Biotechnology

High-throughput genetic screens in brain organoids

Adriana Cherskov & Nenad Sestan

A high-throughput technique has been developed to screen genes implicated in neurodevelopmental diseases in 3D cell cultures. It reveals a mechanism that might be involved in a rare disorder called microcephaly.

Thousands of genetic variants increase the risk that a person will be affected by a major disorder of the nervous system, such as improper brain growth[1–3]. Understanding the effects of these variants could reveal disease mechanisms and point to possible drug targets. But studying each variant individually is a slow and arduous process. Moreover, the complexity of the human brain makes such studies difficult. Writing in *Science*, Esk *et al.*[4] outline a high-throughput *in vitro* screening approach that can overcome these obstacles. The authors then use their screen to analyse genes associated with a rare developmental condition called microcephaly.

In microcephaly, the brain — in particular, its outer part, known as the cerebral cortex — is significantly smaller than usual[1–3]. The condition is the result of decreased proliferation of neural stem cells or increased cell death in the developing brain. Microcephaly has been associated with a range of genes and genetic syndromes[1–3]. Determining whether each of these genes is really involved in microcephaly requires high-throughput technologies, but existing screens have limitations. For instance, those that use animals can be problematic because equivalent mutations in different animal models often have different effects[5], and because genes associated with neurodevelopmental disorders can be expressed differently in different species. And 2D cell-culture systems cannot fully capture processes such as interactions between the various types of neuron and glial cell found in 3D tissues, which are crucial for normal cell proliferation.

But 3D cell-culture systems called organoids, in which stem cells can produce brain-like structures, provide a powerful way to overcome the problems of using animal models and 2D cultures[6]. Organoids form brain-like tissues and can recapitulate key cellular and gene-expression features of human cortical development from early to mid-gestation[6,7]. However, mimicking complex tissues in 3D is a double-edged sword for high-throughput screens. Variability in growth rates in a diverse population of cells makes it difficult to determine whether differences in cell survival or proliferation are due to the nature of the experiment or to the inherent variability of the organoids.

Esk *et al.* set out to overcome this issue by combining brain organoids with a new method based on the gene-editing technique CRISPR–Cas9. In CRISPR–Cas9, a guide RNA (gRNA) is designed to bind to the DNA sequence that is to be edited. The Cas9 enzyme then binds to the gRNA and makes a break in the DNA. Often, this DNA break is incorrectly repaired by the cell, which can result in gene 'knockout', meaning that the gene of interest is no longer translated into a functioning protein. Esk and colleagues designed gRNAs to target 173 genes that are implicated in microcephaly, and tagged every gRNA with a unique DNA sequence called a lineage barcode. They then infected stem cells with these barcoded gRNAs. Each cell that receives a given gRNA, and all its descendants, carry the same barcode, making it easy to identify cell lineages. The stem cells were cultured in 3D for up to 40 days, to form a brain organoid, and the DNA from all the cells in the organoid was then extracted and pooled.

The aim of the screen was to count the number of lineage barcodes present for each gene of interest, and to compare them with the number of lineage barcodes from a control gRNA that did not mutate any gene. Depletion of lineage barcodes for a candidate gene compared with the control would indicate that this gene is necessary for proper cell proliferation. However, it is difficult to detect small numbers of lineage barcodes (that is, those from small lineages) during analysis. Greater resolution is required to be sure of detecting cases of the moderate cell loss that is typical of microcephaly. Moreover, in a 3D brain organoid, baseline lineage sizes vary widely, as discussed above.

To overcome these problems, Esk *et al.* added a second barcode to each cell during DNA processing, thereby labelling the DNA of each cell in a lineage individually (Fig. 1). Barcoding cells individually allowed the researchers to track exactly how many cells are in each lineage. Together, the dual barcodes account for the variability in organoid-cell growth (multiple gRNAs are used for each gene, enabling trends to be reliably detected), and barcoding each cell makes it possible to detect changes in proliferation for small lineages, so it is easier to identify

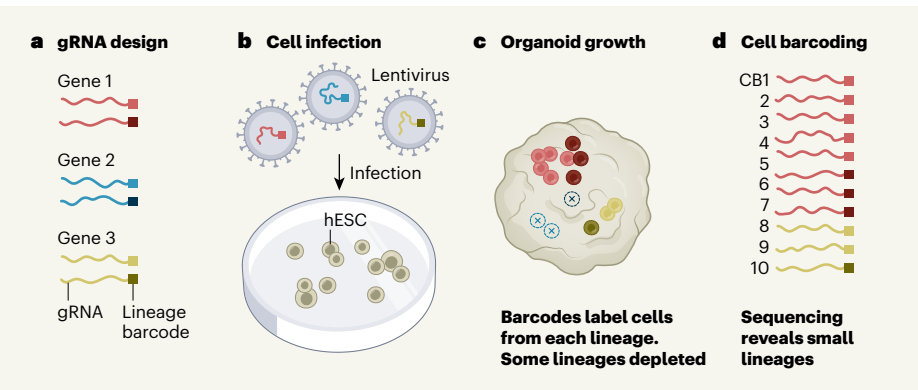

Figure 1 | A gene-editing and lineage-tracing approach called CRISPR-LICHT. Esk *et al.*[4] used their CRISPR-LICHT approach to analyse how genetic mutations can alter cell numbers in the brain. **a**, The authors designed guide RNA (gRNA) molecules that each bind to one of 173 genes of interest. They labelled each gRNA molecule with a unique DNA sequence called a lineage barcode (LB). **b**, Each gRNA was then packaged into a lentivirus (only three are shown here, for simplicity), and the lentivirus pool was used to infect human embryonic stem cells (hESCs). **c**, The cells were placed in 3D culture and the enzyme Cas9 induced (this enzyme targets the gRNAs, causing them to delete genes in each infected cell; not shown). The cells were cultured for up to 40 days to produce complex 3D brain organoids. Some lineages grew rapidly, whereas others were depleted (crosses). **d**, The organoids were collected and the cells containing Cas9 selected (not shown). The DNA from these cells was amplified, and a second unique DNA barcode (a cell barcode, CB) was added. The DNA was then sequenced for gRNAs and barcodes. The LBs allow accurate measurements of the size of each lineage, and the CBs increase the ability to pick out small lineages. If lineages containing gRNAs for one gene are consistently small (here, genes 2 and 3), the gene is likely to have a role in cell proliferation in the brain.

cases where gene deletions cause moderate cell loss. The group named their technique CRISPR-lineage tracing at cellular resolution in heterogeneous tissue (CRISPR-LICHT).

The researchers ranked the lineage-barcode depletion of the 173 genes thought to pose a risk of microcephaly, and examined the top 32 genes in further detail. They investigated the role of these genes in regulating cell proliferation by using a mixing strategy, in which they grew organoids from a combination of control cells and cells that had been genetically edited to lack the gene, and analysed the ratio of control to edited cells in the fully grown organoids. Finding significantly reduced numbers of edited cells suggests that deletion of the gene causes reduced proliferation. Of the 32 genes studied, 25 were found to be implicated in cell proliferation.

Finally, Esk *et al.* focused on one particular gene of the set, which encodes immediate early response 3 interacting protein 1 (IER3IP1). When cells were engineered to lack this gene, the resulting organoids were smaller than the controls. Analysis of organoids lacking this gene revealed that it seems to regulate the unfolded protein response — a cellular response to stress in an intracellular organelle called the endoplasmic reticulum that leads to reduced protein synthesis. Treatment of cells with a small molecule called the integrated stress response inhibitor, which restores protein synthesis, led to normal size and organization of neural progenitors in the organoids. These findings are particularly interesting because they tie the stress-induced unfolded protein response, which has previously been associated with Zika virus-related microcephaly[8], to a genetic cause of the disorder. The experiments highlight the enormous potential of CRISPR-LICHT to investigate the mechanisms that underlie neurodevelopmental disorders.

Although Esk and colleagues focused specifically on genes associated with microcephaly, their cost-effective and time-saving method will be widely applicable to other neurodevelopmental disorders. The Developmental Brain Disorder Gene Database (go.nature.com/3mezn8d) lists about 600 genes whose deletion is associated with neurodevelopmental disorders such as autism, schizophrenia and epilepsy. These genes can now be screened by CRISPR-LICHT.

The approach could also be used to study genes that are under- or overexpressed in neurodevelopmental disorders, by adapting it to modify, rather than eliminate, gene expression. Furthermore, the dual-barcoding techniques of CRISPR-LICHT could be used to create and track genetically distinct populations of cells, to model the effects of genetic variations among cells in the developing brain[9]. Finally, the approach could be used to analyse genes associated with cancer.

There are some limitations, however. CRISPR-LICHT is most suited to studies of early development or cell proliferation. The cerebral organoids in the current study were grown for 40 days, corresponding to development of the human brain early in the first trimester, so neurons and glia that develop and mature later were not considered. Using longer differentiation times would increase the turnaround time. Furthermore, current protocols for organoid growth might activate cellular stress pathways, impairing cell-type specification and fidelity[10]. Finally, not all of the cortex is the same, with different regions having cell types and connections that serve different functions. More-precise protocols would be required to model this complexity and regionalization.

Beyond clinical studies, the CRISPR-LICHT platform could be applied to study the genetic changes and mechanisms underlying the evolution of the healthy human brain. Evidence indicates that the expansion and folding of the human cortex depend on human-specific properties of early neuronal development[11–13]. Using brain organoids to screen genes and other DNA elements that are unique to humans, or that exhibit a distinct pattern of activity in humans[14], could be a powerful way of connecting genes to traits in the context of human neural development.

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Information technology

Artificial intelligence accelerated by light

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The explosive growth of artificial intelligence calls for rapidly increasing computing power. Two reported photonic processors could meet these power requirements and revolutionize artificial-intelligence hardware. **See p.44 & p.52**

Artificial intelligence (AI) is transforming various fields, such as clinical diagnosis, autonomous driving and speech translation. However, the quickly increasing volume of data in modern society poses great challenges for the electronic computing hardware used in AI, in terms of both computing speed and power consumption. Such issues have become a major bottleneck for AI. On pages 44 and 52, respectively, Xu *et al.*[1] and Feldmann *et al.*[2] report photonic processors that accelerate AI processing by harnessing the distinctive properties of light. These demonstrations could inspire a renaissance of optical computing.

With the rise of AI, conventional electronic computing approaches are gradually reaching their performance limits and lagging behind the rapid growth of data available for processing. Among the various types of AI, artificial neural networks are widely used for AI tasks because of their excellent performance. These networks perform complex mathematical operations using many layers of interconnected artificial neurons[3]. The fundamental operation that uses most of the computational resources is called matrix–vector multiplication.

Various efforts have been made to design and implement specific electronic computing systems to accelerate processing in artificial neural networks. In particular, considerable success has been achieved using custom chips known as application-specific integrated circuits[4], brain-inspired computing[5] and in-memory computing[6], whereby processing is performed *in situ* with an array of memory devices called memristors.

Electrons are the carriers of information in electronic computing, but photons have long been considered an alternative option. Because the spectrum of light covers a wide range of wavelengths, photons of many different