

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# siRNA-Induced RNAi Therapy in Acute Kidney Injury

---

Cheng Yang and Bin Yang

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/61838>

---

## Abstract

siRNA therapy has great potential in humans, and its applications have been significantly improved. The kidney is a comparatively easy target organ of siRNA therapy due to its unique structural and functional characteristics. Here, we reviewed recent achievements in the design, delivery, and utilization of RNAi with a focus on kidney diseases, in particular acute kidney injury. In addition, the perspectives and challenges of siRNA therapy such as increasing its serum stability and immune tolerance, targeting single/double/multiple genes, cell/allele-specific delivery, time-controlled silencing, and siRNA-modified stem cell therapy were also discussed. Finally, selecting target genes and therapeutic time windows were addressed.

**Keywords:** Small interfering RNA, kidney diseases, delivery, off-target effects, compensative responses

---

## 1. Introduction

Acute kidney injury (AKI) is very common and critical in clinical practice. The incidence of hospital-acquired AKI is increasing, and many patients require renal replacement therapy [1]. AKI significantly increases the risk of chronic renal disease, end-stage renal disease (ESRD), and death, presenting a major burden to the patient and the health care system. Because of high metabolic activity in handling and transporting ions, amino acids, and other small molecules, the kidney is highly susceptible to acute injuries from lack of sufficient perfusion, exposure to, and accumulation of nephrotoxic substances. Despite numerous clinical trials, AKI remains a cause of significant morbidity and mortality for which there is no effective intervention [2].

RNA interference (RNAi) is a highly conserved biological phenomenon in all eukaryotes, including renal cells. Although RNAi naturally exists, synthetic artificial siRNA exerts similar

effects as natural endogenous microRNA (miRNA). Both sense and antisense strands of siRNA can be synthesized separately and annealed to form double stranded siRNA duplexes *in vitro*. After the siRNA is delivered into the cytoplasm, the artificial siRNA silences the target gene using similar biological processes as endogenous miRNA. Since the introduction of 21-nucleotide artificial siRNAs that triggered gene silencing in mammalian cells [3], synthetic siRNA has generated much interest in biomedical research, in which the kidney is one of important key players. siRNA as a strategic molecule has been highly expected in the field of innovative therapy. Because siRNA is highly efficient at gene silencing, it is possible to develop specific siRNA-based drugs that could target any genes, including those that have unknown pharmacological antagonists or inhibitors. Different types of synthetic siRNA have been tested for their efficacy in various disease models, including cancer [4], autoimmune disorders [5], cardiovascular injuries [6], and organ transplantation [7], including native and transplanted kidney injuries [8].

As siRNA is a posttranscriptional regulator, it must first be absorbed into the target cells. Therefore, the kidney could be an excellent target organ for siRNA therapy because it benefits from rapid and vast blood flow physically, subsequent glomerular filtration, and tubular absorption. In fact, the systemic administration of siRNA leads to rapid uptake by the kidney, yielding a significant decrease of target protein expression [8]. Consequently, RNAi by siRNA has advantages for the treatment of renal diseases due to the unique urological system [9]. In addition, the preservation of donor kidneys before transplantation also provides a suitable time window for the intervention of siRNA.

In this chapter, we highlighted the design and delivery of siRNA and its therapeutic effects with a focus on kidney diseases. We also discussed future challenges of siRNA therapy, targeting single/double/multiple genes, cell/allele specific delivery, time-controlled silence, and siRNA-modified stem cell therapy.

## 2. Current principle of siRNA design

The design of potent siRNAs has been greatly improved over the past decade. The basic criteria for choosing siRNAs include the consideration of thermodynamic stability, internal repeats, immunostimulatory motifs, such as GC content, secondary structure, base preference at specific positions in the sense strand, and appropriate length [10].

Chemical modifications significantly enhance the stability and uptake of naked siRNAs. Importantly, siRNAs can be directly modified without crippling the silencing ability. Chemical modifications have been rigorously investigated for virtually every part of siRNA molecules, from the termini and backbone to the sugars and bases, with the goal of engineering siRNA to prolong half-life and increase cellular uptake. The most common chemical modification involves modifying the sugar moiety. For example, the incorporation of 2'-fluoro (2'-F), -O methyl, -halogen, -amine, or -deoxy can significantly increase the stability of siRNA in serum.

Locked nucleic acid (LNA) has been also applied to modify siRNA. The commonly used LNA contains a methylene bridge connecting the 2'-oxygen with the 4'-carbon of the ribose ring.

This bridge locks the ribose ring in the 3'-endo conformation characteristic of RNA [11]. Additionally, recent studies, including ours [12], have proven the efficacy of LNA-modified siRNA in terms of prolonged half-life in serum, but without detectable adverse effects, suggesting that the natural RNAi machinery could accommodate a certain degree of alterations in the chemical structure of siRNAs [13].

### 3. siRNA delivery

The biggest obstacle faced by siRNA therapies is the *in vivo* delivery of genetic materials. The systemic delivery of synthetic siRNA has the most medical and commercial potential. This type of delivery, however, remains a major challenge for translating siRNA from the research to the clinic. Overcoming the delivery challenge requires effective siRNA delivery vehicles. The virus-based delivery system, while efficient, may be fatally flawed due to raised safety concerns, such as inducing mutations and triggering immunogenic and inflammatory responses [14]. Therefore, extensive research had been performed to develop efficacious nonviral delivery systems, including direct chemical modification of siRNA (as described above) and/or optimization of delivery materials, such as liposome formulation, nanoparticle conjugation and antibodies that target cellular moieties [14].

To date, studies on synthetic siRNA therapy have been performed in a variety of cell culture and rodent models [15] that produced exciting results and were cost effective but failed to faithfully mimic human diseases. Therefore, large animal models, such as porcine models, are indispensable to compensate for the limitations of rodent models due to their greater similarity to human beings. The investigations on siRNA conducted in our laboratory have reflected this trend in the field [7, 12, 16].

#### 3.1. Direct delivery of synthetic siRNA *in vitro/ex vivo*

The siRNA could be easily transduced into various cells for scientific research. For example, we transfected synthetic caspase-3 siRNA in porcine proximal tubular cells (LLC-PK1) using cationic lipid-based transfection reagent. The caspase-3 siRNA inhibited apoptosis and inflammation in LLC-PK1 cells that were subjected to hydrogen peroxide stimulation [17]. In addition to *in vitro* delivery of siRNA, *ex vivo/in vivo* siRNA delivery to target organs is an indispensable step before its clinical application. If it was directly delivered into the kidneys, siRNA could obtain higher local concentrations, which would result in improved gene silencing efficacy. During kidney transplantation, *ex vivo* local delivery of siRNA into the donor kidney is feasible because it could be facilitated by the unique structure of the kidney and the characteristics of kidney transplantation. We utilized an *ex vivo* isolated porcine kidney reperfusion system to assess the efficacy of naked caspase-3 siRNA. The caspase-3 siRNA was directly infused into the renal artery (locally) and autologous blood perfusate (mimic systemic delivery) before 24-h cold storage (CS), followed by a further reperfusion for 3 h. The results demonstrated that the caspase-3 siRNA improved ischemia reperfusion (IR) injury with reduced caspase-3 expression and apoptosis, better renal oxygenation, and acid-base homeo-

stasis [16]. These promising proof-of-principle observations provide valuable guidance for further development before siRNA used in clinical practice.

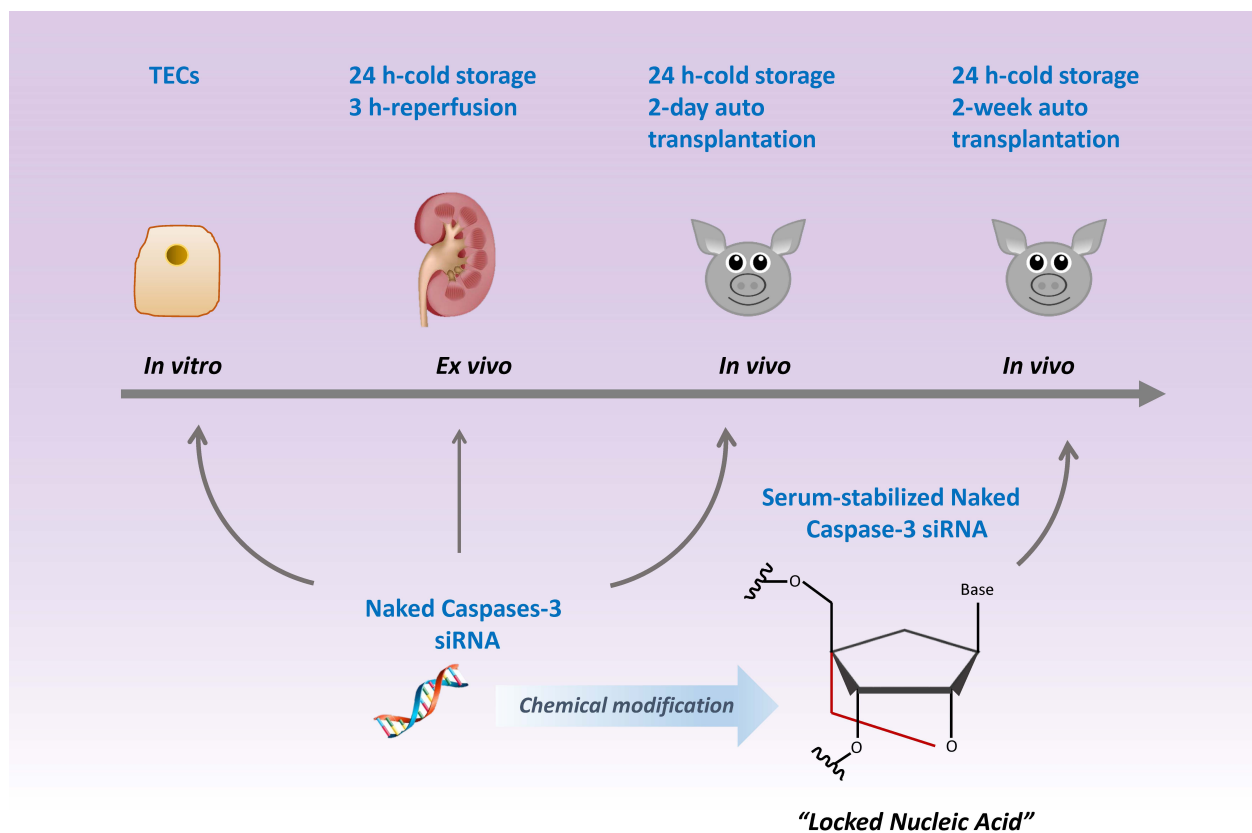
### 3.2. Local or systemic delivery of siRNA *in vivo*

Delivery of siRNA via *ex vivo* route can be applied in donor kidneys, but most renal diseases need *in vivo* delivery. Based on the anatomical and physiological characteristics of the kidney, local delivery can be achieved through several routes: (1) renal artery, first targeting the glomeruli or tubules [18, 19]; (2) renal vein, predominately targeting tubulointerstitium [20]; (3) intraureteral, administered into the renal pelvis and interstitium [21]; and (4) subcapsular administration, achieves intraparenchymal silencing [22]. Due to the rich blood flow through the glomeruli, siRNA injection via the renal artery followed by electroporation could silence specific genes in the glomeruli, such as TGF- $\beta$ 1, which subsequently ameliorates matrix expansion in an experimental glomerulonephritis model [18].

We then used naked caspase-3 siRNA in a porcine kidney autotransplant model for the first time. The left kidney was retrieved from mini pigs and was infused with University of Wisconsin solution, with or without 0.3 mg of naked caspase-3 siRNA, via the renal artery, which was followed by renal artery and renal vein clamping for 24-h cold storage (CS, mimicking donor kidney preservation before transportation in clinic). After right nephrectomy, the left kidney was autotransplanted into the right nephridial pit for 48 h without systemic siRNA treatment (Figure 1). The expression of caspase-3 mRNA and active caspase-3 protein, as well as its precursor, was downregulated by siRNA in the post-CS kidney. In the siRNA preserved posttransplant kidney, however, caspase-3 precursor was further decreased while caspase-3 mRNA, and its activated subunits were upregulated, which resulted in increased apoptosis and inflammation. This study indicated that the naked caspase-3 siRNA was effective for cold preservation but was not effective at protecting posttransplant kidneys, which may be due to systemic compensative responses overcoming local effects. Therefore, to overcome the systemic response and to prolong the therapeutic time window, we subsequently utilized a novel, serum-stable caspase-3 siRNA, both locally as before and systemically via a pretransplantation intravenous injection, and observed the animals for up to 2 weeks posttransplantation. The effectiveness of the novel caspase-3 siRNA was confirmed by downregulated caspase-3 mRNA and protein in the post-CS and/or posttransplant kidneys, as well as reduced apoptosis and inflammation. More importantly, renal function, associated with active caspase-3, HMGB1, apoptosis, inflammation, and tubulointerstitial damage, was improved by this novel, serum-stable caspase-3 siRNA [12].

It has also been revealed that an injection of a single-dose Fas siRNA through the renal vein post ischemia provided a survival advantage in a murine IR model, which was due to the antiapoptosis and antiinflammation effects of the Fas siRNA [20]. Unilateral ureteral obstruction (UUO) is a well-established model for tubulointerstitial fibrosis. Xia et al. injected the siRNA of heat shock protein 47 once via the ureter at the time of UUO preparation, leading to significantly reduced fibrosis-related protein expression and a remarkable alleviation of the accompanying interstitial fibrosis [21]. Subcapsular administration is still used in some experiments due to its unique advantages, although it requires an invasive procedure and has





**Figure 1.** Schematic drawing showed a series of our studies using caspase-3 siRNA. The caspase-3 siRNA was first used to protect porcine renal tubular epithelia cells against hydrogen peroxide-induced injury [17]. The renoprotection of naked caspase-3 siRNA with the same sequences was further validated in a porcine *ex vivo* isolated reperfusion model and showed that the siRNA was effective for cold preservation [16], but not in autotransplanted kidneys without systematic siRNA treatment [7, 12, 16]. Finally, the chemically modified siRNA of caspase-3 via locked nucleic acid stabilized the siRNA in serum and significantly protected autotransplanted kidneys [7, 12, 16].

limitations in clinical practice. Cuevas et al. reported that an infusion of DJ-1 (an antioxidant)-specific siRNA into the subcapsule silenced DJ-1 expression in the renal cortex and increased ROS production [22].

Systemic delivery is a common and convenient clinical practice, although current clinical trials using siRNAs are almost directly administered to the target site, such as the nostril, eye, and lung, thereby avoiding the complexity of systemic delivery [23]. The most common method of systemic siRNA delivery is a hydrodynamic intravenous injection with hydraulic pressure to assist siRNA cell entry. However, the pharmacokinetic metabolism of siRNA is more complicated during systemic delivery because siRNAs can be rapidly degraded by nucleases in the serum and cleared by the kidney and liver. To enhance the *in vivo* efficacy of siRNA treatment, a variety of approaches have been attempted for both siRNA itself and delivery techniques [22–24], as mentioned above.

Due to its anatomical and physiological characteristics, the kidney is the most preferable target organ of systemic siRNA administration. siRNA access to the kidney is thought to be dependent on the filtration and reabsorption functions of the kidney. Proximal tubule cells (PTCs) are

the primary site for rapid and extensive endocytic uptake of siRNA within the kidney following glomerular filtration. In an AKI model, naked synthetic siRNA targeting p53 that was intravenously injected 4 h after renal ischemic injury significantly reduced upregulated p53 expression and protected both the PTCs and kidneys [25]. In another study performed by Zheng et al., siRNA was systematically injected to target complement 3 (C3) and caspase-3 in a murine renal IR injury model. The results showed that the level of serum creatinine and blood urea nitrogen was significantly decreased in the siRNA-treated mice [26]. As most of AKI may be not associated with renal surgery, the systemic siRNA delivery might be a desirable approach. However, for kidney transplantation, in which IR injury is inevitable, local siRNA delivery via any above-mentioned method is feasible and more effective.

### 3.3. Cell-specific delivery

As proposed by precision medicine, individual person should receive customized healthcare including diagnosis and intervention. The dysfunctional cells are the true targets for siRNA delivery. For instance, it is known that p53 in PTCs promotes AKI, whereas p53 in other tubular cells does not [27]. It is also expected that apoptosis-inducing siRNA should be directly delivered into tumor cells rather than the surrounding normal cells. Therefore, the cell-specific delivery method is our key point in the next generation of siRNA development.

Recently, antibody conjugation technology has made tumor-targeting drug delivery systems available. The conjugate can be regarded as a “guided molecular missile” that specifically targets unique antigens [28]. Inspired by cancer therapy strategies, siRNAs have also been “packed” to be delivered to target organs, even cells. Recently, a type of asymmetric liposome particle (ALP) has been developed, which highly efficiently encapsulates siRNA without nonspecific cell penetration. The ALPs protected siRNA from ribonuclease degradation. ALPs without any surface modification elicited almost no uptake into cells, while the polyarginine peptide surface-modified ALPs induced nonspecific cell penetration [29]. Leus et al. delivered siRNA targeting vascular cell adhesion molecule-1 (VCAM-1) into inflammation-activated endothelium using anti-VCAM-1-SAINTPEGarg formulated with additional 2 mol% DOPE-PEG<sub>2000</sub> *in vivo*. The antibody recognizes VCAM-1, which can create specificity for inflammation activated endothelial cells. The siRNA homed to VCAM-1 protein expressing vasculature in TNF- $\alpha$ -treated mice without any kidney and liver toxicity [30]. These results represent great progress in siRNA delivery system development. Antibody-mediated specific recognition rather than virus-mediated recognition may be a mainstream in the future.

### 3.4. Allele-specific RNAi

RNAi, in addition, discriminates between two sequences only differing by one nucleotide conferring a high specificity of RNAi for its target mRNA. This property was used to develop a particular therapeutic strategy called “allele-specific RNAi” devoted to silence the mutated allele of genes causing dominant inherited diseases without affecting the normal allele. Therapeutic benefit was now demonstrated in cells from patients and animal models, and promising results of the first phase Ib clinical trial using siRNA-based allele-specific therapy were reported in pachyonychia congenita, an inherited skin disorder due to dominant

mutations in the *keratin 6* gene [31]. The allele-specific siRNA silencing of the mutant *keratin 12* allele was also applied in corneal limbal epithelial cells grown from patients with Meesmann's epithelial corneal dystrophy [32, 33]. It has also been shown that modified siRNAs conferring allele-specific silencing against disease-causing ALK2 mutants found in fibrodysplasia ossificans progressiva, without affecting normal ALK2 allele [34].

### 3.5. Delivery of siRNA using a cargo system

Although lentivirus vectors as vehicles together with liposome reagents are widely applied in the transduction of siRNA, nanoparticle systems have emerged in last few years as an alternative carrier for advanced diagnostic and therapeutic applications. The nanotechnology offers many merits and overcomes the range of challenges/barriers summarized in the previous section, such as the bioavailability and biodistribution of therapeutic agents. Recent reports have demonstrated that the kidney, the glomerulus especially, is a readily accessible site for nanoparticles. Zuckerman et al. intravenously administered nanoparticles containing polycationic cyclodextrin and siRNA/CDP-NPs, most of which deposited in the glomerular mesangial areas. Furthermore, the cultured mouse and human mesangial cells could rapidly internalize siRNA/CDP-NPs. This process could be accelerated by attaching targeting ligand mannose or transferrin to the nanoparticle surface [35].

Complex nanoparticles, especially cationic polyplexes/lipoplexes and liposomes, dominated the scene in the early days of RNAi therapeutic development. Their main advantage lies in their endosomal release activity and their ability to concentrate multiple RNAi triggers in one particle [36]. Forbes and Peppas cross-linked polycationic nanoparticle formulations using ARGET ATRP or UV-initiated polymerization. The advantage of this method is the one-step, one-pot, and surfactant-stabilized monomer-in-water synthesis, which is simpler and faster compared with traditional complicated multistep techniques involving toxic organic solvents [37].

Regardless of how much each mechanism plays in the transport of the drug, cell entry remains a focus for drug design and discovery. An exciting and relatively new approach to transporting pharmaceutical agents into cells is making use of cell-penetrating peptides (CPPs). CPPs are relatively short peptides, typically less than 30 amino acids, and could be vectors for the delivery of genetic and biologic products. CPPs provide a safe, efficient, and noninvasive mode of transport for various cargos into cells [38]. Recently, van Asbeck et al. discovered that CPP/siRNA complexes with the most negative zeta-potentials in serum were the most resistant to siRNA release over a 20-h incubation period compared to less negatively charged complexes [39]. They also found that the zeta-potential of CPP/siRNA complexes in serum did not correlate with improved cellular association, which might demonstrate the importance of serum proteins or CPP conformation on the ability of CPP/siRNA complexes to associate with the cell membrane. Huang et al. designed a bifunctional peptide named RGD10-10R, by which siRNA was delivered *in vitro* and *in vivo*. Because of their electrostatic interactions with polyarginine (10R), negatively charged siRNAs were readily complexed with RGD10-10R peptides, forming spherical RGD10-10R/siRNA nanoparticles. This is also a novel siRNA delivery tool [40].



Gemini or dimeric lipids (GCLs) are a recent type of amphiphilic molecules that contain two polar headgroups linked by a rigid or flexible spacer that may be hydrophobic or hydrophilic. As each headgroup has a hydrophobic moiety, GCLs may be considered as two conventional monomeric surfactants connected by a spacer group [41]. GCLs have been proved as promising candidates to transfect nucleic acids in gene therapy. The molecular structure of the GCLs offers a high number of alternatives to develop and to improve their capability as transfecting agents.

#### 4. siRNA therapy in AKI

To date, siRNA therapy has been successfully applied in a variety of acute kidney injuries. IR injury is the primary cause of AKI, particularly during kidney transplantation, in which the kidney is exposed to hypoxia and experiences a series of oxidative, inflammatory and apoptotic responses [42, 43]. Consequently, specific siRNAs targeting critical molecules that are involved in the processes of oxidation, inflammation, and apoptosis have been developed.

Caspase-3, which mediates apoptosis and inflammation, is upregulated by IR injury. Multiple pharmacological interventions against caspase-3, including enzyme inhibitors and genetic modification, have been investigated. In recent years, our group studied the delivery and efficacy of caspase-3 siRNA in *in vitro*, *ex vivo*, and *in vivo* kidney injury models. The synthetic caspase-3 siRNA was initially tested in porcine PTCs (LLC-PK1), with or without hydrogen peroxide ( $H_2O_2$ ) stimulation. Apoptotic cells and activated IL-1 $\beta$  protein expression were significantly reduced by the caspase-3 siRNA, with improved cell viability [17]. This outcome led to siRNA application in an isolated organ perfusion system, as described above, and the efficacy of caspase-3 siRNA was further proven, in terms of silenced caspase-3 mRNA and protein expression, attenuated inflammation and apoptosis, and improved renal function and histology [16].

The porcine kidney preserved by caspase-3 siRNA was then autotransplanted in a 2-day model. However, the transplanted kidney was not protected without systemic treatment of the recipient. Moreover, new serum-stabilized caspase-3 siRNAs were applied locally in kidney preservation and intravenously in recipient in a 2-week autotransplant model. The transplanted kidneys were protected without significant off-target effects. These serials of step-by-step studies provided promising evidence to support siRNA treatment to be further applied in clinic.

p53, another pivotal protein in the apoptotic pathway, has been identified as a mediator of transcriptional responses to IR injury [44]. Molitoris et al. revealed that intravenously injected p53 siRNA attenuated ischemic and cisplatin-induced AKI [25]. Fujino et al. also tested the efficacy siRNA targeting p53 via transarterial administration siRNA injected into the left renal artery immediately after ischemia improved tubular injury and downregulated GSK-3 $\beta$  expression [45]. In a diabetic mouse model, p53 inhibition by siRNA also reduced ischemic AKI [46].

Silencing of other important transcription factors or immunity related receptors using siRNAs have also been studied. Renal IR injury and inflammation are related to postsurgical healing and both processes can be influenced by toll-like receptor (TLR) signals. Effective TLR9 silencing by siRNA decreases renal cell apoptosis, mitigates AKI severity, and increases the mice survival [47]. NF- $\kappa$ B, a pro-inflammatory transcription factor induced by TLR and other signals, plays a key role in AKI. NF- $\kappa$ B activation depends on the activation of the inhibitor of  $\kappa$ B kinase  $\beta$  (IKK $\beta$ ). Wan et al. demonstrated that silencing IKK $\beta$  using siRNA diminished inflammation and protected the kidneys against IR injury [19]. These studies clearly demonstrate the therapeutic potential of siRNA-induced silencing of key AKI mediators, which are activated and involved in the pathways of apoptosis, inflammation, immunity, etc.

## 5. Off-target side effects and toxicities of siRNA

The siRNA has been likened to a “magic bullet” due to this potency and specificity, but off-target side effects and toxicities create additional challenges for researchers. The induction of various side effects may be caused by unexpected perturbations between RNAi molecules and cellular components. The off-target effects of siRNA were first reported by Jackson and colleagues in 2003 [48]. Broadly speaking, off-target effects can be siRNA specific or nonspecific. The former are caused by limited siRNA complementarity to nontargeted mRNAs. The latter, resulting in immune- and toxicity-related responses, are due to the construction of the siRNA sequence, its modification, or the delivery vehicle.

The off-target effects associated with siRNA delivery fall into three broad categories: (1) miRNA-like off-target effects, referring to siRNA-induced sequence-dependent regulation of unintended transcripts through partial sequence complementarity to their 3'UTRs; (2) inflammatory responses through the activation of TLR triggered by siRNAs and/or delivery vehicles (such as cationic lipids and viruses); and (3) widespread effects on miRNA processing and function through the saturation of the endogenous RNAi machinery by exogenous siRNAs [49, 50].

### 5.1. miRNA-like off-target effects

The siRNAs and miRNAs share similar machinery downstream of their initial processing. Using several different siRNAs targeting the same gene, microarray profiling showed that each siRNA produced a unique, sequence-dependent signature. Sequence analysis of off-target transcripts revealed that the 3' UTR regions of these transcripts were complementary to the 5' end of the transfected siRNA guide strand [48]. It is now understood that for the off-targeting effects to occur, a perfect complementarity between the seed region of the antisense strand such as nucleotide positions 2–7 or 2–8 and the 3' UTR of the transcript is necessary [49, 51]. Silencing the set of original off-target transcripts could be induced by base mismatches in the 5' end of siRNA guide strands. However, a new set of off-target transcripts within 3' UTRs that were complementary to the mismatched guide strand could be generated [49].

RNAi regulation by miRNAs involves partial complementarity between the targeting RNA and miRNA. Because miRNAs cause gene silencing through mRNA degradation and translation inhibition, the siRNA-mediated off-target effects may also be acting at two levels. For this reason, there should be greater emphasis on improving siRNA design as well as monitoring gene and protein levels following RNAi therapy to account for any off-target effects.

## 5.2. Recognition and stimulation of the innate immune system

The recognition and stimulation of the immune system are nonspecific off-target effects of siRNA therapy. The RNA-sensing pattern recognition receptors (PRRs), localized in endosomes, are the most important components of the innate immune system. The responses of PRRs to siRNAs are either TLR-mediated or non-TLR-mediated. The PRR responses are also associated with siRNA sequence-specific side effects and have recently attracted many attentions from researchers [52]. RNA-sensing TLRs (TLR3 and TLR7) are predominantly located intracellularly and recognize nucleic acids released from invading pathogens. The non-TLR-mediated innate immune responses triggered by siRNA binding are linked to RNA-regulated expression of protein kinase (PKR) and retinoic acid inducible gene 1 (RIG1), which further induce caspase-3 and NF- $\kappa$ B expression, respectively. The activation of PRRs generates excessive cytokine release and subsequent inflammation [53].

Based on this second type of off-target RNAi effects, our group further investigated the mechanism of how short-acting caspase-3 siRNA impaired posttransplanted kidneys. The results suggested that the amplified inflammatory responses in caspase-3 siRNA preserved autotransplant kidneys were associated with TLR3, TLR7, and PKR activation, which may be due to systemic compensative responses, although persistent actions initiated by short-acting caspase-3 siRNA cannot be completely excluded [54]. Other studies have also indicated that the horseshoe-like structure of TLR3 facilitates dsRNA recognition [55, 56]. Interactions between TLR3 and dsRNA were originally reported in 2001 when TLR3-deficient mice exhibited reduced immune responses to dsRNA viruses [57].

Several studies have demonstrated that the immune response to siRNAs is cell type-dependent due to the selective expression of TLRs. siRNAs stimulate monocytes and myeloid dendritic cells through TLR8 to produce proinflammatory cytokines, or activate plasmacytoid dendritic cells through TLR7 to produce type I interferons [58–60]. In addition, the volume of hydrodynamic naked siRNA delivery influences immune activation. Rácz et al. compared the immune responses induced by 50  $\mu$ g siRNA dissolved in either low-volume (1 mL/mouse) or high-volume (10% of body weight, 2.5 mL/mouse in average) physiological salt solution delivered *in vivo*. Low-volume hydrodynamic injection induced slight alanine aminotransferase (ALT) elevation and mild hepatocyte injury, whereas high-volume hydrodynamic injection resulted in higher ALT levels and extensive hepatocyte necrosis. High-volume hydrodynamic injection also led to a time-dependent slight increase in IFN-related gene expression [61]. Collectively, these studies suggest that there is a need for improving siRNA design, establishing experimental controls and carefully interpreting results.

6. From bench to bedside: Clinical trials

The numbers of RNAi-based preclinical studies and clinical trials have grown over the past several years. To date, there have been 27 registered clinical trials using siRNA worldwide. These studies include retinal degeneration, dominantly inherited brain and skin diseases, viral infections, respiratory disorders, metabolic diseases, and of particular note, kidney diseases. In 2011, Quark Pharmaceuticals completed a phase I, randomized, double-blind, dose escalation, safety, and pharmacokinetic study (NCT00554359) on QPI-1002, also designated I5NP, which was a synthetic siRNA that temporarily inhibits p53 expression that is in early development for acute kidney failure therapy. I5NP is the first siRNA to be systemically administered in humans. Based on the preclinical data obtained from animal models, the siRNA was intravenously injected within 4 h to bypass surgery patients. Pharmacokinetic data were collected during the first 24 h, and safety and dose-limiting toxicities were monitored until hospital discharge and 6–12 months after surgery. Recently, Quark initiated a subsequent clinical trial to determine whether a single administration of I5NP can prevent delayed graft function in kidney transplant recipients. Data from this study will be used to identify I5NP doses for follow-on efficacy studies (NCT00802347). Another ongoing phase I trial investigating solid tumors, including Renal cell carcinoma (RCC), was conducted by Calando Pharmaceuticals. The investigators used CALAA-01, whose active ingredient is a type of siRNA, to inhibit tumor growth and/or reduce tumor size. This siRNA inhibits the expression of the M2 subunit of ribonucleotide reductase and resists nuclease degradation by using a stabilized nanoparticle that targets tumor cells (NCT00689065). Besides, there is an ongoing study, in which patients with melanoma, kidney cancer, pancreatic cancer, or other solid tumors that are metastatic or cannot be removed by surgery are treated by APN401, siRNA-transfected autologous peripheral blood mononuclear cells. These cells were modified by siRNA targeting factors inhibiting the killing ability of immune cells in vitro and transfused back into the body, in order to kill more cancer cells (NCT02166255, the above clinical trials can be found at ClinicalTrials.gov, Table 1).

Study	Target/siRNA drug	Status	Disease
NCT00554359	I5NP	Phase I, completed	Kidney injury; acute renal failure
NCT00802347	I5NP	Completed	Delayed graft function in kidney transplantation
NCT00689065	CALAA-01	Phase I, terminated	Solid tumor cancers including RCC
NCT02166255	APN401	Phase I, recruiting	Melanoma, kidney cancer, pancreatic cancer, or other solid

Table 1. Clinical trials of siRNA therapy in kidney diseases.

7. Perspectives and challenges

Despite the enormous potential advantages of siRNA therapy, additional research must be performed before its large-scale clinical application.



### 7.1. Target gene selection

Genome-wide or pathway-specific siRNA libraries have become available using high-throughput screening approaches. Establishing *in vitro* prescreening leads to signaling pathway prediction and target validation in *in vivo* renal disease. However, choosing one or a set of reasonable target genes is the key for designing specific siRNA treatments. The pathophysiological changes during kidney disease, like any other disease, refer to a complex gene and protein regulation network. For example, the network that exists during kidney transplantation involves the original conditions of the donors and the interactions between the donor kidneys and the recipients, which could direct the progression, as well as the recovery, of the injury. Fortunately, transcriptome measurements of the transplanted kidney may provide a comprehensive understanding of gene regulation and would be beneficial for target gene selection.

Mueller et al. analyzed the transcriptome of postreperfusion implant biopsies in living donors (LD) and deceased donors (DD). Hundreds of mRNAs were identified that predicted delayed graft function [62]. In a recent prospective study using human posttransplant kidney biopsies, 20 mRNAs and two miRNAs were identified as molecular signatures of AKI. Elevated secretory leukocyte peptidase inhibitor in AKI allografts was validated and miR-182-5p was identified as a molecular regulator [63]. These genes could be used as potential targets of siRNA therapy. We recently identified 3 times more differentially expressed genes in renal allograft biopsies between living donors and cadaveric donors at 30 min than 3 months posttransplantation. The majority of these differentially expressed genes are responsible for acute responses at 30 min, but also involved in inflammation, nephrotoxicity, and proliferation at 3 months. These divergent transcriptome signatures between two types of donors might be linked with not only the initial injury of the donors, but also the immune responses of the recipients.

Another method for selecting target genes is by identifying their translation product proteins. To find a single or a set of crucial proteins involved in kidney allograft rejection, Wu et al. explored potential transcriptional factors and regulation networks in 352 kidney transplant recipients, of which 85 suffered from acute rejection (AR). The results demonstrated that the dominant processes and responses were associated with inflammation and complement activation in AR. A number of transcription factors were identified in AR patients, including NF- $\kappa$ B, signal transducer, and activator of transcription (STAT) 1 and STAT3 [64]. Their recent study further revealed inflammation-derived kidney allograft injury, such as AR, chronic rejection, and impaired renal function without rejection. Wu et al. 12 common proteins and 11 level-specific proteins from the phenotype-related protein–protein interaction networks [65]. These potential biomarkers also provide valuable targets for siRNA design relating to the treatment of transplant-related injury.

### 7.2. Timely application

Compared with shRNA, an advantage of siRNA for AKI therapy is time-controlled, transient treatment. Silencing the target gene for a short time or a long time should be assessed before RNAi application. The silenced genes may be multifunctional according to the surrounding milieu. For example, caspase-3, generally considered an executor in cellular apoptosis, should



be inhibited in injured tissues. However, it is also a loyal scavenger in malignantly transformed cells, which could be an unavoidable side effect in any caspase-3-targeting siRNA therapy. For AKI, siRNA ineffectiveness is needed after the therapeutic time window. Additionally, siRNA application avoids intracellular traffic. In certain circumstances, shRNA delivery could be harmful to the organ or even fatal.

A study from Grimm et al. investigated the long-term effects of sustained high-level shRNA expression in the livers of adult mice. An evaluation of 49 distinct adeno-associated virus/shRNA vectors, with unique lengths and sequences that were directed against six targets, showed that 36 vectors resulted in dose-dependent liver injury, with 23 ultimately causing death. The observed morbidity was associated with the downregulation of liver-derived miRNAs, indicating possible competition of the latter with shRNAs (through saturation of the endogenous RNAi machinery by the exogenous siRNAs) for the limited cellular factors required for the processing of various small RNAs [66]. Therefore, controlling intracellular shRNA expression levels will be imperative, but siRNA would not influence the endogenous process of RNA degradation mediated by miRNAs.

### **7.3. siRNA targeting single, double or multiple genes**

The knockdown of two or more genes simultaneously using siRNA cocktail has been recently reported. Many applications of siRNA cocktail have demonstrated significant benefits compared with siRNA targeted to a single gene, particularly in anticancer and antiviral therapy [67, 68]. A high concentration of individual siRNAs may represent the key off-target effect in terms of competition for endogenous miRNA biogenesis machinery. Therefore, the other advantage of siRNA cocktail is the relatively low concentration of each siRNA, which may also reduce off-target signatures without sacrificing silencing potency [69].

### **7.4. Cell-specific siRNA targeting**

We have showed that the apoptosis of different types of cells leads to different outcomes. For instance, the apoptosis of inflammatory cells is associated with inflammation clearance and tissue remodeling, whereas the apoptosis of renal parenchymal cells is link to tubular atrophy and renal fibrosis. Therefore, using the genetic material such as siRNA targeting specific cell at particular time frame is crucial to achieve high efficacy of treatment in AKI and also avoid site-effects [12, 54].

It is still challenging to administrate siRNA cell specifically, but it is feasible as there were a few studies showed delivering siRNA into liver cells and antigen-presenting cells [70–72] using carbon nanotubes and mannose-conjugated liposomes. In addition, surface pegylation and cell-specific targeting ligands incorporation in the carriers may improve the pharmacokinetics, biodistribution, and siRNA selectivity. Choosing appropriate siRNA carriers has to consider the safety, effectiveness, ease of manufacturing, off-target effects [12], and innate immune responses. Of course, the efficacy of siRNA is still a most important factor dominating the selection of its carriers [54].

### 7.5. siRNA-modified stem cell therapy

Mesenchymal stem cell (MSC) transplantation has attracted much attention in cell therapy in different organ systems such as myocardial infarction. One of the limitations is the poor survival of grafted cells in the ischemic microenvironment. To tackle this issue, a novel siRNA-mediated prolyl hydroxylase domain protein 2 (PHD2) silencing system has been developed based on arginine-terminated generation 4 poly (amidoamine) nanoparticles. This system, for the first time, exhibited effective and biocompatible siRNA delivery and PHD2 silencing in MSCs *in vitro*. After transplant PHD2 siRNA-modified MSC in myocardial infarction models, MSC survival and paracrine function of IGF-1 were enhanced significantly *in vivo*, with decreased cardiomyocyte apoptosis, scar size, and interstitial fibrosis, and increased angiogenesis in the diseased myocardium, which ultimately attenuated ventricular remodeling and improved heart function. This study demonstrated that a great potential of siRNA-modified stem cells in therapeutic applications, which, of course, might be used in AKI [73].

## 8. Conclusions

The kidney is a comparatively easy target organ for siRNA therapy due to its unique structural and functional characteristics. siRNA intervention is effective, feasible, and has great potential for fighting against kidney diseases. For the next-generation siRNA development, cell-specific precise delivery should be pursued. Although the safety of siRNA therapy has been proven by rapidly emerging clinical studies, off-target and compensative responses still need be overcome via various modification strategies. The time for realizing the therapeutic potential of RNAi has come because optimized siRNA therapy, in conjunction with advanced genetic screening technologies, could facilitate timely and specific treatment of kidney as well as other organ diseases in the near future.

### Author details

Cheng Yang<sup>1,2</sup> and Bin Yang<sup>3,4\*</sup>

\*Address all correspondence to: by5@le.ac.uk

1 Department of Plastic Surgery, Zhongshan Hospital, Fudan University, Shanghai, China

2 Shanghai Key Laboratory of Organ Transplantation, Shanghai, China

3 Medical Research Centre, Medical School of Nantong University; Department of Nephrology, Affiliated Hospital of Nantong University, Nantong, China, United Kingdom

4 Renal Group, Department of Infection, Immunity and Inflammation, University of Leicester, University Hospitals of Leicester, United Kingdom

## References

- [1] Bellomo R, Kellum JA, Ronco C: Acute kidney injury. *Lancet* 2012; 380:756–766. DOI: 10.1016/S0140-6736(11)61454-2
- [2] Palevsky PM, Molitoris BA, Okusa MD, Levin A, Waikar SS, Wald R, Chertow GM, Murray PT, Parikh CR, Shaw AD, et al: Design of clinical trials in acute kidney injury: report from an NIDDK workshop on trial methodology. *Clin J Am Soc Nephrol* 2012; 7:844–850. DOI:10.2215/CJN.12791211
- [3] Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T: Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 2001; 411:494–498. DOI:10.1038/35078107
- [4] Petrocca F, Lieberman J: Promise and challenge of RNA interference-based therapy for cancer. *J Clin Oncol* 2011; 29:747–754. DOI:10.1200/JCO.2009.27.6287
- [5] Jeker LT, Bluestone JA: Small RNA regulators of T cell-mediated autoimmunity. *J Clin Immunol* 2010; 30:347–357. DOI:10.1007/s10875-010-9392-7
- [6] Latronico MV, Condorelli G: RNA silencing: small RNA-mediated posttranscriptional regulation of mRNA and the implications for heart electrophysiology. *J Cardiovasc Electrophysiol* 2009; 20:230–237. DOI:10.1111/j.1540-8167.2008.01357.x
- [7] Yang C, Jia Y, Zhao T, Xue Y, Zhao Z, Zhang J, Wang J, Wang X, Qiu Y, Lin M, et al: Naked caspase 3 small interfering RNA is effective in cold preservation but not in autotransplantation of porcine kidneys. *J Surg Res* 2013; 181:342–354. DOI:10.1016/j.jss.2012.07.015
- [8] Racz Z, Hamar P: RNA interference in research and therapy of renal diseases. *Contrib Nephrol* 2008; 159:78–95. DOI:10.1159/000125587
- [9] Yang C, Zhang C, Zhao Z, Zhu T, Yang B: Fighting against kidney diseases with small interfering RNA: opportunities and challenges. *J Transl Med* 2015; 13:39. DOI: 10.1186/s12967-015-0387-2
- [10] Chaudhary A, Srivastava S, Garg S: Development of a software tool and criteria evaluation for efficient design of small interfering RNA. *Biochem Biophys Res Commun* 2011; 404:313–320. DOI:10.1016/j.bbrc.2010.11.114
- [11] Elmen J, Thonberg H, Ljungberg K, Frieden M, Westergaard M, Xu Y, Wahren B, Liang Z, Orum H, Koch T, Wahlestedt C: Locked nucleic acid (LNA) mediated improvements in siRNA stability and functionality. *Nucleic Acids Res* 2005; 33:439–447. DOI:10.1093/nar/gki193
- [12] Yang C, Zhao T, Zhao Z, Jia Y, Li L, Zhang Y, Song M, Rong R, Xu M, Nicholson ML, et al: Serum-stabilized naked caspase-3 siRNA protects autotransplant kidneys in a porcine model. *Mol Ther* 2014; 22:1817–1828. DOI:10.1038/mt.2014.111

- [13] Mook OR, Baas F, de Wissel MB, Fluiter K: Evaluation of locked nucleic acid-modified small interfering RNA in vitro and in vivo. *Mol Cancer Ther* 2007; 6:833–843. DOI: 10.1158/1535-7163.MCT-06-0195
- [14] Kanasty R, Dorkin JR, Vegas A, Anderson D: Delivery materials for siRNA therapeutics. *Nat Mater* 2013; 12:967–977. DOI:10.1038/nmat3765
- [15] Deng Y, Wang CC, Choy KW, Du Q, Chen J, Wang Q, Li L, Chung TK, Tang T: Therapeutic potentials of gene silencing by RNA interference: principles, challenges, and new strategies. *Gene* 2014; 538:217–227. DOI:10.1016/j.gene.2013.12.019
- [16] Yang B, Hosgood SA, Nicholson ML: Naked small interfering RNA of caspase-3 in preservation solution and autologous blood perfusate protects isolated ischemic porcine kidneys. *Transplantation* 2011; 91:501–507. DOI:10.1097/TP.0b013e318207949f
- [17] Yang B, Elias JE, Bloxham M, Nicholson ML: Synthetic small interfering RNA down-regulates caspase-3 and affects apoptosis, IL-1 beta, and viability of porcine proximal tubular cells. *J Cell Biochem* 2011; 112:1337–1347. DOI:10.1002/jcb.23050
- [18] Takabatake Y, Isaka Y, Mizui M, Kawachi H, Shimizu F, Ito T, Hori M, Imai E: Exploring RNA interference as a therapeutic strategy for renal disease. *Gene Ther* 2005; 12:965–973. DOI:10.1038/sj.gt.3302480
- [19] Wan X, Fan L, Hu B, Yang J, Li X, Chen X, Cao C: Small interfering RNA targeting IKKbeta prevents renal ischemia-reperfusion injury in rats. *Am J Physiol Renal Physiol* 2011; 300:F857–F863. DOI:10.1152/ajprenal.00547.2010
- [20] Hamar P, Song E, Kokeny G, Chen A, Ouyang N, Lieberman J: Small interfering RNA targeting Fas protects mice against renal ischemia-reperfusion injury. *Proc Natl Acad Sci U S A* 2004; 101:14883–14888. DOI:10.1073/pnas.0406421101
- [21] Xia Z, Abe K, Furusu A, Miyazaki M, Obata Y, Tabata Y, Koji T, Kohno S: Suppression of renal tubulointerstitial fibrosis by small interfering RNA targeting heat shock protein 47. *Am J Nephrol* 2008; 28:34–46. DOI:10.1159/000108759
- [22] Cuevas S, Zhang Y, Yang Y, Escano C, Asico L, Jones JE, Armando I, Jose PA: Role of renal DJ-1 in the pathogenesis of hypertension associated with increased reactive oxygen species production. *Hypertension* 2012; 59:446–452. DOI:10.1161/HYPERTENSIONAHA.111.185744
- [23] Gooding M, Browne LP, Quinteiro FM, Selwood DL: siRNA delivery: from lipids to cell-penetrating peptides and their mimics. *Chem Biol Drug Des* 2012; 80:787–809. DOI:10.1111/cbdd.12052
- [24] Braasch DA, Jensen S, Liu Y, Kaur K, Arar K, White MA, Corey DR: RNA interference in mammalian cells by chemically-modified RNA. *Biochemistry* 2003; 42:7967–7975. DOI:10.1021/bi0343774
- [25] Molitoris BA, Dagher PC, Sandoval RM, Campos SB, Ashush H, Fridman E, Brafman A, Faerman A, Atkinson SJ, Thompson JD, et al: siRNA targeted to p53 attenuates is-

chemic and cisplatin-induced acute kidney injury. *J Am Soc Nephrol* 2009; 20:1754–1764. DOI:10.1681/ASN.2008111204

- [26] Zheng X, Zhang X, Sun H, Feng B, Li M, Chen G, Vladau C, Chen D, Suzuki M, Min L, et al: Protection of renal ischemia injury using combination gene silencing of complement 3 and caspase 3 genes. *Transplantation* 2006; 82:1781–1786. DOI:10.1097/01.tp.0000250769.86623.a3
- [27] Zhang D, Liu Y, Wei Q, Huo Y, Liu K, Liu F, Dong Z: Tubular p53 regulates multiple genes to mediate AKI. *J Am Soc Nephrol* 2014; 25:2278–2289. DOI:10.1681/ASN.2013080902
- [28] Ojima I: Guided molecular missiles for tumor-targeting chemotherapy--case studies using the second-generation taxoids as warheads. *Acc Chem Res* 2008; 41:108–119. DOI:10.1021/ar700093f
- [29] Mokhtarieh AA, Cheong S, Kim S, Chung BH, Lee MK: Asymmetric liposome particles with highly efficient encapsulation of siRNA and without nonspecific cell penetration suitable for target-specific delivery. *Biochim Biophys Acta* 2012; 1818:1633–1641. DOI:10.1016/j.bbamem.2012.03.016
- [30] Leus NG, Morselt HW, Zwiers PJ, Kowalski PS, Ruiters MH, Molema G, Kamps JA: VCAM-1 specific PEGylated SAINT-based lipoplexes deliver siRNA to activated endothelium in vivo but do not attenuate target gene expression. *Int J Pharm* 2014; 469:121–131. DOI:10.1016/j.ijpharm.2014.04.041
- [31] Trochet D, Prudhon B, Vassilopoulos S, Bitoun M: Therapy for dominant inherited diseases by allele-specific RNA interference: successes and pitfalls. *Curr Gene Ther* 2015; 15:503–510. DOI:10.2174/1566523215666150812115730
- [32] Courtney DG, Atkinson SD, Allen EH, Moore JE, Walsh CP, Pedrioli DM, MacEwen CJ, Pellegrini G, Maurizi E, Serafini C, et al: siRNA silencing of the mutant keratin 12 allele in corneal limbal epithelial cells grown from patients with Meesmann's epithelial corneal dystrophy. *Invest Ophthalmol Vis Sci* 2014; 55:3352–3360. DOI:10.1167/iovs.13-12957
- [33] Allen EH, Atkinson SD, Liao H, Moore JE, Leslie Pedrioli DM, Smith FJ, McLean WH, Moore CB: Allele-specific siRNA silencing for the common keratin 12 founder mutation in Meesmann epithelial corneal dystrophy. *Invest Ophthalmol Vis Sci* 2013; 54:494–502. DOI:10.1167/iovs.12-10528
- [34] Takahashi M, Katagiri T, Furuya H, Hohjoh H: Disease-causing allele-specific silencing against the ALK2 mutants, R206H and G356D, in fibrodysplasia ossificans progressiva. *Gene Ther* 2012; 19:781–785. DOI:10.1038/gt.2011.193
- [35] Zuckerman JE, Gale A, Wu P, Ma R, Davis ME: siRNA delivery to the glomerular mesangium using polycationic cyclodextrin nanoparticles containing siRNA. *Nucleic Acid Ther* 2015; 25:53–64. DOI:10.1089/nat.2014.0505



- [36] Haussecker D: Current issues of RNAi therapeutics delivery and development. *J Control Release* 2014; 195:49–54. DOI:10.1016/j.jconrel.2014.07.056
- [37] Forbes DC, Peppas NA: Polycationic nanoparticles for siRNA delivery: comparing ARGET ATRP and UV-initiated formulations. *ACS Nano* 2014; 8:2908–2917. DOI: 10.1021/nn500101c
- [38] Li H, Tsui TY, Ma W: Intracellular delivery of molecular cargo using cell-penetrating peptides and the combination strategies. *Int J Mol Sci* 2015; 16:19518–19536. DOI: 10.3390/ijms160819518
- [39] van Asbeck AH, Beyerle A, McNeill H, Bovee-Geurts PH, Lindberg S, Verdurmen WP, Hallbrink M, Langel U, Heidenreich O, Brock R: Molecular parameters of siRNA –cell penetrating peptide nanocomplexes for efficient cellular delivery. *ACS Nano* 2013; 7:3797–3807. DOI:10.1021/nn305754c
- [40] Huang Y, Wang X, Huang W, Cheng Q, Zheng S, Guo S, Cao H, Liang XJ, Du Q, Liang Z: Systemic administration of siRNA via cRGD-containing peptide. *Sci Rep* 2015; 5:12458. DOI:10.1038/srep12458
- [41] Junquera E, Aicart E: Recent progress in gene therapy to deliver nucleic acids with multivalent cationic vectors. *Adv Colloid Interface Sci* 2015. DOI:10.1016/j.cis. 2015.07.003
- [42] Kosieradzki M, Rowinski W: Ischemia/reperfusion injury in kidney transplantation: mechanisms and prevention. *Transplantation Proceedings* 2008; 40:3279–3288. DOI: 10.1016/j.transproceed.2008.10.004
- [43] Eltzschig HK, Eckle T: Ischemia and reperfusion—from mechanism to translation. *Nature Medicine* 2011; 17:1391–1401. DOI:10.1038/nm.2507
- [44] Vaseva AV, Moll UM: The mitochondrial p53 pathway. *Biochim Biophys Acta* 2009; 1787:414–420. DOI:10.1016/j.bbabbio.2008.10.005
- [45] Fujino T, Muhib S, Sato N, Hasebe N: Silencing of p53 RNA through transarterial delivery ameliorates renal tubular injury and downregulates GSK-3 $\beta$  expression after ischemia-reperfusion injury. *Am J Physiol Renal Physiol* 2013; 305:F1617–F1627. DOI:10.1152/ajprenal.00279.2013
- [46] Peng J, Li X, Zhang D, Chen JK, Su Y, Smith SB, Dong Z: Hyperglycemia, p53, and mitochondrial pathway of apoptosis are involved in the susceptibility of diabetic models to ischemic acute kidney injury. *Kidney Int* 2015; 87:137–150. DOI:10.1038/ki. 2014.226
- [47] Liu L, Li Y, Hu Z, Su J, Huo Y, Tan B, Wang X, Liu Y: Small interfering RNA targeting Toll-like receptor 9 protects mice against polymicrobial septic acute kidney injury. *Nephron Exp Nephrol* 2012; 122:51–61. DOI:10.1159/000346953

- [48] Jackson AL, Bartz SR, Schelter J, Kobayashi SV, Burchard J, Mao M, Li B, Cavet G, Linsley PS: Expression profiling reveals off-target gene regulation by RNAi. *Nat Biotechnol* 2003; 21:635–637. DOI:10.1038/nbt831
- [49] Jackson AL, Burchard J, Schelter J, Chau BN, Cleary M, Lim L, Linsley PS: Widespread siRNA “off-target” transcript silencing mediated by seed region sequence complementarity. *RNA* 2006; 12:1179–1187. DOI:10.1261/rna.25706
- [50] Jackson AL, Linsley PS: Recognizing and avoiding siRNA off-target effects for target identification and therapeutic application. *Nat Rev Drug Discov* 2010; 9:57–67. DOI: 10.1038/nrd3010
- [51] Lin X, Ruan X, Anderson MG, McDowell JA, Kroeger PE, Fesik SW, Shen Y: siRNA-mediated off-target gene silencing triggered by a 7 nt complementation. *Nucleic Acids Res* 2005; 33:4527–4535. DOI:10.1093/nar/gki762
- [52] Kabilova TO, Meschaninova MI, Venyaminova AG, Nikolin VP, Zenkova MA, Vlasov VV, Chernolovskaya EL: Short double-stranded RNA with immunostimulatory activity: sequence dependence. *Nucleic Acid Ther* 2012; 22:196–204. DOI:10.1089/nat.2011.0328
- [53] Robbins M, Judge A, MacLachlan I: siRNA and innate immunity. *Oligonucleotides* 2009; 19:89–102. DOI:10.1089/oli.2009.0180
- [54] Yang C, Li L, Xue Y, Zhao Z, Zhao T, Jia Y, Rong R, Xu M, Nicholson ML, Zhu T, Yang B: Innate immunity activation involved in unprotected porcine auto-transplant kidneys preserved by naked caspase-3 siRNA. *J Transl Med* 2013; 11:210. DOI: 10.1186/1479-5876-11-210
- [55] Bell JK, Askins J, Hall PR, Davies DR, Segal DM: The dsRNA binding site of human Toll-like receptor 3. *Proc Natl Acad Sci U S A* 2006; 103:8792–8797. DOI:10.1073/pnas.0603245103
- [56] Choe J, Kelker MS, Wilson IA: Crystal structure of human toll-like receptor 3 (TLR3) ectodomain. *Science* 2005; 309:581–585. DOI:10.1126/science.1115253
- [57] Alexopoulou L, Holt AC, Medzhitov R, Flavell RA: Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* 2001; 413:732–738. DOI:10.1038/35099560
- [58] Hornung V, Guenthner-Biller M, Bourquin C, Ablasser A, Schlee M, Uematsu S, Noronha A, Manoharan M, Akira S, de Fougerolles A, et al: Sequence-specific potent induction of IFN-alpha by short interfering RNA in plasmacytoid dendritic cells through TLR7. *Nat Med* 2005; 11:263–270. DOI:10.1038/nm1191
- [59] Judge AD, Sood V, Shaw JR, Fang D, McClintock K, MacLachlan I: Sequence-dependent stimulation of the mammalian innate immune response by synthetic siRNA. *Nat Biotechnol* 2005; 23:457–462. DOI:10.1038/nbt1081

- [60] Sioud M: Induction of inflammatory cytokines and interferon responses by double-stranded and single-stranded siRNAs is sequence-dependent and requires endosomal localization. *J Mol Biol* 2005; 348:1079–1090. DOI:10.1016/j.jmb.2005.03.013
- [61] Racz Z, Godo M, Revesz C, Hamar P: Immune activation and target organ damage are consequences of hydrodynamic treatment but not delivery of naked siRNAs in mice. *Nucleic Acid Ther* 2011; 21:215–224. DOI:10.1089/nat.2010.0248
- [62] Mueller TF, Reeve J, Jhangri GS, Mengel M, Jacaj Z, Cairo L, Obeidat M, Todd G, Moore R, Famulski KS, et al: The transcriptome of the implant biopsy identifies donor kidneys at increased risk of delayed graft function. *Am J Transplant* 2008; 8:78–85. DOI:10.1111/j.1600-6143.2007.02032.x
- [63] Wilflingseder J, Sunzenauer J, Toronyi E, Heinzl A, Kainz A, Mayer B, Perco P, Telkes G, Langer RM, Oberbauer R: Molecular pathogenesis of post-transplant acute kidney injury: assessment of whole-genome mRNA and MiRNA profiles. *PLoS One* 2014; 9:e104164. DOI:10.1371/journal.pone.0104164
- [64] Wu D, Zhu D, Xu M, Rong R, Tang Q, Wang X, Zhu T: Analysis of transcriptional factors and regulation networks in patients with acute renal allograft rejection. *J Proteome Res* 2011; 10:175–181. DOI:10.1021/pr100473w
- [65] Wu D, Liu X, Liu C, Liu Z, Xu M, Rong R, Qian M, Chen L, Zhu T: Network analysis reveals roles of inflammatory factors in different phenotypes of kidney transplant patients. *J Theor Biol* 2014; 362:62–68. DOI:10.1016/j.jtbi.2014.03.006
- [66] Grimm D, Streetz KL, Jopling CL, Storm TA, Pandey K, Davis CR, Marion P, Salazar F, Kay MA: Fatality in mice due to oversaturation of cellular microRNA/short hairpin RNA pathways. *Nature* 2006; 441:537–541. DOI:10.1038/nature04791
- [67] Liu K, Chen H, You Q, Shi H, Wang Z: The siRNA cocktail targeting VEGF and HER2 inhibition on the proliferation and induced apoptosis of gastric cancer cell. *Mol Cell Biochem* 2014; 386:117–124. DOI:10.1007/s11010-013-1850-0
- [68] Zhou J, Neff CP, Liu X, Zhang J, Li H, Smith DD, Swiderski P, Aboellail T, Huang Y, Du Q, et al: Systemic administration of combinatorial dsRNAs via nanoparticles efficiently suppresses HIV-1 infection in humanized mice. *Mol Ther* 2011; 19:2228–2238. DOI:10.1038/mt.2011.207
- [69] Ge Q, Xu JJ, Evans DM, Mixson AJ, Yang HY, Lu PY: Leveraging therapeutic potential of multi-targeted siRNA inhibitors. *Future Med Chem* 2009; 1:1671–1681. DOI:10.4155/fmc.09.131
- [70] Jiang N, Zhang X, Zheng X, Chen D, Siu K, Wang H, Ichim TE, Quan D, McAlister V, Chen G, Min WP: A novel in vivo siRNA delivery system specifically targeting liver cells for protection of ConA-induced fulminant hepatitis. *PLoS One* 2012; 7:e44138. DOI:10.1371/journal.pone.0044138
- [71] Siu KS, Chen D, Zheng X, Zhang X, Johnston N, Liu Y, Yuan K, Koropatnick J, Gillies ER, Min WP: Non-covalently functionalized single-walled carbon nanotube for topi-

cal siRNA delivery into melanoma. *Biomaterials* 2014; 35:3435–3442. DOI:10.1016/j.biomaterials.2013.12.079

- [72] Chen D, Koropatnick J, Jiang N, Zheng X, Zhang X, Wang H, Yuan K, Siu KS, Shun-nar A, Way C, Min WP: Targeted siRNA silencing of indoleamine 2, 3-dioxygenase in antigen-presenting cells using mannose-conjugated liposomes: a novel strategy for treatment of melanoma. *J Immunother* 2014; 37:123–134. DOI:10.1097/CJI.0000000000000022
- [73] Zhu K, Lai H, Guo C, Li J, Wang Y, Wang L, Wang C: Nanovector-based prolyl hydroxylase domain 2 silencing system enhances the efficiency of stem cell transplantation for infarcted myocardium repair. *Int J Nanomedicine* 2014; 9:5203–5215. DOI: 10.2147/IJN.S71586

IntechOpen

