of tumour subtypes was available, neuro-oncologists knew that people with a type of glioma called oligodendroglioma tend to respond better to chemotherapy, and have a better prognosis overall, than do those with another type of glioma called astrocytoma. Initially, these two gliomas were differentiated by their appearance and other clinical characteristics: for example, oligodendrogliomas comprise cells with a distinctive shape that is reminiscent of a fried egg, and astrocytomas tend to occur in younger patients.

But making this distinction is more of an art than a science. Gliomas can contain cells that share some characteristics of both. They can also contain a mix of oligodendrocytoma-like and astrocytoma-like cells. Different neuropathologists had different habits of diagnosis: some rarely assigned tumours to the oligodendrocytoma category, whereas others did so readily.

Then, in the late 2000s, researchers led by Bert Vogelstein at Johns Hopkins University in Baltimore, Maryland, discovered mutations in the gene *IDH1* in about 12% of people with a type of glioma known as glioblastoma multiforme (GBM)[1] — usually one of the most aggressive forms of brain cancer. Those who had such mutations were more likely to buck the disease's dismal trend for long-term survival. Propelled by this finding, the team looked at other types of glioma and were again able to identify *IDH1* mutations in a proportion of those tested.

"We found that the patients who have better survival all have the *IDH1* mutations," says Hai Yan, a neuro-oncologist at Duke University School of Medicine in Durham, North Carolina. Soon, mutations in a closely related gene, *IDH2*, were also found to portend longer survival times.

Meanwhile, another predictive marker for glioma was emerging from studies of abnormalities that affect large chunks of the genome. In the early 1990s, researchers led by Jenkins discovered that some gliomas lacked a portion of chromosome 19 (ref. 2). Around the same time, an international group of researchers identified a portion of chromosome 1 that was sometimes missing in such tumours[3]. Both alterations were associated with a better prognosis.

A couple of years later, researchers established that the two almost always occurred together[4] — a pattern that became known as 1p and 19q co-deletion. Jenkins also worked with a team of researchers from the United States and Canada to demonstrate that people with glioma who responded to procarbazine–lomustine–vincristine chemotherapy tended to have tumours that carried the 1p and 19q co-deletion. This became some of the first evidence

that molecular markers in brain cancer could be used to guide decisions on treatment.

CLASSIFICATION CONUNDRUM

The discovery of these molecular markers revolutionized glioma diagnosis and sharpened the once-fuzzy categories that are used to help determine prognosis. "Instead of classifying the patient based on how their slides looked, we can classify them with much greater certainty by molecular alteration," says Cameron Brennan, a neurosurgeon at Memorial Sloan Kettering Cancer Center in New York City.

The updated WHO scheme still takes into account the appearance of cells, but uses genetic markers as a more-precise way of differentiating between tumour types. Generally, oligodendrogliomas must carry both an *IDH* mutation and the 1p and 19q co-deletion. And gliomas with an *IDH* mutation but intact 1p and 19q genomic regions are classified as astrocytomas, as are those that lack an *IDH* mutation.

The molecular classification enables oncologists to more confidently prescribe chemotherapy, radiotherapy or a combination — even if a person's tumour has an intermediate appearance. And, by giving clues about how aggressive a tumour is likely to be, the scheme helps doctors to weigh up the risks and benefits of various surgical strategies.

Confusingly, *IDH* mutation seems to both set gliomas in motion and mitigate their severity. Because several types of brain cancer have *IDH* mutations in common, this alteration could be an early event in tumour development. "*IDH* is probably the gatekeeper gene of brain tumours," says Yan.

However, researchers are not sure how *IDH* mutation contributes to making cells malignant. They know that mutant IDH enzymes spur the massive overproduction of a metabolite called 2-hydroxyglutarate. This compound broadly alters patterns on DNA of epigenetic markers — molecular 'switches' that can turn genes on or off. But with so many such changes occurring in tumours, it can be difficult to tease out those that are most important for tumour formation, let alone determine which factors push some tumours with an *IDH* mutation to become oligodendrogliomas but others astrocytomas.

Nevertheless, the importance of *IDH* mutation in glioma and other cancers has spurred efforts to develop drugs that block the mutant IDH enzymes. The hope is that such drugs could help to prevent the subsequent epigenetic changes, enabling cells to differentiate normally. A drug that targets mutant IDH2 was approved for use in people with acute myeloid leukaemia in 2017. So far, solid tumours have proved more difficult to address, but phase I and phase II trials of at least five drugs directed at IDH1 or IDH2 in people with brain tumours are under way.

TALE OF THE TELOMERE

Large-scale genomics studies have identified hundreds of genetic alterations in brain cancer. "So now, we have a large collection of knowledge of what you can find in the patient's tumour," says Sidi Chen, a geneticist at Yale School of Medicine in West Haven, Connecticut. He and his colleagues are now trying to work out the importance of those mutations, so that they might be used to guide personalized treatment decisions.

Chen is applying the gene-editing tool CRISPR–Cas9 to a mouse model for his investigations. The technology, says Chen, enables him to "zoom in to which genes and genetic combinations are more important than the others". In 2017, Chen's team reported that mutations in two genes, *Zc3h13* and *Pten*, can make cancer cells resistant to a common chemotherapy drug[5]. It also identified pairs of mutations that are sufficient to cause GBM.

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Cancer cells must have a mechanism for maintaining structures known as telomeres, which are found at the ends of chromosomes and have a role in cell ageing. In normal cells, telomeres shorten with time, until the cells are no longer able to divide. But in tumours, telomeres remain long, conferring immortality on cells.

Some GBM tumours carry mutations in a region of DNA called the TERT promoter. These mutations lead to overexpression of the catalytic subunit of telomerase, an enzyme that adds DNA repeats to the ends of telomeres to keep them intact. Others carry a mutation in the gene *ATRX*, which leads to a phenomenon known as alternative lengthening of telomeres (ALT).

These two mechanisms contribute to telomere maintenance in many forms of cancer. However, scientists were unsure how telomeres are maintained in GBM tumours that do not carry mutations in the TERT promoter or *ATRX*. This year, Yan's group found that such tumours can have chromosomal rearrangements that disrupt the gene *TERT* (ref. 6), producing another route to telomerase overexpression. They also uncovered another gene, called *SMARCAL1*, that can drive the ALT process when mutated.

Together, these four genetic alterations can explain telomere maintenance in almost all GBM tumours. And Yan thinks that his results will open up the possibility of personalized treatments that can target each tumour's specific telomere-related genetic abnormality. "Every patient: you have an answer for them," he says. "It's amazing."

FAMILY HEIRLOOMS

A further piece of the brain-cancer genetics puzzle is inherited risk. Genome-wide association studies have identified specific variations that are associated with developing the disease. About 40% of people with oligodendrogliomas or astrocytomas with an *IDH* mutation carry a variation known as a single nucleotide polymorphism (SNP) in the 8q24 region of the genome. Another SNP that seems to increase the risk of *IDH*-mutant brain cancer is found in the 11q23 region. But little is known about the mechanisms behind these associations.

About 5–8% of gliomas are familial, which means that they occur in people with at least one other close biological relative who has had a glioma, says Melissa Bondy, an epidemiologist at Baylor College of Medicine. In 2014, Bondy's team identified the first gene to be associated with familial glioma, *POT1* (ref. 7). Her team has found *POT1* mutations in 6 of the almost 300 families with glioma that it has studied, a finding that Bondy describes as a "partial home run". It has also identified almost 20 other genes that might contribute to inherited risk.

Familial and non-familial gliomas seem to involve similar disease mechanisms. "It's occurring at slightly younger ages" in people with a family history of the cancer, Bondy says. But, "When we look at the mechanism that's involved in tumorigenesis of glioma, it looks like there's not a difference."

Bondy hopes to recruit more families affected by glioma to find out how *POT1* and the other genes she has identified influence a person's prognosis and response to treatment. To learn more about how these genes contribute to brain-tumour formation, she is collaborating with Deneen, who has developed a CRISPR–Cas9 mouse model to evaluate the effects of various mutations in the same gene on the development of glioma.

Deneen says that this research challenges the 'hotspot' model of cancer genomics, in which genes that are mutated in many forms of cancer are presumed to be important for all of them. Instead, "Variants that drive in one form of cancer don't necessarily drive in other forms of cancer."

Moreover, two variants of the same gene might behave in very different ways — one might be important for making a cell become malignant, whereas the other is just along for the ride. Or two variants in the same gene might produce cancers with divergent characteristics. "We can look at, basically, a single amino-acid difference and we can see drastic changes," Deneen says. That suggests that an era of even deeper decoding of glioma-associated mutations lies ahead. ■

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