CRISPR-CAS9 AND ITS CLINICAL APPLICATIONS

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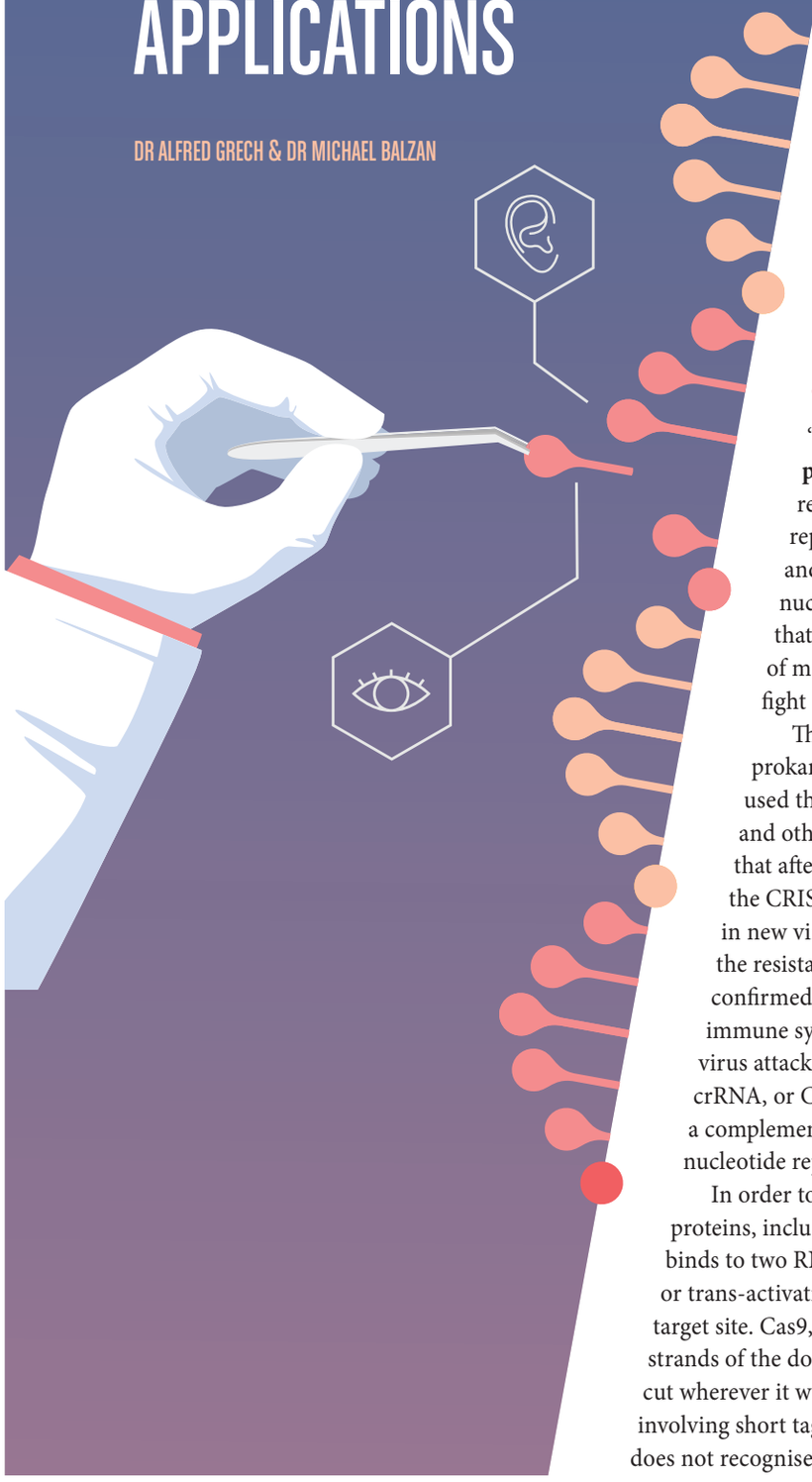

ABSTRACT

CRISPR-Cas9 is a powerful and simple tool for editing genomes. It allows researchers to alter DNA sequences and gene function. Its potential applications include correcting genetic defects and treating and preventing the spread of diseases. In China, clinical human trials using CRISPR-Cas9 are now in progress.

INTRODUCTION

CRISPR (pronounced "crisper") and its therapeutic use in human cell lines is revolutionising biomedical research. In particular, this is because CRISPR makes it straightforward to edit or inactivate genes in a cell line. It also simplifies the approach of creating animal models that are then used to explore diseases. Indeed, research work, which took months or years, can now be completed in weeks.

THE KEY PLAYERS

"CRISPR" stands for "**c**lusters of **r**egularly **i**nterspaced **s**hort **p**alindromic **r**epeat". In detail, CRISPR is a bacterial DNA region made up of two specialised characteristics: (i) nucleotide repeats that are distributed throughout the CRISPR region, and (ii) spacers, bits of DNA that are interspersed among the nucleotide repeats. In general, the spacers are taken from viruses that have attacked the bacteria. Indeed, spacers are used as a bank of memories, therefore allowing bacteria to recognise viruses and fight off future attacks.

That CRISPR provides acquired resistance against viruses in prokaryotes was demonstrated over a decade ago.[1] Here, researchers used the *Streptococcus thermophilus* bacterium, found in yoghurt and other dairy products, as their model. Barrangou *et al.* showed that after a virus attacks a bacterium, new spacers are introduced into the CRISPR region. In addition, by taking out the spacers or putting in new viral DNA sequences, the researchers were able to change the resistance of the bacterium against a particular virus. It was thus confirmed that CRISPRs have a role in the regulation of the bacterial immune system. Indeed, after introducing a spacer, a subsequent virus attack causes a portion of the CRISPR to be transcribed into crRNA, or CRISPR RNA. In this case, the CRISPR template produces a complementary sequence of RNA, with each crRNA consisting of a nucleotide repeat and a spacer portion.[2]

In order to stop foreign attacks, bacteria use CRISPR RNA and Cas proteins, including Cas9. Cas9 is an enzyme that cuts foreign DNA. It first binds to two RNA molecules: the crRNA, and another one called tracrRNA, or trans-activating crRNA. Both RNAs then guide Cas9 to the cleavage target site. Cas9, using two separate domains on its structure, cuts both strands of the double helix, resulting in a double strand break.[2] Cas9 does not cut wherever it wants in the genome. Instead, there is a built-in mechanism involving short tags known as PAMs ("protospacer adjacent motifs"): if Cas9 does not recognise a PAM next to its target site, it does not cut.

GENOME EDITING TOOL

Genomic DNA holds instructions for all living things. CRISPR-Cas9 provides the means to alter these instructions. It does this through a break or a cut in the DNA therefore circumventing the repair mechanisms and introducing the alterations. It was this knowledge that fuelled the spiralling interest in CRISPR-Cas9. In principle, the revolution began in 2012 with two research papers describing how the bacterial CRISPR-Cas9 can be transformed into a simple genome-editing tool. Jinek *et al.*[3] and Gasiunas *et al.*[4] concluded that Cas9 could cut any DNA region if the nucleotide sequence of the crRNA is changed. Jinek *et al.* went further and fused crRNA and tracrRNA to create a single guide RNA (gRNA). Overall, genome editing requires the Cas9 protein and a gRNA.

Operationally, a stretch of 20 base pairs that matches the gene to be edited is designed. Subsequently, an RNA molecule that is complementary to the 20 bp stretch is constructed. Just like a pair of scissors, Cas9 and the RNA will then cut the DNA. In the end, the cell's natural repair mechanisms will work (e.g. through non-homologous end joining, or NHEJ) to introduce the changes in the genome.

CLINICAL APPLICATIONS

It was a matter of time until the bacterial CRISPR-Cas9 was used in a clinical setting.[5] So far, it has been used to ameliorate muscle function in mice with Duchenne muscular dystrophy.[6] In particular, using CRISPR, researchers deleted the defective gene, allowing the mice to produce one of the main proteins in the muscles. CRISPR is also being exploited to treat HIV,[7] since it can attack multiple regions of the viral DNA, therefore making the development of virus resistance harder. In turn, this can decrease the chance of viral escape and resistance to treatment. In the field of cancer, CRISPR is being applied to turn on and off genes implicated in the development of cancer, to inspect the protein domains involved in cancer, and also, to evaluate the drug targets.[8]

In vitro and animal models of human disease have also demonstrated that CRISPR-Cas9 can be effective in the correction of genetic defects, thus paving the way for clinical therapeutic applications in humans. Yuan *et al.*,[9] for example, investigated the role of αA-crystallin in rabbits. αA-crystallin increases cellular stress tolerance and prevents precipitation of denatured proteins.[10] It is these functions that maintain eye lens transparency and prevent cataracts.[11] Yuan *et al.* demonstrate that mutations in αA-crystallin are linked to the formation of cataracts. In detail, it was shown that a CRISPR-Cas9 mutation of αA-crystallin causes congenital cataracts, failed differentiation of lens fibres, microphthalmia, and obscurity. In light of this, further investigations should pursue the association between mutations in the αA-crystallin gene and congenital cataracts in humans.

CRISPR-Cas9 is also being used to understand cystic fibrosis, an autosomal recessive, chronic, genetic disease of the lung caused by mutations in the cystic fibrosis transmembrane regulator (CFTR). Sanz *et al.*,[12] for instance, describe an efficient method for editing three different and rare CFTR mutations, which together account for 3% of patients suffering from cystic fibrosis. In a similar method, Schwank *et al.*[13] used CRISPR-Cas9 to correct the CFTR locus through homologous recombination in intestinal stem cell organoids of patients.

In addition to cataracts and cystic fibrosis, CRISPR-Cas9 studies have also been carried out on Fanconi anaemia,[14] hearing loss,[15] haemophilia,[16] leishmaniasis[17] and malaria.[18] For example, hearing loss affects about 1 in 500 newborns. It is known that genetic deafness is often due to mutations of the inner ear genes. Using CRISPR-Cas9, the roles of these genes can be studied through the disruption of normal gene alleles via the NHEJ mechanism. In particular for genetic hearing loss, CRISPR-Cas9 can disrupt mutations through NHEJ, or repair mutations via homology-directed-repair (HDR), both of which could restore hearing. Zou *et al.*[15] have shown that genome editing is an efficient tool in the mammalian inner ear in vivo.

In situ genome editing has also resulted in successful correction of haemophilia, an X-linked genetic bleeding disorder due to a lack in coagulator factor IX (haemophilia B). In the human F9 gene, Guan *et al.*[16] identified a new mutation (Y371D) in haemophilia B. Using CRISPR-Cas9 to generate transgenic mice, they confirmed that this novel mutation results in severe haemophilia. Guan *et al.* proceeded to develop therapeutic strategies targeting this mutation.

In regard to the parasitic *Leishmania donovani* that causes leishmaniasis disease, Zhang *et al.*[17] exploited the CRISPR-Cas9 tool to specifically mutate the parasitic genome. In this case, high-throughput functional analysis can be used to understand its functional genes, thus promoting the discovery of future therapeutic strategies. In a similar approach, but for a different parasite, Hammond *et al.*[18] used CRISPR-Cas9 to target female reproduction in the malaria mosquito vector *Anopheles gambiae*. In addition, in the agricultural and food industries, CRISPR-Cas9 has been applied to vaccinate industrial cultures (e.g. for yoghurt) against viruses.[19] Phage infection of starter cultures is a widespread and significant problem in the dairy industry. The process of applying CRISPR-Cas9 is simply to select those bacterial strains that make the best yogurt, expose them to

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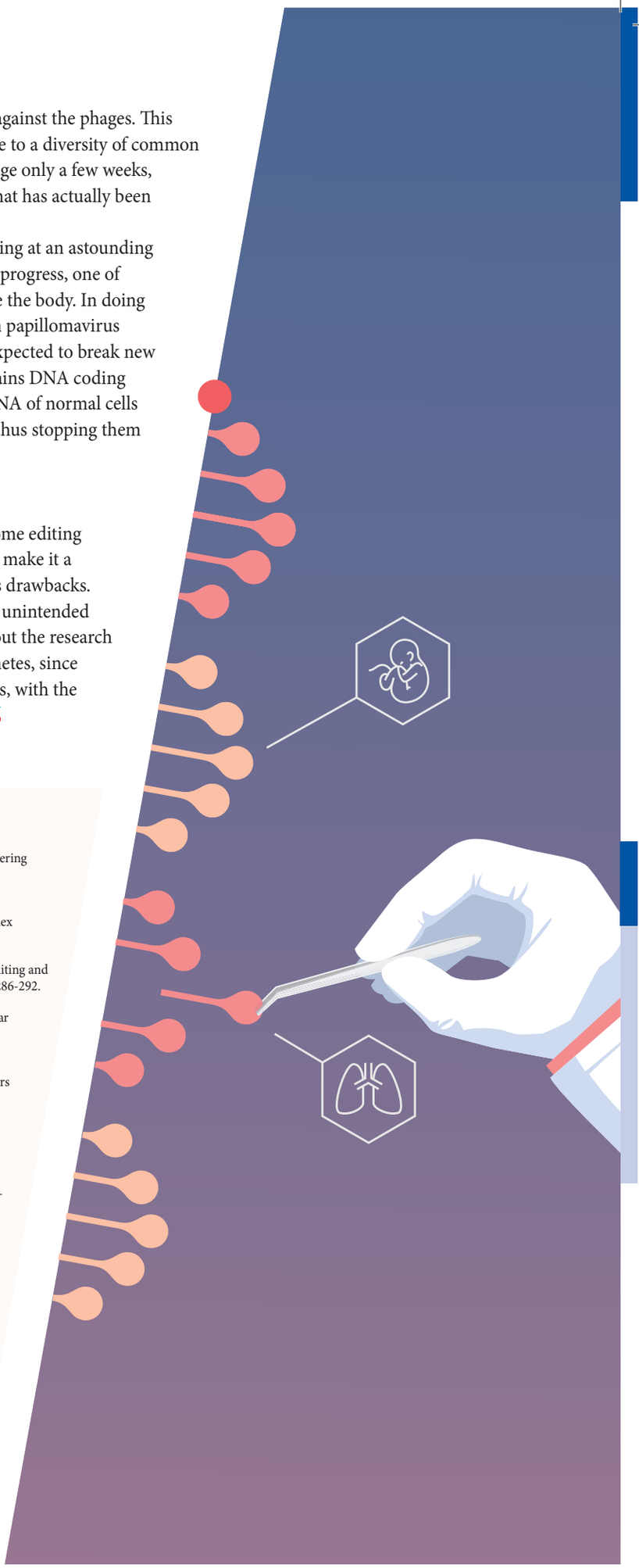
phages and screen for strains that become naturally vaccinated against the phages. This process is repeated until you end up with a strain that is immune to a diversity of common phages. In view of the fact that this entire process takes on average only a few weeks, many people have possibly unknowingly consumed a product that has actually been manufactured using CRISPR-enhanced starter cultures!

In general, the CRISPR genome editing revolution is advancing at an astounding pace. In China, for instance, 20 clinical human trials are now in progress, one of which will use CRISPR, for the first time ever, to edit cells inside the body. In doing so, the aim is to prevent cervical cancers by targeting the human papillomavirus (HPV) genes that cause the tumour to grow. This HPV trial is expected to break new ground. Instead of editing cells outside the body, a gel that contains DNA coding for CRISPR is applied to the cervix. CRISPR should leave the DNA of normal cells untouched, however, it should destroy cells infected with HPV, thus stopping them from turning cancerous.

CONCLUSION

Overall, the robustness and simplicity of the CRISPR-Cas9 genome editing in human cells and model organisms such as mice and primates make it a promising tool in clinical research. However, it is not without its drawbacks. Indeed, if the DNA is cut at sites other than the intended target, unintended mutations are introduced. CRISPR has also raised questions about the research ethics of human genome editing, especially in embryos and gametes, since these can be passed on to subsequent generations. In spite of this, with the progress seen to date, it is clear that this is just the beginning.



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