CRISPR Gene Editing in the Kidney

Nelly M. Cruz and Benjamin S. Freedman

CRISPR is a nuclease guidance system that enables rapid and efficient gene editing of specific DNA sequences within genomes. We review applications of CRISPR for the study and treatment of kidney disease. CRISPR enables functional experiments in cell lines and model organisms to validate candidate genes arising from genetic studies. CRISPR has furthermore been used to establish the first models of genetic disease in human kidney organoids derived from pluripotent stem cells. These gene-edited organoids are providing new insight into the cellular mechanisms of polycystic kidney disease and nephrotic syndrome. CRISPR-engineered cell therapies are currently in clinical trials for cancers and immunologic syndromes, an approach that may be applicable to inflammatory conditions such as lupus nephritis. Use of CRISPR in large domestic species such as pigs raises the possibility of farming kidneys for transplantation to alleviate the shortage of donor organs. However, significant challenges remain, including how to effectively deliver CRISPR to kidneys and how to control gene editing events within the genome. Thorough testing of CRISPR in preclinical models will be critical to the safe and efficacious translation of this powerful young technology into therapies.

Complete author and article information provided before references.

Am J Kidney Dis. 71(6): 874-883. Published online March 30, 2018.

doi: 10.1053/j.ajkd.2018.02.347

Note from Editors: This article was commissioned to celebrate the selection of CRIPSR-Cas9 as a finalist in NephMadness 2017. NephMadness is an educational project styled as a tournament in which key concepts in nephrology "compete" to determine which deserves to be crowned the most notable recent advance in the field.

The Discovery of CRISPR Gene Editing

In 2012, two landmark articles described an enzyme that could be "programmed" with a customizable RNA input to cleave specific DNA sequences of interest.[1,2] The versatility and ease of use of this system suggested that it could be useful for a variety of gene editing and gene targeting applications.[1,2] The technique was rapidly shown to work in mammalian (including human) cells, raising the possibility of using it for gene therapy in the clinic.[3,4]

This gene editing system was called CRISPR (clustered regularly interspaced short palindromic repeats; see Box 1 for expansion and definitions of other key terms), after the bacterial DNA sequences that originally led to its discovery.[5-9] As shown in Figure 1A, it has 2 main components: a CRISPR-associated system (Cas) endonuclease and a guide RNA (gRNA). The gRNA combines an invariant "scaffold" sequence that binds to Cas and a variable "spacer" sequence that provides sequence specificity. The spacer is approximately 20 nucleotides in length and terminates immediately upstream of a characteristic NGG sequence called the protospacer adjacent motif. Because there are 4 possible nucleotides for each position in the gRNA, the spacer provides up to 4^{20} possible sequence combinations (>1 trillion). This greatly exceeds the number of base pairs in any known genome, providing sufficient sequence specificity to identify unique sites.

Cas forms a ternary complex with the gRNA and a genomic DNA sequence complementary to the spacer and introduces a double-stranded break at that site. A commonly used Cas nuclease, Cas9, typically cuts both strands of DNA between the third and fourth nucleotides upstream of the protospacer adjacent motif.[1,2] There are then 2 main ways that DNA can be repaired by the cell. The first is nonhomologous end joining (NHEJ), an error-prone process involving trimming of the severed DNA followed by direct ligation. NHEJ occurs throughout the cell cycle and is the primary pathway by which CRISPR-induced lesions are repaired. NHEJ typically leads to indel (insertion or deletion) mutations in the original sequence, which can disrupt open reading frames (Fig 1B).

The second repair mechanism is homology-directed repair (HDR), in which DNA is corrected based on a template from undamaged DNA of similar sequence. This template DNA may be supplied by the experimentalist or alternatively may reside elsewhere in the genome, for instance, in an undamaged sister chromatid following DNA replication.[3,4,10] In the presence of such a template, CRISPR can be used to engineer specific mutations in the genome through the HDR pathway, which provides a mechanism for more accurate repair of DNA than NHEJ (Fig 1C). In certain cell types, rates of both NHEJ and HDR can be moderately enhanced by synchronizing the cells at either the replication or division stages of the cell cycle.[11] However, HDR remains inefficient compared to NHEJ, which predominates even in the presence of template DNA.[3,4,11]

The discovery of CRISPR opens new avenues for our ability to modify genomes. This has important ramifications for genetic disease and bioengineering applications. CRISPR builds on previous gene editing technologies, including homologous recombination, zinc-finger nucleases, TALEN (transcription activator-like effector nucleases), and AAV (adeno-associated virus).[12-16] No gene editing technology is 100% specific, and all have the potential to introduce off-target mutations. Also, CRISPR is not necessarily more efficient than some of these earlier systems. However, CRISPR has significant advantages over

Box 1. Definitions of Key Terms

- Adeno-associated virus (AAV): a small virus that infects human cells and can be used as a vector for gene therapy
- Chimeric antigen receptors (CAR) T cells (CAR-T): T lymphocytes with receptors that have been engineered to recognize specific antigens
- CRISPR (clustered regularly interspaced short palindromic repeats): a gene editing system combining a programmable nuclease with a customizable input RNA; can also refer to the bacterial DNA sequences that led to the discovery of this system
- CRISPR-associated system (Cas): a family of nucleases used for CRISPR gene editing
- Gene editing: introduction of targeted mutations into a specific sequence of DNA within the genome
- Guide RNA (gRNA): a short RNA sequence that directs Cas to complementary sites within the genome
- Indel: mutations featuring the insertion or deletion of base pairs, commonly introduced during DNA repair processes
- Interspecies blastocyst complementation (IBC): establishment of a genetic niche within a host embryo to grow a tissue from another species
- Kidney organoids: multicellular units in vitro containing podocytes, proximal tubules, and distal tubules in nephron-like patterns
- Pluripotent stem cells (PSCs): cells at an early embryonic stage that can give rise to the entire body

other systems in terms of cost and ease of use.[17] This has led to widespread adoption of CRISPR in research laboratories and, more recently, in clinical trials. We discuss applications of CRISPR relevant to the study and treatment of kidney disease.

Studying Kidney Disease With CRISPR

A significant proportion of kidney disease is genetic, with up to 15% of all cases deriving directly from a Mendelian mutation and many more involving more complex inheritance patterns.[18] For instance, polycystic kidney disease (PKD), which is predominantly caused by hereditary mutations, is the primary diagnosis in ~10% of patients requiring renal replacement therapy.[19] The influence of genetics in kidney disease is partially masked by the high incidences of hypertension and diabetes, which together account for up to 75% of kidney failure, but primarily affect other organ systems.[18] Genetic causes therefore contribute disproportionately to cases of kidney disease associated with primary defects in the kidneys themselves.

Although many of the genes that cause kidney disease have been identified, functional experiments are required to validate candidate genes arising from genetic studies and determine how mutations cause disease at the cellular and tissue level. In addition, many genes involved in kidney disease remain unknown. CRISPR enables researchers to perform targeted experiments to address these questions. The general approach has been to knock out candidate genes and inspect the mutants for phenotypic differences compared with isogenic (ie, having a uniform genetic background) controls that were not modified by CRISPR. Sequencing of DNA amplified from the target region, followed by protein analysis (using immunoblot and immunofluorescence) to confirm the absence of the gene product, are the standard methods used to verify gene knockout.

CRISPR carries a risk for inadvertent edits to DNA sequences that are similar but not identical to the gRNA. To minimize the likelihood of these "off-target" effects, gRNAs can be selected based on computational algorithms that maximize sequence specificity.[3,4] Minimizing the duration and concentration of the genome's exposure to Cas9 is one way to reduce the risk for accumulated mutations. "Nickase" mutants of Cas9 that require 2 gRNAs (on opposite sides of the target) to efficiently cleave DNA can also provide an additional measure of specificity.[20] However, this approach is more complicated than single-site CRISPR, may be less efficient, and increases the number of off-target sites that need to be considered. To verify that the appropriate modifications have been made, whole-genome sequencing would ideally be performed for every CRISPR product,[21,22] although this may not be economically feasible or efficiently capture certain types of mutations such as copy number variations and large deletions. At a minimum, to reduce the possibility of off-target effects, multiple separate knockouts created by different gRNAs are typically compared for each genotype and phenotype, because these would not be expected to produce the same off-target effects.[23] When CRISPR is being used to generate clonal cell lines, it is similarly important to isolate and characterize multiple clones for each genotype to reduce the possibility of clonal idiosyncrasies.

Following this approach, CRISPR has been applied to kidney epithelial cell lines to model features of tubular physiology and disease. For instance, knockout of multidrug resistance protein 1 was observed to reduce efflux of transporter substrates in Madin-Darby canine kidney cells, whereas knockout of a tight junction scaffolding protein was shown to increase paracellular flux.[24,25] In mouse inner medullary collecting duct cells, knockout of A-kinase anchoring protein 220 was found to result in actin organization defects and buildup of aquaporin 2 at the apical plasma membrane, corresponding to reduced urine-diluting capacity in animal models.[26] In human renal cortical tubular epithelial cells, knockout of *GANAB*, a candidate gene for PKD, was observed to result in failed trafficking of polycystin proteins to primary cilia, which is associated with PKD.[27]

More complex models of disease are possible with human pluripotent stem cells (hPSCs), a cell type that includes embryonic stem cells derived from embryos and induced pluripotent stem cells reprogrammed from somatic cells.[28,29] hPSCs have emerged as a valuable system for studying mechanisms of kidney disease in vitro.[23,30-33] Early work established a cohort of hPSCs from patients with PKD, including both autosomal dominant and autosomal recessive forms of the disease.[30] hPSCs with