WHITE PAPER

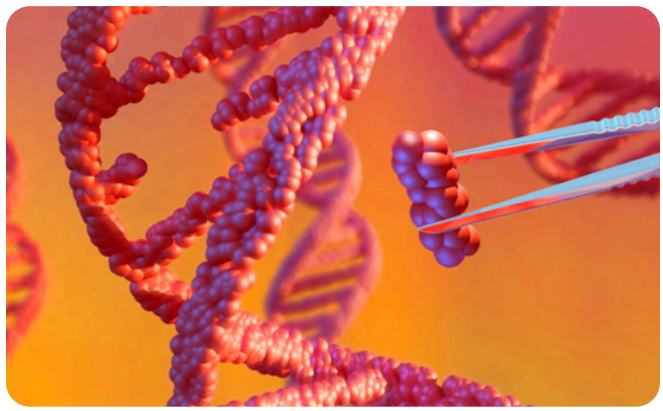
CRISPR-derived base editing technology: The what, the how, and the why

Introduction

Base editing and CRISPR are two genetic engineering techniques that are utilized to modify the DNA sequence of a cell. CRISPR-Cas9 is a gene editing technology that uses the Cas9 enzyme to cleave the DNA at a specific location, creating a double-stranded break (DSB) that can lead to unpredictable and unwanted mutations. While CRISPR-Cas9 has a high success rate in introducing new sequences into the genome, it is also associated with unpredictability and unintended mutations.

On the other hand, base editing is a more precise method that allows for the direct modification of single nucleotides within the DNA sequence without requiring a DSB. This technique uses a deaminase enzyme to chemically modify a particular base, converting one nucleotide to another. Base editing allows for the modification of a single base pair and does not involve the addition or deletion of any nucleotides, making it a reliable technique for precise genetic engineering.

Base editing has several advantages over traditional gene editing approaches, including greater precision and efficiency, reduced off-target effects, and a lower risk of causing unintended mutations, translocations, or damage to the genome. Genetic conditions including sickle cell anemia, Hutchinson-Gilford progeria syndrome, and Leber congenital amaurosis all stem from a single point mutation allowing base editing to be a useful approach for understanding these conditions.[1] The evolution of CRISPR-based techniques such as base editing has helped to address the historic lack of curative intervention within the gene therapy space.



What is base editing?

Base editing was discovered in 2016 by two separate research groups, one led by Dr. David Liu at the Broad Institute and Harvard University, and the other led by Dr. Akihido Kondo at Kobe University.[2] There are two primary classes of base editors: cytosine base editors (CBEs) and adenine base editors (ABEs).[3] CBEs were the earliest base editing technology and used cytosine deaminase to convert cytosine to uracil, which has the base pairing properties of thymine. ABEs, which were developed after successful CBE testing, use adenine deaminase to alter adenine to inosine, which has the pairing properties of guanine. Together, these classes of base editors offer the potential to correct genetic errors associated with all forms of transitional mutations, which could have far-reaching implications for cell and gene therapy.

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Recent studies have demonstrated the feasibility of base editing in mammalian cells, including in human embryos.[4] One advantage of base editing over traditional gene editing tools like CRISPR-Cas9 is that it avoids the potential for off-target effects, which can lead to unintended changes in the genome.[1] However, further research is needed to improve the specificity and efficiency of base editing, as well as to address safety concerns related to the potential for unintended consequences.[5] Base editing is a relatively new technology, and its potential applications are still being explored.

How does base editing work?

Base editing enables precise changes to be made to a single DNA base, without the need to cut both strands of DNA. Base editing employs key components of the CRISPR system, including a guide RNA and a catalytically impaired Cas9, and a deaminase enzyme, to facilitate the base edit. Instead of a wildtype Cas9 enzyme that makes a DSB, base editors create a single-stranded break (nick) at the target site, resulting in more precise edits.[4] The nicked DNA is then repaired in a way that eliminates the need for endogenous non-homologous end joining (NHEJ) and homology-directed repair pathways, which can sometimes lead to non-specific outcomes from indels.[5]

The guide RNA serves as a molecular guide to direct the deaminase enzyme to the specific location within the DNA sequence that needs to be modified.[4] It is designed to bind to a specific DNA sequence at the target nucleotide and forms a complex with the Cas-deaminase fusion.

Once the target sequence is located, the deaminase enzyme chemically converts a specific nucleotide into a different one, such as from cytidine to thymidine while the opposite DNA strand is nicked. The deaminase enzyme achieves the modification by removing an amine group from the cytosine base, which converts it to uracil. Uracil is not a natural base in DNA but is a natural base in RNA.[4] Thus, the DNA repair machinery recognizes it as an error and replaces it with the correct base. This process results in a precise, single-nucleotide change within the DNA sequence without creating a DSB. By adjusting specific base pairs, base editors can make targeted, isolated changes in the DNA sequence with higher specificity than the CRISPR-Cas9 system.

Application of base editing in gene and cell-based therapies

The versatility of base editors applies to both dividing and terminally differentiated cell types. It is estimated that for SNV or point mutations, 37% or 4,000 genetic disorders could be corrected using base editing technology.[1] CBEs are capable of gene editing in conditions including, phenylketonuria, deafness, Niemann-Pick disease, hereditary hypercholesterolemia, sickle cell anemia, and beta thalassemia.[1] ABEs may be effective for the treatment of Duchenne muscular dystrophy, cystic fibrosis, progeria, sickle cell anemia, and beta thalassemia. Both base editors can help to make more efficient cell therapies against cancer.[1] *In vitro* and studies in animal models have illustrated proof-of-concept and offer the potential for one-time treatment correcting single nucleotide variant (SNV) errors that give rise to a myriad of diseases of genetic origin.

Base editing has been used in human cell culture experiments to alter mitochondrial DNA, a capability CRISPR-Cas9 lacked due to the absence of an RNA transport mechanism.[1] The process of base editing has potential application in treating Leber's hereditary optic neuropathy, a form of hereditary blindness characterized by the loss of retinal cells, through the correction of mitochondrial DNA mutations.[1] Furthermore, certain genetic mutations that result in elevated levels of cholesterol and triglycerides have been linked to an inherited form of hypercholesterolemia, which in turn increases the risk of cardiovascular disease. Base editing could potentially correct such mutations and provide a means of treating this condition.[5] Researchers have discovered that inhibiting two genes, PCSK9 and ANGPL3, resulted in lower circulating levels of cholesterol in a non-human primate study, and are therefore being investigated as potential targets for genetic intervention.[5]

Genetic corrections in sickle cell anemia and beta-thalassemia have been performed using base editing technology to alter hematopoietic stem cells and progenitor cells in fetal development without the need for DSB editing capabilities.[1]

A challenge for cell and gene therapy is the mechanism of delivery, which depending on the disorder of interest, will pose different challenges requiring creative solutions to ensure the safety and efficacy of treatment in a clinical setting. Still, base editing as a technological advancement built on the foundation of CRISPR-Cas9 brings the promise of potentially curative solutions for complex and point mutation-derived genetic diseases.

Conclusion

The development of base editing technologies has generated hope for more highly specific and efficacious gene editing treatments to be developed in the cell and gene therapy space. The high specificity of base editing has unlocked a greater potential for safe genetic intervention, building upon the foundation of CRISPR-based therapeutics with increased gene editing opportunities compared to its originator. The need for further safety and efficacy testing to evaluate the clinical implications of its capabilities is paramount to moving toward clinical testing.

References

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Revvity's Pin-point™ Base Editing Platform

Revvity's Pin-point™ base editing platform is a genetic engineering tool that enables precise and efficient base editing of the genome. The Pin-point platform offers flexibility and versatility for a wide range of research and therapeutic applications. Furthermore, the Pin-point system uniquely enables and simplifies challenging multiplex knockout/knock-in projects in a single transfection.

With Pin-point base editing, researchers can introduce precise single-base changes to the genome without relying on double-stranded breaks in the DNA. These changes can result in gene correction, activation, or silencing. The platform offers high editing efficiency and minimizes off-target effects. The base editor can be customized to target specific genomic loci, and users can choose from a variety of deaminase enzymes to achieve the desired nucleotide conversion.

The Pin-point platform is compatible with a broad range of applications and can be delivered using various methods, including electroporation, lipid-mediated transfection, or lentiviral transduction.[6] The platform also allows for the simultaneous targeting of multiple sites, making it suitable for applications such as gene regulation and synthetic biology. Pin-point base editing is supported by Revvity's scientific expertise and customer support. Whether for basic research or developing new therapies for genetic diseases, the Pin-point platform provides the necessary precision, efficiency, and flexibility to succeed.

Learn more

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