Oligonucleotide-Based Therapies

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The global burden of chronic kidney disease (CKD) is increasing every year and represents a great cost for public healthcare systems, as the majority of these diseases are progressive. Therefore, there is an urgent need to develop new therapies. Oligonucleotide-based drugs are emerging as novel and promising alternatives to traditional drugs. Their expansion corresponds with new knowledge regarding the molecular basis underlying CKD, and they are already showing encouraging preclinical results, with two candidates being evaluated in clinical trials. However, despite recent technological advances, efficient kidney delivery remains challenging, and the presence of off-targets and sideeffects precludes development and translation to the clinic.

Keywords: chronic kidney disease ; kidney ; oligonucleotide therapeutics ; kidney delivery ; nanocarrier ; nanoparticle

1. Introduction

Recent studies have estimated that chronic kidney diseases (CKDs) affect around 850 million people worldwide (one in ten adults). The global burden of CKD is increasing and is projected to become the fifth most common cause of years of life lost globally by 2040 [1]. Since CKD is mostly irreversible and progressive, patients who advance to end-stage renal disease (ESRD) require dialysis or renal transplantation, which negatively affect quality of life and have a large impact on healthcare systems. It has been estimated that the costs of dialysis and transplantation consume 2–4% of annual healthcare budgets in high-income countries [1][2]. Moreover, kidney transplantation is associated with a high risk of morbidity and mortality, after rejection, infections, and cancer development, as a consequence of the continuous immunosuppression required [3]. Therefore, kidney disease has a major effect on global health and deserves greater attention for the development and improvement of new detection methods and treatments.

Oligonucleotide (ON) therapeutics, such as those based on antisense oligonucleotides (ASOs), small interfering RNA (siRNA), microRNA (miRNA), aptamers, and decoys, are promising agents that have gained importance during the last decades. As of January 2020, ten oligonucleotide drugs have received regulatory approval from the United States Food and Drug Administration (FDA) and dozens are under clinical trials [4][5]. However, a major obstacle that still hampers the development of new oligonucleotide-based therapies is the difficulty in directing them to specific organs. The kidneys are highly vascularized organs that receive up to 25% of cardiac output, and are susceptible to targeting by most systemic administration routes. Additionally, the glomerular filtration barrier has evolved to filter molecules smaller than 50 kDa in size, which includes the majority of oligonucleotides commonly used in therapeutics, allowing their access to the tubular epithelium. However, this route mostly favors targeting of the liver and other peripheral organs, such as the spleen, due to its vascularized anatomy and scavenging functions. Indeed, at least half of the approved oligonucleotide-based drugs have been developed for liver therapy [4][5]. The unresolved problem of non-specific and off-target effects is a second major obstacle yet to be overcome by improving delivery methods. Importantly, toxicity, and side-effects of oligonucleotides have already been described, including inhibition of unspecific genes, oversaturation of the endogenous small RNA processing pathways, or non-complementary binding of the oligonucleotide to unintended RNAs with a sequence similar to the target RNA [6][7][8][9][10].

2. Oligonucleotides Used in Therapeutics

Oligonucleotide therapies have received considerable attention in recent years, mainly because of their advantages over conventional treatments. Contrary to traditional small molecule drugs, which typically combat disease pathology by modulating the downstream pathways of a disease-causing gene, oligonucleotide-based therapies may directly modify the gene encoding intermediates at fault by targeting DNA or mRNA precursors. Therefore, ONs can also be effective against a wide variety of targets, including proteins and posttranslational protein modifications, carbohydrates, lipids, and metabolites, by directly targeting them with aptamers, or by modulating gene expression. Their versatility is derived from their simple structure, easy synthesis, and the possibility of a rational design. In contrast to conventional drugs that are usually limited to binding specific protein pockets or active sites, DNA and RNA targeting is mostly based on sequence recognition or the presence of unique three-dimensional conformations that allow ONs to potentially bind any target molecule [11][12]. To date, many different oligonucleotide-based therapeutic strategies have been designed, including both DNA and RNA ONs. However, the active roles that RNAs play in cell biology and metabolism, together with our increasing understanding of their role in gene expression and endogenous regulatory machinery, make RNA-based therapeutics the preferred option for their use in medicine [13].

One of the major drawbacks of using extra-cellular oligonucleotides, especially those based on RNA, is their susceptibility to degradation by nucleases and poor pharmacokinetics. To overcome these limitations, such drugs often include chemical modifications of their backbone and nucleobases that increase stability, enzymatic resistance, and efficacy, which will be explained in further detail in the next chapter of this review. A notable advantage of targeting the kidney is that oligonucleotide therapies are rapidly cleared from the circulation via renal filtration, favoring their biodistribution in the kidney over other organs [14]. Here, we will outline the main strategies employed in the development of oligonucleotide-based therapies and the most recent advances for their use in renal diseases, which are also summarized in Table 1.

Table 1. Summary of preclinical studies targeting the kidney with oligonucleotide-based therapies.

STRATEGY	REFERENCE	RENAL TARGETS (Target; Cell/Tissue Type; Animal Model)	SEQUENCE (5′-3′)	CARRIER AN ROUTE OF ADMINISTRA
siRNA	Molitoris et al., 2009 [15]	*Trp53*; PTECs; cisplatin-induced and ischemic AKI models in rats	GAAGAAAAUUUCCGCAAAA	Naked; I
	Takabatake et al., 2009 [16]	*Egfp* and *Tgfb1*; glomeruli; glomerulonephritis model in rats	*Egfp*—GGCUACGUCCAGGAGCGCA *Tgfb1*—GUCAACUGUGGAGCAACACdTdT	Naked, R
	Shimizu et al., 2010 [17]	*Mapk1;* glomeruli; glomerulonephritis model in mice and rats	UGCUGACUCCAAAGCUCUGdTdT	Polyion con nanocarrier
	Gao et al., 2014 [18]	*Aqp1*; PTECs; mice	CGCAACUUCUCAAACCACUTT	Chitosan NP
	Morishita et al., 2014 [19]	*Smad4*; tubulointerstitium and tubule epithelial cells; renal fibrosis model in mice	GAUGAAUUGGAUUCUUUAATT	Naked; I
	Yang et al., 2015 [20]	*Cox2*; peritoneal macrophages recruited to the kidney; UUO model in mice	GGAUUUGACCAGUAUAAGUTT	Chitosan NP
	Zuckerman et al., 2015 [21]	*Egfp;* glomerular mesangium; mice	GGCUACGUCCAGGAGCGCACC	Polycatio cyclodextrir functionali with mann and transfe IV
	Zheng et al., 2016 [22]	*Fas*, *C3* and *RelB*; glomeruli and medullar tubule cells; ischemic AKI in mice	*Fas*—GUGCAAGUGCAA ACCAGAC *C3*—GUGCAAGACUUCCUAAAGA *RelB*—GGAAUCGAGAGCAAACGAA	Naked; R
	Alidori et al., 2016 [23]	*Ctr1, Trp53* and *Mep1b*; cortex and PTECs; AKI model in mice	*Ctr1*—GGCAUGAACAUGUGAAUUGCUGGTT *Trp53*—AGGAGUCACAGUCGGAUAUCAGCCT *Mep1b*—GGAAUUGACCAAGACAUAUUU GATA	Fibrillar car nanotube (fCNT); I
	Eadon et al., 2017 [24]	*Lrp2*; PTECs, mice		Naked or li base transfectio
	Narváez et al., 2019 [25]	*Cd40*; tubulointerstitium; UUO model in mice	GUGUGUUACGUGCAGUGACUU	Naked; I
	Wang et al., 2020 [26]	*p38α* MapK and *p65*; glomerular mesangium and peritubular endothelial cells; glomerulonephritis model in mice	*p38α*—GGUCACUGGAGGAAUUC *p65*—GCGACAAGGUGCAGAAAGA	Liposomal IV
	Thai et al., 2020 [27]	*Trp53*; tubular epithelial cells; AKI model in mice	GAGAAUAUUUCACCCUUCA	DNA nanostructu

STRATEGY	REFERENCE	RENAL TARGETS (Target; Cell/Tissue Type; Animal Model)	SEQUENCE (5′-3′)	CARRIER AN ROUTE OF ADMINISTRA
shRNA plasmid	Wang et al., 2006 [28]	*Mr;* cortical tubule cells; renal hypertension and damage model in rats	CCAACAAGGAAGCCTGAGC	AAV; IV
	Shou et al., 2009 [29]	*Sharp2*; T-cells; transplantation model in rats	ACCCGAACATCTCAAACTTA	Lentivirus vivo perfus
	Zhou et al., 2011 [30]	*Coll*; cortex; rats	GCAACCTGGATGCCATCAA	Lentivirus;
	Fujino et al., 2013 [31]	*Trp53*: cortex and medullar tubule cells; ischemic AKI model in mice		Cationic poly RA
	Espana-Agusti et al., 2015 [32]	*Tsc1* and *Luc*; PTECs, DTECs and interstitium; mice	*Tsc1*—CGGAAGAAGCTGCAATATCTAA *Luc*—CCGCCTGAAGTCTCTGATTAA	Lentivirus;
	Xu et al., 2020 [33]	*Yap* and *Klf4*; renal tubules; ischemic AKI model in mice		Adenovirus
saRNA	Zeng et al., 2018 [34]	*Trpv5*; medullar tubule cells; calcium crystal formation model in rats	AAGGGTCTCATGATTTCTCTA	Naked; R
miRNA antagomir	Chau et al., 2012 [35]	miR21; PTECs; UUO model in mice		Naked; I
	Putta et al., 2012 [36]	miR192; cortex and glomeruli; DN model in mice	GGCTGTCAATTCATAGGTCAG	Naked; S
	Li et al., 2014 [37]	miR204; cortex and medulla; candidemia-induced AKI model in mice	AGGCAUAGGAUGACAAAGGGAA	Naked; I
	Gomez et al., 2015 [38]	miR21; PTECs, Alport nephropathy mouse model		Naked, S
	Wang et al., 2017 [39]	miR107; peritubular endothelial cells; septic AKI model in mice		Complexed atelocollage
	Wilflingseder et al., 2017 [40]	miR182-5p; cortex and medulla; ischemic AKI model in mice, rats and pigs		Naked; IV (I and rats), ex perfusion (
	Wei et al., 2016 [41]	miR489; tubular epithelial cells; ischemic AKI model in mice		Naked; I
	Wei et al., 2018 [42]	miR668; tubular epithelial cells; ischemic AKI model in mice		Naked; I
	Lee et al., 2019 [43]	miR17; PTECs; ADPKD mouse model	GUUUCACGA	Naked; S
	Luan et al., 2020 [44]	miR150; cortex and medulla; renal fibrosis model in mice	UACAAGGGUUGGGAG	Naked; I

STRATEGY	REFERENCE	RENAL TARGETS (Target; Cell/Tissue Type; Animal Model)	SEQUENCE (5′-3′)	CARRIER AN ROUTE OF ADMINISTRA
miRNA mimic	Li et al., 2014 [37]	miR204 and miR211; cortex and medulla; candidemia-induced AKI in mice	miR204—UCCCGGUAAUCCCUUACCUGGUU CCCUUCCUU miR211—UCCCGGCUUUCCCUUACCUGGUUUUCCCCCUU	Naked, I
	Wei et al., 2018 [42]	miR668; cortex and medulla; ischemic AKI model in mice		Lipid-bas transfecti reagent;
	Zhu et al., 2019 [45]	miR199a-3p; tubular epithelial cells; ischemic model AKI in mice		Exosomes
ASO	Shi and Siemann [46]	*Vegf*; Caki-I RCC cell line; xenograft model in mice	CTCACCCGTCCATGAGCCCG	Naked; I
	Daniel et al., 2003 [47]	*Tsp1*; glomeruli; glomerulonephritis model in mice	*Tsp1-1*—TTCTCCGTTGTGATTGAA *Tsp1-2*—CACCTCCAATGAGTT	Naked b electroporati HVJ-liposo RA
	Kausch et al., 2004 [48]	*Ki67*; Renca cells; RCC orthotopic model in mice	ACCAGGTGAGCCGAGGACGCCAT	Naked, I
	Guha et al., 2007 [49]	*Ctgf*; PTECs and mesangial cells; DN model in mice	CCACAAGCTGTCCAGTCTAA	Naked; S
	Wang et al., 2012 [50]	*Kras*; tubular epithelial cells; UUO model in rats	*Kras-1*—ATTCACATGACTATACACCT *Kras-2*—CACACTTATTCCCTACTAGG	Naked; S
	Ravichandran et al., 2014 [51]	*mTORC*; tubular epithelial cells; PKD mouse model	TCCACTTTTCACAGCACTGC	Naked, I
	Ravichandran et al., 2015 [52]	*Agt*; tubular epithelial cells; PKD mouse model	TCTTCCACCCTGTCACAGCC	Naked, I
TFD	Chae et al., 2006 [53]	*Sp1*; tubulointerstitium; UUO model in rats	GGGGCGGGGC	HVJ-liposo RV
	Tomita et al., 2007 [54]	*E2f*; glomeruli; rats		HVJ-liposo RA
Aptamers	Matsui et al., 2017 [55]	RAGE; kidney, heart, eyes, testis; DN model in rats	CCTGATATGGTGTCACCGCCGCCTTAGTATTGGTGTCTAC	Naked; I
	Um et al., 2017 [56]	Periostin; medulla; DN model in mice		PEG-conjug IP
	Taguchi et al., 2018 [57]	RAGE; glomeruli; hypertensive mouse model	CATTCTTAGATTTTTGTCTCACTTAGGTGTAGATGGTGAT	Naked; S
	Zhang et al., 2018 [58]	RCC 786-O cells; xenograft model in mice	ACTCATAGGGTTAGGGGCTGCTGGCCAGATATTCAGATGGTAGGGTTACTATGA	Naked; I

Abbreviations: proximal tubule epithelial cells (PTECs); distal tubule epithelial cells (DTECs); diabetic nephropathy (DN); unilateral ureteral obstruction (UUO); acute kidney injury (AKI); renal cell carcinoma (RCC); intravenous administration (IV); subcutaneous administration (SC); intraperitoneal administration (IP); Renal artery administration (RA); retrograde renal vein administration (RV); renal parenchyma administration (RP); retrograde ureteral administration (RU); nanoparticles (NPs); adeno-associated virus (AAV); hemagglutinating virus of Japan (HVJ); antisense oligonucleotide (ASO); transcription factor decoy (TFD); sequences that are not listed within the table were not specified or could not be found within the corresponding article, or are under the protection of a patent.

2.1. RNA-Based Strategies

2.1.1. siRNA

Short interference RNAs are short (20–27 nucleotides) double-stranded RNAs that target and degrade mRNA in a sequence-specific manner. The guide (antisense strand) is loaded onto Argonaute 2 protein (AGO2), forming the RNA induced silencing complex (RISC), whereas the sense strand is cleaved. The guide strand targets the specific mRNA by complete complementarity and AGO2 catalyzed mRNA cleavage. The RISC and guide strand can be recycled to target multiple mRNA molecules leading to efficient gene silencing [11][13][59]. Alternatively, siRNAs can also be designed to target long non-coding RNAs (lncRNAs), often involved in transcriptional repression, reversing the effects of this negative regulation [4]. siRNAs can sometimes be encoded in the form of short-hairpin RNAs (shRNAs), which are usually delivered to the cell by transduction with viral vectors and, in some cases, integrated into the host genome. shRNA is first expressed as a miRNA and processed into a siRNA duplex by the enzymes Drosha and Dicer, which then follows the same interference mechanism previously described [13]. Two siRNA-based therapies, Patisiran and Givosiran, have already been approved by the regulatory agencies in May 2020, both targeting the liver [4][5]. Interestingly, siRNA technology has also been explored for kidney diseases, showing potential as a therapeutic agent as well as contributing to the understanding of the molecular mechanisms of renal diseases (Table 1) [60]. One of the earliest studies successfully demonstrated the feasibility of using a siRNA-based therapy to ameliorate glomerular sclerosis in a mouse model of glomerulonephritis, by modulating the transforming growth factor beta (TGFβ) pathway as result of *Mapk1* silencing [17]. Likewise, Morishita et al. [19] prevented renal fibrosis by using a siRNA against *Smad4,* suggesting it could be a crucial therapeutic target for renal fibrosis in vivo. Other studies using siRNA-based drugs have focused on reducing the extent of acute kidney injury (AKI), an unavoidable side effect of numerous medical treatments and surgical procedures which deprive the kidney of oxygen. For instance, Glebova et al. [61] evaluated the potential of 53 different siRNA targets, mainly related to apoptosis, inflammation and immune rejection pathways after ischemia-reperfusion caused by transplantation. This approach is still under development but has already shown promising results in a mouse model [22]. In a similar study, the authors effectively evaluated the prophylactic role of siRNA targeting meprin-1β and p53 expression in a cisplatin-induced murine model of AKI. These two proteins play key roles in depolarization and apoptosis after kidney injury [23]. Narváez et al. [25] also demonstrated that the administration of a siRNA therapy against *Cd40* in a mouse model of AKI induced by unilateral ureteral obstruction (UUO) significantly reduced inflammation and promoted kidney repair. Importantly, upon demonstration of a lack of reduction in megalin protein expression in vivo using a siRNA, another recent study has pointed out that the use of siRNA-based therapies in kidney diseases might, indeed, be more suited to prevention of upregulation than reduction of constitutive baseline mRNA expression [24]. Although there is still scope for improvement, siRNA-based therapies in the kidney have shown promising results, especially in the prevention of AKI where some clinical trials have already been carried out [60].

2.1.2. saRNA

Small activating RNAs are double-stranded RNAs of 21 nucleotides in length that possess two nucleotide overhangs on both ends. Comparable to siRNA, saRNAs are loaded onto AGO2, where the sense strand in cleaved. Then, the saRNA–AGO2 complex is translocated to the nucleus, binding to complementary promoter regions and recruiting key elements for transcription initiation [62]. Thus, saRNA has an identical structure and chemical components as siRNA, but its biological function is the opposite of siRNA, since it acts to enhance gene transcription. The use of saRNA with therapeutic purposes has been recently tested in an in vivo model of ethylene glycol (EG)-induced calcium oxalate (CaOx) kidney crystal formation in rats. Using this approach, the authors significantly enhanced the expression of *Trpv5*, a key protein mediating calcium transport and reabsorption in the kidney, and achieved a reduction in CaOx crystal formation by promoting calcium reabsorption [34].

2.1.3. miRNA

miRNA constitute a class of single-stranded non-coding RNAs (ncRNAs) with a length of approximately 22 nucleotides after maturation. Their natural biogenesis starts in the nucleus, where they are transcribed as pri-miRNAs and cleaved into pre-miRNAs (~70 nucleotides in length) by a multiprotein complex mainly comprised of the Drosha and Dcrg8 proteins. Double-stranded pre-miRNAs are then exported to the cytoplasm and processed by Dicer into mature miRNAs (~22 nucleotides in length) [63]. For their mechanism of action, miRNAs are loaded onto AGO2 to form RISC, guiding the complex to its complementary binding site in the target transcript, commonly found in the 3′ untranslated region (UTR). In contrast with siRNAs, miRNAs typically bind with partial complementarity and usually promote translational repression by triggering mRNA decay through deadenylation and decapping. Different miRNAs can bind to the same transcript by overlapping or non-overlapping sites [11][13]. In general, there are three approaches to developing miRNA-based therapeutics: (1) anti-miRNA oligonucleotides (AMOs) or miRNA antagomirs, also known as antisense oligonucleotides (ASOs), which antagonize endogenous miRNAs by steric blocking of the miRNA within the RISC complex [4][11]; (2) miRNA sponges, which are genetically engineered competitive miRNA inhibitors designed by insertion in tandem of multiple binding sites of targets of analogous miRNAs or mRNAs [64]; (3) miRNA mimics, which are engineered double-stranded miRNAs that replace, improve or supplement the function of native miRNAs [11][59]; (4) target site blockers (TSB), designed to recognize and mask the regulatory sequences of miRNAs within a specific mRNA, with the potential to maintain the rest of the mRNA network unaffected [4].

miRNAs play an important role in the negative regulation of post-transcriptional gene expression. Consequently, aberrant miRNA expression is implicated in the development and progression of numerous diseases, and multiple families of miRNAs are shown to be dysregulated in kidney disease. The role of miRNAs in molecular pathology has already been reported in AKI and kidney transplantation [65][66], polycystic kidney disease (PKD) [67][68], and renal fibrosis [69][70], which represents the final outcome and most relevant pathological event of CKD. This, together with the fact that miRNAs can be detected in exosomes in plasma and urine, indicate them as suitable and attractive new biomarkers for diagnostic purposes and disease monitoring [71][72]. Importantly, up/downregulated miRNAs also represent novel therapeutic targets for kidney diseases, whose potential has already begun to be explored in animal models (Table 1). One of the earlier studies was carried out by Chau et al. [35], who successfully used an anti-miR21 antagomir, which limited injury and kidney fibrosis in two murine models of AKI. Similarly, renal fibrosis was also ameliorated by an anti-miR192 antagomir in a mouse model of diabetic nephropathy (DN) [36]. The use of miR204 and miR211 mimics has also proved effective at reducing the severity of kidney injury in a mouse model of systemic candidiasis [37] and inhibition of miR107 using an antagomir significantly prevented tubular cell injury in a mouse model of AKI induced by sepsis [39]. Moreover, the use of miRNA-based approaches has also shown potential in the treatment and understanding of the basis of ischemic-kidney injury, as demonstrated by a collection of recent studies (Table 1). One of the most common causes of ischemic AKI is transplantation. In that regard, a recent study has demonstrated the applicability of using antisense technology against the miR182-5p target to improve kidney function and morphology, employing a model of ischemic AKI in rats [40]. In addition, Wei et al. [41][42] elucidated the molecular mechanisms of miR489 and miR668 in the protection of the kidney during ischemia, indicating the possibility of using these miRNA or miRNA mimetics as therapeutic agents. Similarly, another study demonstrated the protective role of miR199a-3p in an ischemia-reperfusion model in vivo [45]. Taken together, these findings are promising for future evaluation of the clinical utility of miRNA mimetics and inhibitors targeting key pathologic renal pathways. However, translation of preclinical findings is sometimes complicated, as a deep understanding of the miRNA regulatory networks underlying the disease is needed. In some cases, targeting key points in the same network may prove more effective. Additionally, most miRNAs are regulated in a cell-type or organ-specific manner; thus, the possibility of off-target and undesired effects in unrelated organs is high. This problem could explain why few investigations using miRNAs-based therapies move forward to the clinical stage. In fact, only four miRNA-based therapies have reached clinical development, two of them dedicated to renal disease [66]. One of the drugs was developed for Alport nephropathy, a genetic disorder characterized by chronic glomerulonephritis that progresses to end-stage renal disease in young adult life. The antagomir against miR21 was effectively evaluated in a mouse model, where the animals displayed substantially milder disease and significantly improved survival after treatment [38]. The drug RG012 targeting miR21 is currently undergoing a phase 2 clinical trial (clinical trial identifier NCT02855268). The second drug is RGLS4326, an antagomir inhibiting miR17 developed for the treatment of autosomal dominant PKD (ADPKD), a genetic disorder caused by mutations in either *PKD1* or *PKD2* genes resulting in hyperproliferation of the renal tubular cells and cyst formation [43]. Treatment with RGLS4326 attenuated cyst growth in several PKD mouse models and human ADPKD models in vitro and is now in a phase 1 clinical trial (clinical trial identifier NCT04536688).

2.2. RNA/Protein-Based Strategies (CRISPR)

The development of the revolutionary CRISPR/Cas9 gene editing technology is driving the progress of RNA therapeutics forward in a similar way. This system, initially discovered as a form of acquired immunity in bacteria and archaea, consists of a protein (CRISPR-associated nuclease Cas9) and an oligonucleotide guide RNA (sgRNA). While the gRNA directs the nuclease to a specific genomic location adjacent to a protospacer adjacent motif (PAM) sequence, correct base-pairing activates Cas9 nuclease domains, which, cut the DNA, resulting in a double strand-break. In an attempt to repair the damage, eukaryotic cells can use two different mechanisms, nonhomologous end joining (NHEJ) and homology-directed repair (HDR). The NHEJ repair mechanism is prone to introduce small insertion or deletion errors, causing frameshift mutations and leading to gene knockout by disruption of the open reading frame. Conversely, when a donor template is introduced, it can be utilized by the HDR mechanism and harnessed to introduce a new sequence bearing a mutational correction or sequence knock-in in the desired loci [73][74]. A great advantage of the system, as consequence of its modularity, is that it allows for the testing of many different potential sgRNA while maintaining the protein component invariant. Importantly, although this system was initially developed to target DNA, RNA-targeting and interference has also been possible due to the development of new engineered nucleases such as Cas13 and RNA-targeting Cas9 (rCas9). Similarly, nuclease deficient Cas9 variants (dCas9) restricted from generating DNA breaks have been fused to transcriptional activation (VPR) [75], silencing proteins (KRAB) [76], or epigenetic modifiers [73][74], which can then be targeted to specific gene promoters, regulating gene expression. Catalytically inactive Cas9 and Cas13 variants have also been fused to other types of functional domains, such as base-pair editors or deaminases able to catalyze A-T to G-C transitions, allowing for single-base edition at the DNA and RNA level without the need to generate double or single-strand breaks [74], or reporter proteins, to visualize DNA or RNA [73]. Nonetheless, despite the fact that the CRISPR/Cas9 system has emerged as a promising platform with a wide variety of applications in biology research and therapeutics of human disease, efficient and safe delivery of its components to target cells in vivo remains challenging [77].

The use of the CRISPR/Cas9 system as a therapeutic approach for renal diseases has great potential, as a significant proportion of these diseases, such as autosomal dominant PKD or Alport syndrome, arise as consequence of genetic mutations. Nevertheless, gene editing in solid organs still faces the challenge of effective delivery to specific cells or

tissues. Thus, the use of this tool in kidney research has so far been limited to its application in the development of novel in vitro (using human organoids) and in vivo models of renal disease. Such models are very useful in understanding the molecular mechanisms underlying renal diseases, as well as in the identification of new genes responsible for their progression and that could represent potential therapeutic targets [78][79]. Another exciting potential use of CRISPR/Cas9 technology is focused on expanding the available sources of kidneys for transplantation. Some authors have proposed the possibility of transplanting organs from other species such as pigs (xenotransplantation), an approach that, to this day, would lead to an extreme human immune response and rejection of the donor organ. However, CRISPR/Cas9 has appeared as a promising tool that could circumvent this limitation. In this direction, some authors have already employed CRISPR/Cas9 to genetically modify swine eggs in order to generate animals lacking carbohydrate xenoantigen, whose recognition by human and non-human primate antibodies was effectively diminished [80], or to generate I MHC null pigs [81]. The first demonstration of the feasibility of this approach was published by Higginbotham et al. [82], where the authors achieved effective pig-to-primate long-term transplantation (>125 days).

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