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REVIEW

Gene Editing: An Effective Tool for the Future Treatment of Kidney Disease

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Abstract: Gene editing technology involves modifying target genes to alter genetic traits and generate new phenotypes. Beginning with zinc-finger nucleases (ZFN) and transcription activator-like effector nucleases (TALEN), the field has evolved through the advent of clustered regularly interspaced short palindromic repeats and CRISPR-associated protein (CRISPR-Cas) systems, and more recently to base editors (BE) and prime editors (PE). These innovations have provided deep insights into the molecular mechanisms of complex biological processes and have paved the way for novel therapeutic strategies for a range of diseases. Gene editing is now being applied in the treatment of both genetic and acquired kidney diseases, as well as in kidney transplantation and the correction of genetic mutations. This review explores the current applications of mainstream gene editing technologies in biology, with a particular emphasis on their roles in kidney disease research and treatment of. It also addresses the limitations and challenges associated with these technologies, while offering perspectives on their future potential in this field.

Keywords: clear cell renal cell carcinoma, ccRCC, clustered regularly interspaced short palindromic repeats, CRISPR, gene editing, genetic kidney diseases, polycystic kidney disease

Introduction

Gene editing technology modifies target genes to alter genetic traits and generate new phenotypes. Since its introduction in 2012, this technology has experienced global growth, marking a pivotal advancement in molecular biology and driving rapid progress in bioengineering, agriculture, and medicine. Following the traditional gene editing, the development of base editor (BE) and primer editor (PE) has solved the limitations of earlier technologies. By improving the structure of deaminase, optimizing RNA design, and using RNP methods to reduce off-target effects, they have improved their efficiency and stability. Demonstrating the potential of new technologies for the ability to edit non-dividing cells is a major advance.

Chinese scientists conducted the world's first clinical trial of clustered regularly interspaced short palindromic repeats and CRISPR-associated protein (CRISPR-Cas) technology in 2016 and confirmed its safety in 2020. That same year, Jennifer A. Doudna was awarded the Nobel Prize for her groundbreaking contributions to gene editing, underscoring the significance of this technology and its vast potential for diverse applications.

Kidney diseases have remained a major challenge for the medical community when it comes to treatment, the main refractory causes include genetic factors, neoplastic diseases, immune diseases, metabolic diseases, etc. These patients often enter chronic kidney disease (CKD) early or even rapidly progress to end-stage renal disease (ESRD). In 2017, the prevalence of CKD was estimated to be 9.1%.[1] The Global Burden of Disease Study 2019 reported that there were about

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697 million CKD patients worldwide.[2] By 2020, the WHO report showed that CKD had become one of the top ten causes of death in the world.[3] Current treatments for kidney disease include drugs, dialysis, and kidney transplantation. However, due to the limited efficacy of these measures and the frequent complications, new treatments for kidney disease are needed. Among them, complex kidney diseases represented by hereditary kidney disease (such as polycystic kidney disease) and neoplastic kidney disease (such as clear cell renal cell carcinoma) have always lacked effective treatment methods. Conventional treatment can only delay the progression of renal failure or barely maintain life, which is difficult to fundamentally improve the quality of life or prolong the life of patients. Therefore, the emergence of gene editing technology has undoubtedly brought subversive changes to the field of kidney disease research and treatment, and also kindling a new survival hope for kidney disease patients. The rapid advancement of gene editing technology in recent years has introduced novel strategies for treating these conditions. This article is structured to explore the following four aspects: (1) the principles of current mainstream gene editing technologies and their applications in biology and medicine; (2) a detailed review of the research progress of gene editing technologies in the context of genetic and tumorous kidney diseases; (3) a discussion of the areas that need improvement in current gene editing technologies; and (4) an outlook on future developments and research directions in gene editing.

In recent years, the development of gene editing technology represented by CRISPR has provided new possibilities for the treatment of kidney diseases, especially for hereditary and neoplastic kidney diseases, and has promoted the process of personalized precision treatment.

Gene Editing Overview

Gene editing technology enables the precise modification of an organism's genome by deleting, replacing, or adding specific DNA sequences, thereby altering its genetic traits. This technology allows scientists to directly modify genetic material at the molecular level. The principle underlying gene editing involves creating site-specific double-strand breaks (DSBs) at targeted locations within the genome. These DSBs are then repaired through non-homologous end joining (NHEJ) or homologous recombination (HR), completing the gene editing process.[4]

Homology-Directed Repair (HDR)

HDR utilizes homologous sequences as templates to repair DSBs in DNA.[5] When a DSB occurs, the MRE11-RAD50-NBS1 (MRN) complex and CtBP interacting protein (CtIP) collaborate to generate 3' overhang single-stranded DNA (ssDNA), which is then bound by proteins such as Rad51.[6–8] This ssDNA invades a homologous DNA strand, forming a "Holliday Junction (HJ)" structure, and uses this strand as a template for repair via DNA polymerase.[8] Once DNA synthesis is complete, the HJ structure is resolved through pathways involving the GEN1 nuclease, the SLX1-SLX4 protein complex, or the BLM protein pathway, among others.[8–11] Finally, DNA ligase joins the newly synthesized DNA fragments to the original DNA strands, completing the HDR process.

NHEJ is Categorized Into Two Pathways

Canonical non-homologous end joining (c-NHEJ) and alternative end-joining (a-EJ). The c-NHEJ pathway is initiated by a cascade reaction involving the combined action of Ku70/80 and DNA-dependent protein kinase (DNA-PK).[12] This is followed by the processing of damaged ends by proteins such as Artemis, Polλ, Polμ, polynucleotide kinase 3'-phosphatase (PNKP), and tyrosyl-DNA phosphodiesterase 1 (TDP1), which complete repairing the DSB.[10] In contrast, the a-EJ pathway, which is more error-prone, begins with a cascade reaction triggered by the recognition and binding of poly (ADP-ribose) polymerase 1 (PARP1) to the DSB site.[13] Subsequently, DNA repair-related proteins are recruited to process the damaged ends.

Experimental Techniques and Methods for Gene Editing

Zinc-Finger Nuclease (ZFN)

ZFNs consist of two primary components: a DNA recognition domain and a cleavage domain, known as FokI.[14] The DNA recognition domain is made up of zinc fingers (ZFPs), which are protein structures each comprising approximately

30 amino acids. These zinc fingers adopt a specific three-dimensional structure stabilized by a zinc ion. A ZFP typically consists of three or more tandemly arranged zinc finger modules, each folding into an α-β-β structure from C-terminal to N-terminal.[15] The cleavage domain, FokI endonuclease, is responsible for introducing DSBs in the DNA. Two ZFN monomers bind to the complementary forward and reverse strands of the target DNA. When these domains come into proximity, they form a dimer that cleaves the DNA.[16] The cell then repairs the DSBs through either NHEJ or HDR.

Transcription Activator-Like Effector Nuclease (TALEN)

TALENs (Transcription Activator-Like Effector Nucleases) are composed of DNA-binding domains and the FokI endonuclease, with the FokI endonuclease functioning similarly to that in ZFNs. The DNA-binding domain of TALENs is the transcription activator-like effector (TALE) protein. The specificity of TALE proteins is determined by repeat-variable diresidues (RVDs), with each RVD pair recognizing a specific nucleotide. By designing and assembling various RVDs, TALENs can be engineered to bind to a particular DNA sequence. Unlike ZFNs, which may suffer from context-dependent effects and inefficient recognition due to interactions between zinc finger proteins, TALENs benefit from RVDs that individually recognize single nucleotides, reducing these issues. Consequently, TALENs offer higher accuracy, efficiency, and lower off-target rates compared to ZFNs.[17]

Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-Associated Protein 9 (CRISPR/Cas9)

CRISPR/Cas9 is an RNA-guided endonuclease used for targeted DNA modification. It consists of CRISPR sequences and Cas genes. The CRISPR sequences contain exogenous genes, while the Cas genes, which flank the CRISPR sequences, encode proteins with endonuclease-related structures and functions.[18]

Using CRISPR/Cas9 as an example, when an exogenous virus infects bacteria, the Cas protein cleaves the viral DNA and integrates repeating sequences into the CRISPR array, serving as a genetic memory (including the protospacer adjacent motif [PAM]).[19] Upon subsequent viral infections, the CRISPR array and Cas9 gene are transcribed. The Cas9 protein is synthesized, and the transcribed tracrRNA and pre-crRNA pair complementarily and are processed by RNase to produce mature gRNA. This gRNA-Cas9-tracrRNA complex directs Cas9 to the target DNA sequence for cleavage.[20] For Cas9 to distinguish between viral and bacterial DNA, it must first bind to a specific PAM sequence on the target DNA. The presence of PAM at a precise location is crucial for Cas9 to recognize and cleave the target DNA[21] (Figure 1).

To solve this problem, James K Nunez et al[22] developed a CRISPR-based epigenome editing system named CRISPRoff. This technique can control the expression of genes by adding or removing methylation in them. It is formed by the fusion of Cas9 protein with loss of cleavage activity (dCas9) and DNA methyltransferase (DNMT3A). They reduced Tau expression in neurons through CRISPRoff, which in turn led to Alzheimer's disease symptoms. Tau protein is closely related to Alzheimer's disease.[23] The discovery of this technology not only provides new ideas for the treatment of Alzheimer's disease, but also provides a new tool for the exploration of epigenetic mechanisms.

In 2016, David R Liu et al[24] developed BE technology. They found a protein named rAPOBEC1 in mice that can complete the editing of C to U in single-stranded DNA, and compared it with dCas9 (dead Cas9, only used for positioning, $P < 0.05$).[25] No shearing activity) was linked to XTEN (a polypeptide) as a complex, designated BE1. The substitution of C > T without DNA double-strand break and editing template is achieved.[26]

Currently available gene editing technologies have many limitations. For example, BE1 has a low deamination effect in animal cells, only 0.8–7.7% in cells,[24] and the intracellular uracil DNA glycosylase (UNG) can recognize the mismatched U-G base pair and repair the U-G produced by BE1 to restore the C-G pairing through the base mismatch repair pathway (BER).[27] In order to inhibit the function of UNG and improve the efficiency of C > T conversion, researchers fused uracil DNA glycosylase inhibitor (UGI) derived from phage PBS to the C terminus of BE1 to develop BE2 (rAPOBEC1-XTEN- dCas9-UGI). On the basis of BE2, dCas9 was replaced by nCas9 to develop BE3 (rAPOBEC1-XTEN-nCas9-UGI). Based on BE3, a base editor BE4 (RapoBEC1-xten - nCas9-Ugi-Ugi) was developed by adding one copy of UGI at the C terminus and increasing the length of the linker short peptide between rAPOBEC1 and nCas9 (16 aa to 32 aa). The editing efficiency of bases was improved.[28] The addition of one copy of UGI to the

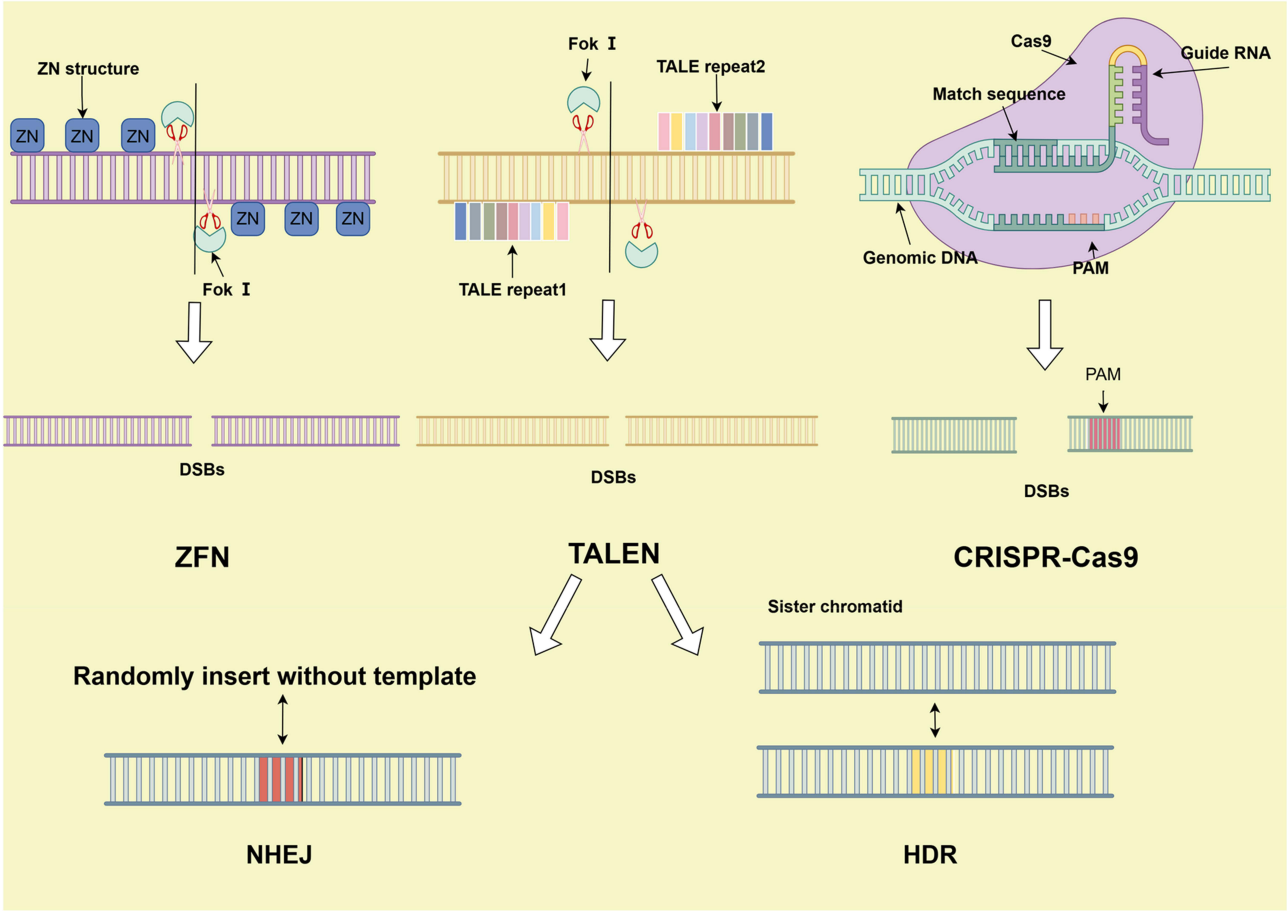

Figure 1 Comparison of three major gene editing technologies: ZFN, TALEN, and CRISPR-Cas. This figure illustrates the working principles of each technology and their mechanisms for repairing genomic DSBs through NHEJ or HDR.

structure of BE3 by BE4 improved the purity of editing products in cell lines containing UNG. However, it will lead to the increase of C > T mutation rate in the whole cell and the decrease of the purity of editing products.[29] CRISPR-Cas technology has similar risks. Gregoire Cullot et al[30] used two different cancer cell lines and immortalized fibroblasts with inactivated P53 gene to detect a single double-strand break (DSB) mediated by Cas9 enzyme. A high frequency of chromosome terminal deletions was observed in 10% cancer cell lines and 7.7% fibroblasts. Among them, 5 tumor suppressor genes that inhibit leukemia were lost. The loss of these tumor suppressor genes is likely to lead to an increased risk of cell carcinogenesis. The study of Emma Haapaniemi et al[31] also showed that the cells after CRISPR/Cas9 gene editing were often the cells with p53 gene mutation, which had the possibility of becoming cancer cells.

Applications of Gene Editing in Biology and Medicine

Applications in Cell Cycle and Gene Expression

Gene editing technology has enabled the exploration and regulation of molecular mechanisms such as cell metabolism and the cell cycle.[32] Chavez et al developed the dead Cas9 (dCas9)-VPR system by combining dCas9 with transcriptional activation domains from VP64, p65, and Rta.[33] Using this system, Kearns et al and Chakraborty et al successfully upregulated specific genes and uncovered new pathways for the directed differentiation of stem cells.[34,35] Tanenbaum et al created the SUperNovaTag (Suntag) system by fusing multiple peptide clusters to the C-terminus of dCas9, which allowed for efficient activation and regulation of genes such as CXCR4.[36] Konermann et al developed the synergistic activation mediator (SAM) system by fusing proteins to single-guide RNA (sgRNA), enabling the activation of endogenous and non-coding genes and conducting a genome-wide screen for BRAF inhibitors.[37] Liao et al optimized

the SAM system and used adeno-associated virus (AAV) to deliver targeted sgRNA sequences and wild-type Cas9 into Duchenne muscular dystrophy (DMD) mice, resulting in successful upregulation of the DMD gene and significant improvement in muscle function.[38] Chang et al employed their CRISPR-AID (TAM) base editor to construct a DMD mouse model, utilizing CRISPR-AID for induced DNA damage and TAM for targeted AID-mediated mutagenesis.[39,40] Liu et al achieved stable activation of the fragile X mental retardation protein (FMRP) gene in induced pluripotent stem cells (iPSCs) from patients with fragile X syndrome by using a dCas9-Tet1 fusion protein (Tet1: ten-eleven translocation methylcytosine dioxygenase 1).[41]

Utilizing Gene Editing Technology to Increase Production Efficiency

Cobb et al successfully enhanced the microbial strain of *Streptomyces lividans* by knocking out both the redN and actVA-ORF5 genes.[42] Zhang et al utilized the CRISPR/Cas9 system to produce antifungal substances from *Streptomyces roseosporus* and antimalarial substances from *Streptomyces venezuelae*.[43] Kong et al increased algal biofuel production through gene editing.[44] Wu et al made significant advancements in crop variety improvement by using an adenine base editor (ABE) with N-methylpurine DNA glycosylase (MPGv3) for efficient A-to-K base editing in rice.[45] The CRISPR-BEST system developed by Tong et al allows for the rapid identification of unknown biosynthetic pathways by simultaneously knocking out multiple genes.[46]

Utilizing Gene Editing Technology to Record Biological Information

Leveraging DNA's responsiveness to light signals, metal ions, and other stimuli, genome editing technologies can be employed in conjunction with instruments to record cellular bioinformation.[47] The DNA origami microfluidics operation (DOMINO) technology developed by Lu et al utilizes a controllable base editor to achieve site-specific editing in response to detected signals.[47] Cai et al employed the single-molecule RNA fluorescence in situ hybridization (smFISH) technique to record intracellular events.[48] Using mammalian synthetic cellular recorder integrating biological events (mSCRIBE) technology, Sun et al recorded nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway activation and lipopolysaccharide (LPS)-mediated inflammatory responses by utilizing various mutations generated through sgRNA self-cleavage as signals.[49] Bagheri et al utilized Toehold Switch DNA hairpins to control PAM design in CRISPR as a "switch" to regulate the biological function of Cas12a.[50]

Research Progress of Gene Editing in Mitochondrial DNA (mtDNA)

Due to factors such as reactive oxygen species, the mutation rate of mitochondrial DNA (mtDNA) is approximately 100 times higher than that of nuclear DNA (nDNA), which significantly increases the likelihood of mitochondrial metabolic enzyme defects and subsequent organ dysfunction.[51] To address this, researchers have developed various base editors for mtDNA. Mok et al employed DddA-derived cytosine base editors (DdCBEs) to achieve cytosine-to-thymine (C-T) conversions in mtDNA.[52] To overcome the challenge of high off-target rates, Lee et al introduced uracil glycosylase inhibitor (UGI), which enhanced editing efficiency by eightfold.[53] Cho et al demonstrated high-precision adenine-to-guanine (A-G) base conversion using DdCBEs, with efficiency increasing from 51% to 99%.[54,55] Yi et al utilized mitochondrial base editors (mitoBEs) to achieve A-G and thymine-to-cytosine (T-C) substitutions in mtDNA with an efficiency of 77%.[56] Zhao et al developed a copolymer (methionine/n-propyl methacrylate [Met/n-PMA]) capable of self-assembling with Cas9-mRNA/sgRNA to effectively traverse the mitochondrial membrane.[57] This therapy resulted in a 20% reduction in blood low-density lipoprotein (LDL) levels in mice with hypercholesterolemia. Additionally, Jiang et al utilized silica nanoparticles-Cas9 complexes to target cells overexpressing cluster of differentiation 44 (CD44), achieving precise mtDNA editing.[58]

Applications of Gene Editing in Genetic Diseases

In 2016, China conducted the world's first CRISPR clinical trial, establishing itself as a leader in the clinical application of gene editing technology for tumor treatment.[59] Olson et al employed CRISPR-Cas9 base editing to remove oxidative activation sites of Ca2+/calmodulin-dependent protein kinase II delta (CaMKIIδ), thereby protecting the heart from ischemia-reperfusion injury.[60] Wilson et al used gene editing to inactivate proprotein convertase subtilisin/kexin type 9

(PCSK9), and injected the PCSK9-inactivated substance into monkey liver cells for culture, resulting in a notable reduction in LDL levels.[61,62] Luna et al designed sgRNA for Cas9 and incorporated it into CD34+ hematopoietic stem and progenitor cells (HSPCs), demonstrating that edited HSPCs could enhance red blood cell proliferation, thus showing promise for anemia treatment.[63] Gao et al regulated the transcription of the CRISPR-Cas13a system using the NF-κB transcription factor, effectively inhibiting cancer cell growth.[64] Newby et al utilized ABE8e-NRCH to convert the sickle cell disease (SCD) allele (HBBS) to β-globin (HBBG [Makassar β-globin]), and with targeted delivery methods, introduced mRNA into hematopoietic stem cells of patients with SCD, showcasing the potential of gene editing technology combined with stem cell transplantation (Figure 2).[65]

Research on Gene Editing in Kidney Diseases

Research on Gene Editing in Genetic Kidney Diseases

Inherited kidney diseases (IKD) are the fifth most common cause of end-stage renal disease, characterized by structural or functional abnormalities of the kidneys resulting from genetic factors.[66] Data indicate that among patients undergoing renal replacement therapy, approximately 10–15% of adults and the majority of pediatric patients have IKD.[67,68]

Polycystic Kidney Disease

In 2015, Freedman et al pioneered the introduction of biallelic mutations in PKD1 or PKD2 into human pluripotent stem cells (hPSCs) using CRISPR/Cas9 genome editing, and subsequently differentiated these cells into kidney organoids featuring cyst structures derived from cloned knockout induced pluripotent stem cells (iPSCs).[69] Building on this model, Cruz et al discovered that modifying the extracellular matrix material environment significantly influences cyst

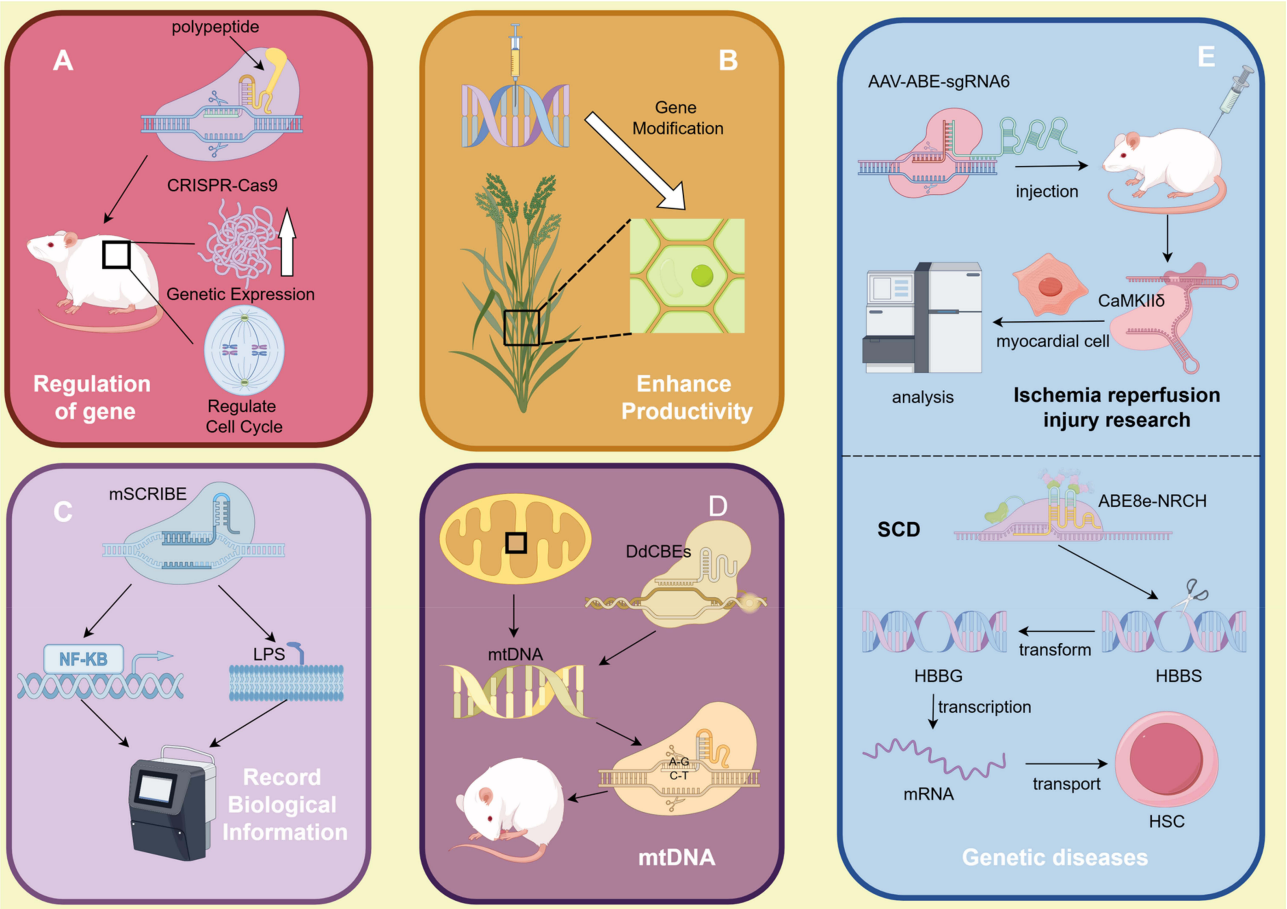

Figure 2 Applications of gene editing technology in biology. This figure depicts how gene editing can regulate the cell cycle (**A**), enhance production efficiency (**B**), record biological information (**C**), advance mitochondrial research (**D**), and study genetic diseases (**E**).

formation.[70] In kidney development, nephron progenitor cells contribute to the formation of tubules and glomeruli, while ureteric buds (UBs) develop into collecting ducts. Although cysts in polycystic kidney disease (PKD) organoids mainly originate from renal tubules, research has shown that large cysts often arise from collecting ducts.[71] Using CRISPR/Cas9, Kuraoka et al created kidney and UB organoids with PKD1 mutations. These organoids exhibited varied responses to cystogenic stimuli, laying the groundwork for accurate modeling and drug screening in PKD.[72] In 2018, Czerniecki et al became the first to apply high-throughput sequencing (HTS) to gene-edited PKD organoids.[73] They also identified blebbistatin, a specific inhibitor of non-muscle myosin II (NMII), as a relevant compound for PKD research.[74] Yasaman et al advanced Freedman's work by enhancing the genome editing efficiency of human induced pluripotent stem cells (hiPSCs) through doxycycline induction. Their CRISPR-edited stem cell pool maintained pluripotency and differentiation capabilities, facilitating the rapid development of screening platforms.[75] Tran et al used CRISPR/Cas9 to edit PKD1 and PKD2 genes in H9 human embryonic stem cells (hESCs), observing cyst growth in organoids derived from these edited cells.[76] Studies have shown that small molecule analogs of cyclic adenosine monophosphate (cAMP) and forskolin can elevate intracellular cAMP levels, promoting cyst formation.[72,73] Researchers developed a scalable organoid platform for high-throughput screening of protein kinase inhibitors that suppress cyst growth. This screening identified novel cyst inhibitors such as UCN-01 (7-hydroxyastrosporin), UCN-02 (7-epi-Hydroxystaurosporine), and QNZ (quinazoline derivative).[77] Using the PKD2 R186X mutation as a model, Vishy et al established hPSC models and applied base editors to correct the mutated bases, leading to a significant reduction in the incidence and size of organoid cysts. They also found that treating PKD organoids with eukaryotic ribosomal selective glycosides (ERSGs) to induce readthrough could dose-dependently reverse cyst formation.[78]

Additionally, using the CRISPR/Cas9 system, Chumley et al created eight distinct truncated mutant cell lines to explore the relationship between polycystin deficiency and changes in energy metabolism. Their findings revealed that truncated mutations lead to alterations in mitochondrial morphology and energy metabolism.[79] Kim et al developed a PKD2-targeted knockout pig fibroblast cell line through gene editing. RNA-seq analysis identified osteopontin (OPN) as the most significantly differentially expressed gene in PKD2-/- cells. OPN expression was also reduced in kidney cyst tissues and urine of patients with autosomal dominant polycystic kidney disease (ADPKD), and showed a negative correlation with disease severity, suggesting it as a potential urinary biomarker for predicting ADPKD progression[80] (Figure 3).

Other Genetic Kidney Diseases

As early as 2001, Heikkil a et al tried to use adenovirus vector to deliver human COL4A5 cDNA into the pig kidney by renal artery perfusion. The experiment showed that COL4A5 cDNA expression was observed in the glomeruli, and COL4A5 was effectively deposited in the glomerular basement membrane.[81]

Using an enhanced method for urinary podocyte extraction, Daga et al isolated urine-derived podocytes from two patients with Alport syndrome (AS) who had single base mutations. They employed a self-inactivating dual-plasmid system based on CRISPR/Cas9 for gene editing, achieving a final HDR rate of up to 58.8% for the two AS mutants.[82,83] Yamamura et al used an antisense oligonucleotide (ASO) to target a truncating exon 21 variant in the COL4A5 gene that causes AS to convert it to an in-frame deletion. In the X-linked Alport syndrome mouse model, this exon skipping enabled the production of functional collagen type IV α345 trimer in the renal basement membrane, which significantly alleviated the pathological changes and delayed the progression of AS to ESRD.[84] Cohort studies have indicated that nephronophthisis (NPH) is a prevalent monogenic cause of adult end-stage renal disease (ESRD).[85] Li et al developed the first mouse model of nephronophthisis 1 (Nphp1) with human kidney phenotypes by using CRISPR/Cas9 to knockout specific exons of the Nphp1 gene.[86] Zheng et al took advantage of the multiple genome editing characteristics of Cpf1 to simultaneously target two genes related to hepatic oxalate synthesis, Hao1 and Ldha, and screen the most effective crRNA with high specificity to deliver in vivo drug delivery to rats through integrated AAV8. The results showed that the treatment reduced urinary oxalate levels, protected renal function, and reduced renal CaOx deposition in PH1 rats, with no off-target effects in the genome or organ.[87] Chen et al utilized CRISPR-mediated delivery of adeno-associated virus (AAV) carrying ABE8e to repair 13% of the pathogenic genes in primary hyperoxaluria type 1 (PH1) AgxtQ84X rats, partially restoring alanine-glyoxylate aminotransferase (AGT) expression and reducing calcium oxalate crystal deposition

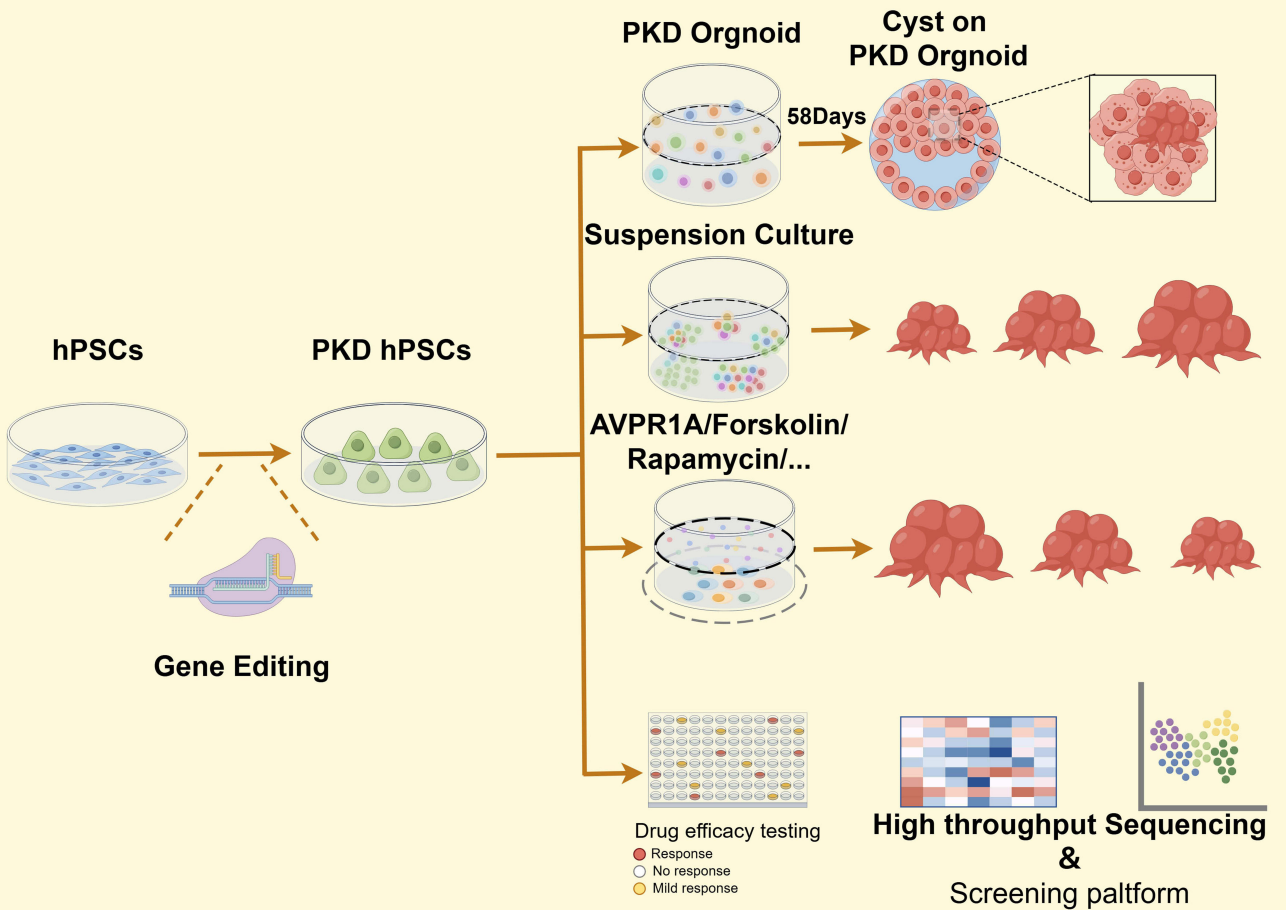

Figure 3 Research on gene editing in PKD. The figure shows how gene editing transforms hPSCs into PKD hPSCs that can decompose cystic structures and differentiate into PKD organoids. It also demonstrates how gene-edited PKD models can affect cyst formation by altering the material environment or culture conditions and how these models can be used in high-throughput sequencing for drug and target screening.

in vivo.[88,89] Zhang et al successfully created a cystinuria rat model by knocking out 7 bp in the Slc7a9 gene using CRISPR/Cas9. Their high-throughput RNA sequencing suggested that the L-Glutathione (GSH) metabolic pathway might be involved in cystinuria.[90,91]

Sphingosine-1phosphate lyase insufficiency syndrome is a metabolic disease caused by SGPL1 gene mutation. It is clinically manifested as steroid-resistant nephrotic syndrome. Patients often progress rapidly to renal failure, accompanied by endocrine, neurological, and hematopoietic damage. Zhao et al found that in SGPL1 knockout neonatal mice, the temporal vein injection of AAV9 containing human SGPL1 can significantly prolong the survival period of mice and prevent the occurrence and development of renal failure.[92] Subsequent studies by Khan et al found that AAV9-SGPL1 in the above study was evolved into AAV9-SGPL1 2.0, which was to replace the cytomegalovirus early enhancer/chicken β-actin promoter with the cytomegalovirus promoter. SGPL1 knockout mice can significantly delay the appearance of renal phenotype and survive for at least 5 months.[93] Fabry disease is an X-linked recessive metabolic systemic disease caused by mutations in the GLA gene encoding the lysosomal enzyme α-galactosidase A (α-gal A). Patients with Fabry disease often die of systemic complications such as progressive renal failure, heart failure and stroke. Nakamura et al were the first to rapidly inject A solution containing DNA encoding human α-gal A naked plasmid into the left kidney of Fabry mice by retrograde renal intravenous injection. α-gal A activity increased in kidney, liver, heart and plasma after injection, and Gb3 activity decreased in kidney, contralateral kidney, liver, heart and spleen after injection, which laid the foundation for the opening of gene therapy for Fabry disease.[94]

Research on Gene Editing in Tumorous Kidney Diseases

Clear cell renal cell carcinoma (ccRCC) is the most prevalent histological type of renal cell carcinoma. Guo et al discovered that serum cystatin C (Cys-C) levels are negatively correlated with survival in patients with ccRCC.[95] In a study involving a Cys-C knockout ccRCC cell line created using CRISPR/Cas9, it was found that Cys-C inactivation could reduce cell proliferation and invasion by inhibiting the extracellular signal-regulated kinase 1/2 (ERK1/2) and signal transducer and activator of transcription-3 (STAT-3) pathways while activating the c-Jun N-terminal kinase (JNK) pathway.[96] Neumann et al compared ccRCC tumor tissues with normal tissues and identified that membrane-bound O-acyltransferase domain-containing protein 7 (MBOAT7) correlates with tumor severity. They used CRISPR/Cas9 to create an MBOAT7 knockout cell line and found that MBOAT7 deficiency reduced AA-PI levels and cell proliferation in ccRCC cells, indicating a connection between metastatic ccRCC and changes in AA-PI metabolism.[97] Kaplun et al introduced a Kaiso frameshift mutation in the Caki1 cell line using CRISPR/Cas9. Kaiso is known to protect regions with low histone modification from hypermethylation and regulate genomic methylation in renal cancer cells.[98] Van et al successfully created somatic genome-edited animal models (genetically engineered mouse models [GEMMs]) of BRCA1-associated protein 1 (BAP1) and recombinant polybromo 1 (PBRM1) using an inducible CRISPR-Cas9 system.[99] Brockett et al established a homologous mutation model in Drosophila for E902Q, a novel single-base mutation in SET domain containing 2 (SETD2), a gene commonly mutated in ccRCC. This genetic model could advance individualized and precise cancer treatments.[100]

CRISPR technology is also valuable for high-throughput screening. Zou et al conducted a genome-wide CRISPR screen in human renal cancer cells and identified the peroxisome as a critical factor influencing sensitivity to ferroptosis.[101] Xu et al used the CRISPR/Cas9 system to create BAP1 knockout cells to screen for epigenetic compounds, discovering that OTX015 inhibits the transcription of several proliferation-related genes in these BAP1 knockout cells.[102]

In recent decades, immunotherapy has transformed cancer treatment, significantly enhanced patient survival rates and alleviating the burden of the disease. Hu et al developed a mouse renal cell carcinoma (RCC) model using a non-integrating lentivirus (NIL) vector combined with CRISPR/Cas9 technology.[103] This model can continuously grow and metastasize in immunocompetent hosts, providing a valuable tool for investigating the role of immunity in RCC metastasis and for advancing immunotherapy approaches.[104] Paloma et al used CRISPR/Cas9 to create various RCC models with different levels of human leucocyte antigen-G (HLA-G) downregulation.[105] Co-culturing these RCC models with NK cells revealed that HLA-G expression was reduced, which in turn activated immune cells and stimulated the host immune system to target and eliminate tumor cells.

Internal tandem duplications (ITDs) in the BCOR gene are identified as a driver mutation in over 80% of clear cell sarcoma of the kidney (CCSK) cases. Whole exome sequencing (WES) of nine CCSK patients with positive BCL6 co-repressor-ITD (BCOR-ITD) revealed that fibroblast growth factor 3 (FGF3) was significantly expressed in those with metastatic CCSK. Using CRISPR/Cas9, Fiore et al introduced ITDs into BCOR in human embryonic kidney 293 (HEK293) cells.[106] They discovered that FGF3 markedly enhanced cell migration, highlighting a new potential therapeutic target for aggressive tumors.

Research on Gene Editing in Other Kidney Diseases

Using CRISPR/Cas9-mediated Keap1 knockout (KO), Kurzhagen et al enhanced the antioxidant potential of nuclear factor erythroid 2-related factor 2 (Nrf2) in CD4+ T cells.[107] In a mouse model of renal ischemia-reperfusion injury (IRI), adoptive transfer immunotherapy with Keap1-KO CD4+ T cells significantly improved renal function in mice with acute kidney injury (AKI). Additionally, Tomoko et al employed CRISPR/Cas9 to create a CD2-associated protein (CD2AP) p.S198fs mouse model with gene mutations from a patient, discovering that this frameshift mutation accurately replicated the focal segmental glomerulosclerosis (FSGS) and ESRD phenotypes observed in patients.[108] Whole exome sequencing (WES) of 94 FSGS patients suggested that MYO9A could be a candidate pathogenic gene for FSGS. Li et al demonstrated that MYO9A regulates RhoA activity and podocyte function, and introducing the MYO9A p.Arg701 mutation into mice via CRISPR reproduced the FSGS phenotype seen in the proband. Moreover, multiple sequencing analyses indicated that kidney-related genetic variants identified in eGFR genome-wide association studies (GWAS)

might be located in the Dachshund homolog 1 (DACH1) regulatory region in the distal renal tubule. Doke et al used CRISPR/Cas9 to delete the intronic risk region containing eGFR GWAS variants in HEK293 cells, validating the causal role of eGFR GWAS SNPs (single nucleotide polymorphisms) in regulating DACH1 expression. The deletion of DACH1 was associated with renal tubular cell phenotypes and cytokine release.[109,110]

As genome sequencing becomes more widespread, gene editing has increasingly been applied to various aspects of kidney diseases, leading to the development of novel gene therapies, especially for inherited conditions. Despite these advancements, there are significant challenges which must be overcome before gene editing can be effectively translated into clinical practice for kidney diseases (Figure 4 and Table 1).

Challenges and Prospects of Gene Editing in Kidney Diseases

Limitations of Existing Research

Although ZFNs and TALENs possess comparable genome editing capabilities as demonstrated in previous research, CRISPR quickly established an unshakable position in various fields due to its simplicity and efficiency.[111,112] Over the past decade, CRISPR and other gene editing technologies have advanced significantly, with several methods now being tested in human clinical trials for treating genetic diseases. In the renal system, CRISPR is a powerful tool for developing gene therapies and improving organ transplantation.

However, the primary challenge when applying CRISPR is the off-target effect, which occurs when the Cas protein binds to and cuts unintended genomic sites. This can lead to serious genomic issues, including large-scale deletions,

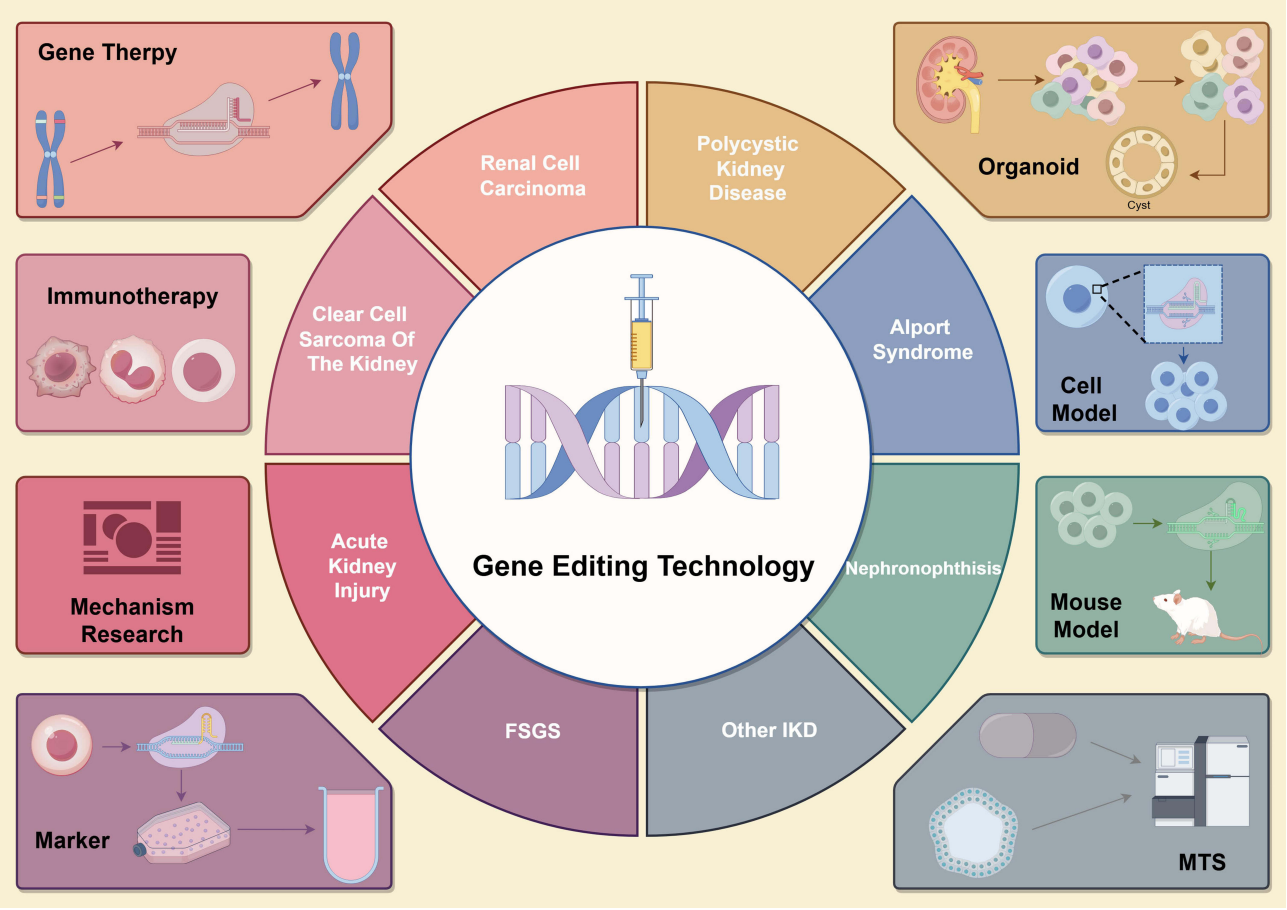

Figure 4 Applications of gene editing in kidney diseases. This figure outlines the involvement of gene editing techniques in researching various kidney diseases, including PKD, Alport syndrome, nephronophthisis, clear cell renal cell carcinoma, renal cell carcinoma, acute kidney injury, and FSGS. It highlights the use of gene editing to construct various cells, organoids, and animal models; develop screening strategies and high-throughput research platforms; explore disease mechanisms; identify therapeutic targets and biomarkers; and advance clinical immunotherapy and gene therapy.

Table 1 Application of Gene-Editing Tools in Kidney Disease

Disease	Mutant Gene	Gene-editing Tools	Purpose	Animal Model	Disease
PKD	PKD1, PKD2	CRISPR/Cas9, BE	Research	Yes	AAV
Alport syndrome	COL4A3, COL4A4, COL4A5	CRISPR/Cas9	Research	Yes	AAV
NPHP	NPHP1-18, NPHP1L, NPHP2L	CRISPR/Cas9	Research	Yes	AAV
PH	AGXT, GRHPR, HOGA1	BE	Research	Yes	AAV
Cystinuria	SLC3A1, SLC7A9	CRISPR/Cas9	Research	Yes	AAV
SPLIS	SGPL1	-	Research	Yes	AAV
Fabry Disease	GLA	-	Research	Yes	Plasmid
RCC	VHL, PBRM1, BAP1, SETD2	CRISPR/Cas9	Research	Yes	NIL
AKI	-	CRISPR/Cas9	Research	Yes	-
FSGS	MYO9A	CRISPR/Cas9	Research	Yes	-

Note: The table summarizes the diseases, mutated genes, tool versions, animal models, and delivery methods for which the gene-editing tool was used.

genomic rearrangements, and potentially fatal gene mutations.[113] Researchers are actively developing new strategies to minimize these off-target effects. For example, Fu et al demonstrated that truncating several nucleotides from sgRNA can reduce off-target mutations significantly.[114] Mali et al designed two sgRNAs to guide two separate Cas9 proteins to the same locus, converting both Cas9 proteins into nickases to enhance specificity and reduce off-target effects.[115] Kleinstiver et al introduced 3 to 4 mutations into Cas9 to simultaneously improve specificity and reduce off-target effects.[116] Kim et al discovered Cpf1 (CRISPR-associated endonuclease Cpf1), an enzyme with higher specificity than Cas9, capable of performing multiplex gene editing with a single CRISPR RNA (crRNA) and exhibiting lower off-target effects and a smaller size.[117,118] Additional strategies include using light-activated Cas9 variants, split Cas9 variants, small molecule-induced Cas9, and engineered allosterically regulated Cas9 to reduce off-target rates.[119–122] Park et al developed a system to dual-regulate Cas protein expression and exposure time to minimize off-target effects.[123]

In hyperoxaluria research, the Cpf1 system has been utilized for multiplex editing of hydroxyacid oxidase 1 (Hao1) and lactate dehydrogenase A (Ldha).[87] Huang et al found that nephron progenitor cell-derived organoids exhibited fewer off-target cells and a higher proportion of podocytes compared to hPSC-derived kidney organoids.[123] In the in vivo study of ABE8e-edited PH1 disease model, deep sequencing of targeted liver tissue showed no increase in indels among the predicted top 20 off-target sites, and whole-genome sequencing showed a similar distribution of mutation types in treated and untreated groups. Editing efficiency also remained below 1% for testing samples from different organs (testis, spleen, lung, heart, kidney, and skeletal muscle).[89] HAO1 gene is involved in encoding hepatic glycolate oxidase (GO) and thus affects the pathogenesis of PH1. When targeting the mouse HAO1 gene, the investigators observed that at a dose of 0.3 mg/kg, deep sequencing revealed no greater frequency of indels observed at off-target sites than in controls. At the 1 mg/kg dose, the frequency of indels was below 0.5% at most loci. The only off-target sites with an indel frequency greater than 0.5% were located in intergenic regions with no significant known biological function. When targeting the human HAO1 gene, the investigators detected only one off-target site among 52 predicted sites, which, although not of predicted functional significance, suggested that the off-target activity could be reduced by employing high-fidelity Cas9 variants or by developing more efficient nucleases. In addition, optimizing delivery systems and dosing regimens may help reduce off-target effects.[124]

Once in vitro gene editing effectiveness is established, the next challenge is delivering the editing components in vivo. CRISPR/Cas9 can be delivered in various forms, including nanoparticles, viral vectors, and electroporation. Viral vectors, while widely used for their transport efficiency and tissue specificity, have limitations such as small capacity and potential off-target effects or immune responses due to prolonged gene expression.[125,126] Nanoparticles can be divided into lipid-mediated and cationic-mediated types, with the former having some cytotoxicity.[127]

Electroporation uses high-voltage electrical pulses to create transient nano-sized pores in the cell membrane, allowing negatively charged target elements to enter the cell. Although effective, electroporation's immunogenicity and risk of degradation, limit its use in vivo, making it more common in vitro. Considering the above factors, adeno-associated virus

(AAV) is considered to be the most suitable choice for human gene therapy. AAV is a nonpathogenic parvovirus with a 4.7-kb genome that can transinfect both proliferating and differentiating cells. There are different serotypes of AAV, each with a preference for a different tissue. Unlike the liver, where hepatocytes account for more than 80% of the tissue, the kidney is a complex organ containing at least 26 specialized cell types. Compared with other tissue systems, the pace of gene therapy in the kidney system has been relatively slow. Based on this, it is particularly important to broaden the tropism of AAV and screen highly specific AAV capsids for the treatment of kidney diseases.[128] In 2018, researchers found that Anc80 can efficiently transduce renal interstitial and mesangial cells,[129] and the combination of AAV9 vector and segmental specific gene promoter of retrograde ureteral perfusion can also target nephron segmental specific genes.[130] Ding et al demonstrated that the AAV-LK03 serotype is capable of efficiently transducing human podocytes in vitro and that podocyte AAV2/9 is even more efficient in vivo and verified the effectiveness of the kidney-specific promoter NPHS1. AAV2/9 delivery of inducible foot protein in a mouse model of pediatric hereditary nephrotic syndrome led to improvements in albuminuria, plasma creatinine, plasma urea, plasma cholesterol, histologic changes, and long-term survival.[131] Transduction specificity and limited efficiency of native AAV serotypes in various renal cells.[132] Wu et al developed an evolutionarily directed selection strategy that targeted the glomerulus and resulted in the selection of a novel AAV vector, AAV2-GEC. The vector can efficiently and specifically target glomerular endothelial cells (GECs) after systemic administration, showing stable GEC tropism in both normal control and disease rodent models. The researchers delivered the IgG-degrading enzyme of the bacterial cysteine protease Streptococcus pyogenes (Ide S) to the GEC.[133,134] In this study, the potential of AAV2-GEC for renal targeted therapy was confirmed.[135] In addition, Jiang et al not only used lipid nanoparticles (LNPs) to deliver CRISPR-Cas9 to embryos to rapidly generate PH1 mouse models, but also used the LNP-CRISPR-Cas9 system to safely and effectively target the Hao1 gene, which significantly reduced urine oxalate levels and improved kidney function in PH1 mice. This effect persisted for up to 12 months without significant off-target effects, hepatotoxicity, or immune response.[124] Rodriguez et al also investigated the use of solid lipid nanoparticles (SLN) for intravenous delivery of α-Gal A to A mouse model of FD. Sln-based delivery improved α-Gal an activity by 10–20% in the liver, spleen, heart and kidney, respectively, compared with the wild type, without hepatotoxicity. This study reinforces the potential of lipid nanoparticles as carriers for gene therapy.[136]

Future Research Directions

Undoubtedly, the advent of gene editing technology has opened new possibilities for manipulating the genomes of various organisms. This technology is not only assisting researchers in constructing diverse models, developing screening strategies, and identifying therapeutic targets in basic research but is also paving the way for precision medicine in clinical applications. For single-gene inherited diseases such as sickle cell disease and muscular dystrophy, genome editing has demonstrated significant therapeutic potential. Despite the promise, the research and development process can be expensive, and patient responses can vary due to individual differences. Some inherited diseases such as Alport syndrome, are predominantly caused by mutations in just three genes: collagen type IV alpha 3 (COL4A3), collagen type IV alpha 4 (COL4A4), and collagen type IV alpha 5 (COL4A5). However, the specific mutations and their combinations can vary widely among patients globally. This variability poses a challenge for single editing therapies, which may not be effective for all genetic mutations. Therefore, personalized gene therapies tailored to the specific genetic profiles of individual families may become a key focus for future research in genetic diseases.

Furthermore, while current research on somatic cell editing addresses issues in the affected individual, human germline editing has the potential to address genetic problems across entire family lineages. This area of research holds promise but requires further exploration and development.

Conclusion

Gene editing technology, often hailed as the ability of “rewrite the code of life”, has revolutionized fields such as biology, medicine, and agriculture due to its vast potential. This article provides an overview of the principles and history of gene editing technologies, as well as their applications in both biology and medicine. Utilizing tools like ZFNs, TALENs, and CRISPR/Cas systems, researchers can regulate cell cycle, metabolism, and gene expression; enhance product yields;

record intracellular biological information; explore mitochondrial genomes and associated genetic disorders; and apply these technologies to clinical diagnosis and treatment.

In the field of nephrology, gene editing technology is being investigated for various conditions, including genetic kidney diseases such as polycystic kidney disease and Alport syndrome, kidney tumors like clear cell renal cell carcinoma and clear cell renal sarcoma, as well as other kidney disorders including acute kidney injury, focal segmental glomerulosclerosis (FSGS), and kidney fibrosis. Applications in kidney diseases include constructing diverse cell lines, organoids, and animal models; developing screening strategies and high-throughput research platforms; identifying therapeutic targets; and advancing clinical precision gene therapy.

Despite its transformative impact, gene editing technology still faces significant challenges, such as minimizing off-target effects, enhancing specificity and targeting, improving delivery methods, and increasing in vivo editing efficiency. These challenges require ongoing research and optimization. Nevertheless, gene editing remains one of the most important advancements in molecular biology and holds promise for diagnosing and treating kidney diseases.

Abbreviations

CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; Cas9, CRISPR-associated protein 9; BE, Base Editor; PE, Prime Editor; DSB, Double Strand Breaks; CKD, chronic kidney disease; NHEL, Non-homologous End Joining; HR, Homologous recombination; HDR, Homology directed repair; MRN, MRE11-RAD50-NBS1; CtIP, CtBP interacting protein; ssDNA, Single-stranded DNA; SLX1, SLX1 structure-specific endonuclease subunit; SLX4, SLX4 structure-specific endonuclease subunit; PNKP, Polynucleotide Kinase 3'-Phosphatase; TDP1, Tyrosyl-DNA Phosphodiesterase 1; ZFN, Zinc-finger nuclease; ZFP, Zinc-finger Protein; TALEN, transcription activator-like effector nuclease; TALE, TAL effector; RVD, Repeat-variable Diresidue; PAM, Protospacer Adjacent Motif; dCas9, Dead Cas9; DNMT3A: DNA methyltransferase; XTEN, Extended recombinant polypeptide; Suntag, SUperNovaTag; CXCR4, C-X-C chemokine receptor type 4; SAM, Synergistic Activation Mediator; BRAF, V-Raf murine sarcoma viral oncogene homolog B; AAV, Adeno-associated Virus; PH1, primary hyperoxaluria 1; AGT, Alanine-glyoxylate-aminotransferase; DMD, Duchenne muscular dystrophy; CRISPR-AID, CRISPR associated induced DNA damage; FMRP, fragile X mental retardation protein; DOMINO, DNA Origami Microfluidics Operation; mSCRIBE, Mammalian Synthetic Cellular Recorder Integrating Biological Events; LPS, Lipopolysaccharides; mtDNA, Mitochondrial DNA; nDNA, Nuclear DNA; DdCBEs, DddA-derived Cytosine Base Editors; UGI, Uracil Glycosylase Inhibitor; mitoBEs, Mitochondrial DNA base editor; CaMKIIδ, Ca2+/calmodulin Dependent Protein Kinase II delta; PCSK9, Proprotein convertase subtilisin/kexin type 9; HSPC, Hematopoietic Stem and Progenitor Cells; SCD, Sickle Cell Disease; HBBG, Makassar β-globin; IKD, Inherited Kidney Diseases; iPSC, induced Pluripotent Stem Cells; UBs, Ureteric Buds; PKD, Polycystic Kidney Disease; HTS, High Throughput Sequencing; NM II, non-muscle myosin II; hESC, Human Embryonic Stem Cell; cAMP, Cyclic Adenosine Monophosphate; UCN-01, 7-hydroxyastrosporin; UCN-02, 7-epi-Hydroxystaurosporine; QNZ, Quinazoline Derivative; ERSGs, Eukaryotic ribosome selective glycosides; OPN, Osteopontin; ADPKD, Autosomal Dominant Polycystic Kidney Disease; ASO, antisense oligonucleotide; ESRD, End Stage Renal Disease; Nphp1, nephronophthisis 1; GSH, L-Glutathione; ccRCC, Clear Cell Renal Cell Carcinoma; Cys-C, Cystatin C; ERK1/2, Extracellular Signal-Regulated Kinase 1/2; STAT-3, Signal Transducer and Activator of Transcription 3; JNK, c-Jun N-terminal kinase; MBOAT7, Membrane-bound O-acyltransferase domain 7; AA-PI, arachidonic acid-enriched phosphatidylinositols; BAP1, BRCA1 Associated Protein 1; PBRM1, Recombinant Polybromo 1; GEMMs, Genetically Engineered Mouse Models; SETD2, SET Domain Containing 2; NIL, Non-integrating Lentivirus; HLA-G, human leucocyte antigen-G; BCOR, BCL6 co-repressor; ITD, Internal Tandem Duplication; CCSK, Clear Cell Sarcoma of Kidney; WES, Whole Exome Sequencing; FGF3, Fibroblast Growth Factor 3; Nrf2, Nuclear Factor erythroid 2-Related Factor 2; IRI, Ischemia Reperfusion Injury; FSGS, focal segmental glomerulosclerosis; CD2AP, CD2-associated protein; MYO9A, Myosin IXA; eGFR, Estimated Glomerular Filtration Rate; GWAS, Genome-wide Association Study; DACH1, Dachshund homolog 1; SNPs, Single Nucleotide Polymorphisms; Cpf1, CRISPR-associated endonuclease Cpf1; crRNA, CRISPR RNA; Hao1, Hydroxyacid Oxidase 1; Ldha, Lactate Dehydrogenase A; SGPL1, Sphingosine phosphate lyase 1; GLA, Gamma-Linolenic Acid; α-gal A, α-galactosidase A; RNP, ribonucleoprotein; GO, glycolate oxidase; Indels: insertion-deletions; GEC: glomerular

endothelial cells; Ide S: Streptococcus pyogenes cysteine protease; SLN: solid lipid nanoparticles; COL4A3, Collagen Type IV Alpha 3; COL4A4, Collagen Type IV Alpha 4; COL4A5, Collagen Type IV Alpha 5.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Disclosure

The authors declare that they have no conflict of interests.

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